Differential effect of vascularity between long- and short-term survivors with IDH1/2 wild-type glioblastoma

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Introduction: IDH1/2 wt glioblastoma (GB) represents the most lethal tumour of the central nervous system. Tumour vascularity is associated with overall survival (OS), and the clinical relevance of vascular markers, such as rCBV, has already been validated. Nevertheless, molecular and clinical factors may have different influences on the beneficial effect of a favourable vascular signature.

Purpose: To evaluate the association between the rCBV and OS of IDH1/2 wt GB patients for long-term survivors (LTSs) and short-term survivors (STSs). Given that initial high rCBV may affect the patient’s OS in follow-up stages, we will assess whether a moderate vascularity is beneficial for OS in both groups of patients.

Materials and methods: Ninety-nine IDH1/2 wt GB patients were divided into LTSs (OS ≥ 400 days) and STSs (OS < 400 days). Mann-Whitney and Fisher, uni- and multi-parametric Cox, Aalen’s additive regression and Kaplan-Meier tests were carried out. Tumour vascularity was represented by the mean rCBV of the high angiogenic tumour (HAT) habitat computed through the haemodynamic tissue signature methodology (available on the ONCOhabitats platform).

Results: For LTSs, we found a significant association between a moderate value of rCBVmean and higher OS (uni- and multi-parametric Cox and Aalen’s regression) (\(p = 0.0140\), HR = 1.19; \(p = 0.0085\), HR = 1.22) and significant stratification capability \(p = 0.0343\). For the STS group, no association between rCBVmean and survival was observed. Moreover, no significant differences \(p > 0.05\) in gender, age, resection status, chemoradiation, or MGMT methylation were observed between LTSs and STSs.

Conclusion: We have found different prognostic and stratification effects of the vascular marker for the LTS and STS groups. We propose the use of rCBVmean at HAT as a vascular marker clinically relevant for LTSs with IDH1/2 wt GB and maybe as a potential target for randomized clinical trials focused on this group of patients.
1 | INTRODUCTION

Glioblastoma with isocitrate dehydrogenase 1/2 wild type (IDH1/2 wt GB) is the most lethal and common tumour of the central nervous system,\(^1\) with patient median survival rates of 13-14 months,\(^1\) and represents about 90% of the cases of GB.\(^2\) Inter-patient tumour heterogeneity makes notable differences to the overall survival (OS) of GB patients, with angiogenesis being one of the most relevant processes involved in tumour heterogeneity.\(^3\)–\(^5\)

This process can be studied using non-invasive techniques, such as MRI, from the time of tumour diagnosis.\(^6\)–\(^11\) Hence, functional MRI techniques allow researchers and clinicians to study relevant vascular markers with prognostic, predictive and stratification capabilities. In particular, the relative cerebral blood volume (rCBV) which is the most consistently recognized independent predictor of survival.\(^6\)–\(^11\) For this, the haemodynamic tissue signature (HTS), a machine learning technology, is able to automatically define regions of interest within the tumour and the oedema based on the morphologic and perfusion analysis of the MRI of the patient. It also allows calculation of perfusion metrics within each habitat to study associations with patient OS.\(^12\)–\(^13\) These habitats are regions with different vascular behaviours that represent the high angiogenic tumour (HAT) part of the enhancing tumour, the low angiogenic tumour (LAT) part of the enhancing tumour, the infiltrated peripheral part of the oedema (IPE) and the pure vasogenic peripheral oedema (VPE). On the other hand, despite having demonstrated the robustness of these vascular markers,\(^13\) biological and clinical factors, which are not static with time, can affect the effectiveness of the prognostic image markers. We hypothesize that the influence of vascularity on the aggressiveness of the tumour can vary during the treatment of the GB. To address these issues, we consider it relevant to evaluate the usefulness and accuracy of these vascular markers in different groups of patients with time, to assess for any potential clinical benefit. In particular, we have focused on the HAT habitat, ie that region of the active tumour with higher levels of blood volume and flow, since it is the habitat that has been shown to be most capable of predicting survival in previous studies.\(^12\)–\(^13\)

Different studies have evaluated the molecular differences between long-term survivor (LTS) and short-term survivor (STS) groups of GB patients in relation to tumour angiogenesis, because of their relevance to improve patient prognosis and to decide on the correct therapeutic target.\(^5\) Burgenske et al.\(^14\) analysed the gene expression profile of IDH1/2 wt GB patients and split their patients into LTSs and STSs to elucidate which variables were associated with differences in survival. Their results showed apparent similarities between the two groups.

Despite those results, it was observed that LTSs presented a higher proportion of methylated O-6-methylguanine-DNA methyltransferase (MGMT), and for that group an enrichment of the genes of sphingomyelin metabolism was detected, which has been related to a decrease in tumour growth and angiogenesis.\(^14\) In addition, Michaelsen et al analysed the molecular profile of LTS and STS to identify cluster of differentiation 34 (CD34) mRNA level (regulator of GB angiogenesis by promoting new blood vessel networks\(^15\)) as prognostic for GB patient survival.\(^16\) Moreover, clinical factors, such as initial performance score,\(^17\) tumour size and location\(^18\) and completeness of tumour resection\(^19\) may also determine the likelihood of a patient becoming an LTS.

The evident efficacy of the rCBV has already been proven when dealing with clinical challenges such as diagnosis, non-invasive characterization of molecular profile and prediction of prognosis, amongst others,\(^20\) but the influence of this marker for LTSs and STSs has not yet been fully assessed. To elucidate this, we analyse in our work the prognostic and stratification capabilities of rCBV\(_{\text{mean}}\) at the HAT habitat for the LTS and STS groups, independently. In the current study, we evaluate whether the beneficial effect of having moderate and functional vascularity is constant with time and if it is presented in both LTS and STS groups.

2 | MATERIALS AND METHODS

2.1 | Patient information

This is a sub-study of the approved multicentre retrospective clinical trial NCT03439332. For this study, 99 IDH1/2 wt GB patients from five clinical centres were included. Participating centres were Hospital Universitario de La Ribera, Alzira, Spain; Hospital Clinic, Barcelona, Spain; Hospital Universitario Vall d’Hebron, Barcelona, Spain; Azienda Ospedaliero-Universitaria di Parma, Parma, Italy and Oslo University Hospital, Oslo, Norway.

A material transfer agreement was approved by all the participating centres and an acceptance report was issued by the ethical committee of each centre. The managing institution (Universitat Politècnica de València, Valencia, Spain) review board also approved this study.
The criteria to include patients in this study were: (a) adult patients (age > 18 years) with histopathological confirmation of IDH1/2 wt GB^{2} diagnosed between 1 January 2012 and 1 January 2018; (b) access to the complete preoperative MRI studies, including pre- and post-gadolinium T_{1}-weighted, T_{2}-weighted, fluid-attenuated inversion recovery (FLAIR) and dynamic susceptibility contrast (DSC) T_{2*-}weighted perfusion sequences; and (c) patients with a minimum survival of 30 days (since shorter survival is generally associated with incidents during the surgery or previous severe pathologies, and could generate misleading results).

The study cohort was divided in two groups as previously reported\textsuperscript{21,22}: LTSs were defined as those patients with an OS equal to or greater than 400 days and STSs were defined as those patients with an OS less than 400 days. Because this value is close to the median survival of the study population (384 days), it allows the number of patients in the two groups to be balanced. Patients still alive at readout were considered removed observations. The date of removal was the last date of contact with the patient or, if not available, the date of the last MRI examination.

### 2.2 MRI

Standard of care MR examinations were obtained for each patient before surgery, including pre- and post-gadolinium-based contrast-agent-enhanced T_{1}-weighted MRI, as well as T_{2}-weighted, FLAIR T_{2}-weighted and DSC T_{2*} perfusion MRI. A detailed description of the acquisition parameters used at each institution is shown in Table 1.

### 2.3 Processing of MRI and vascular markers

We used the HTS method, freely accessible at the ONCOhabitats platform at www.oncohabitats.upv.es, to process MR images and calculate the rCBV. The HTS is an automated unsupervised method to describe the heterogeneity of the enhancing tumour and oedema tissues by delineating four vascular habitats.

| TABLE 1 | Summary of the MRI acquisition parameters for each centre |
|---------|------------------------------------------------------------|
|         | MFS (T) | T_{R} (ms) | T_{E} (ms) | Matrix (mm) | Slice thickness (mm) | FOV (cm\textsuperscript{2}) | Number of dynamics |
| H. Ribera\textsuperscript{a} | 1.5 | T1 | 25 | 4.6 | 268 x 268 | 0.9 | 24 x 24 | – |
|         |       | T2 | 2000 | 120 | 320 x 199 | 5.0 | 23 x 18.3 | – |
|         |       | FLAIR | 1100 | 140 | 256 x 164 | 6.0 | 23 x 18.3 | – |
|         |       | DSC | 1650 | 40 | 116 x 116 | 2.2 | 24 x 24 | 80 |
| C. Barcelona\textsuperscript{b} | 3.0 | T1 | 12 | 4.68 | 256 x 256 | 1.0 | 24 x 24 | – |
|         |       | T2 | 3000 | 80 | 256 x 256 | 5.0 | 24 x 24 | – |
|         |       | FLAIR | 9000 | 164 | 256 x 256 | 5.0 | 24 x 24 | – |
|         |       | DSC | 1550 | 32 | 128 x 128 | 5.0 | 24 x 24 | 50 |
| H. Vall d’Hebron\textsuperscript{c} | 3.0 | T1 | 253 | 2.64 | 320 x 180 | 4.0 | 22 x 16.5 | – |
|         |       | T2 | 6100 | 91 | 512 x 326 | 4.0 | 22 x 17.5 | – |
|         |       | FLAIR | 9000 | 68 | 320 x 288 | 4.0 | 22 x 19.8 | – |
|         |       | DSC | 1450 | 45 | 128 x 128 | 5.0 | 23 x 23 | 60 |
| A. O. Parma\textsuperscript{d} | 3.0 | T1 | 8.18 | 8.18 | 256 x 256 | 1.0 | 24 x 24 | – |
|         |       | T2 | 6500 | 65.90 | 160 x 160 | 4.0 | 24 x 24 | – |
|         |       | FLAIR | 12000 | 96.72 | 384 x 224 | 4.0 | 24 x 24 | – |
|         |       | DSC | 1500 | 30 | 128 x 128 | 4.0 | 24 x 24 | 60 |
| Oslo U. H.\textsuperscript{e} | 3.0 | T1 | 5.2 | 2.3 | 512 x 512 | 1.0 | 25.6 x 25.6 | – |
|         |       | T2 | 3800 | 84 | 896 x 896 | 3.0 | 22.0 x 22.0 | – |
|         |       | FLAIR | 4800 | 325 | 512 x 512 | 0.9 | 25.6 x 25.6 | – |
|         |       | DSC | 1500 | 25/105 | 128 x 128 | 5.0 | 25.6 x 25.6 | 100 |

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\textsuperscript{b}Clinic de Barcelona
\textsuperscript{c}Hospital Vall d’Hebron
\textsuperscript{d}Azienda Ospedaliero-Universitaria di Parma
\textsuperscript{e}Oslo University Hospital

MFS, magnetic field strength; T_{R}, repetition time; T_{E}, echo time; FOV, field of view; T1, pre-gadolinium T_{1} weighted; T2, T_{2} weighted.
The HTS method includes four phases (Figure 1).

a. **Preprocessing**, incorporating correction of usual MRI artefacts such as magnetic field inhomogeneities and noise, multimodal registration, brain extraction, or motion correction.

b. **GB segmentation**, which implements a state-of-the-art deep learning 3D convolutional neural network that differentiates the enhancing tumour, the oedema, and the necrotic tissue. It is based on the directional class adaptive spatially varying finite mixture model (DCA-SVFMM), which is a clustering algorithm that combines Gaussian mixture modelling with continuous Markov random fields to make use of the self-similarity and local redundancy of the images. The methodology includes the unenhanced and GBCA-enhanced $T_1$-weighted sequences, the $T_2$-weighted sequence, and the fluid-attenuated inversion-recovery $T_2$-weighted sequence combined with atlas-based prior knowledge of healthy tissues to delineate the segmentation.

c. **DSC perfusion quantification**, which calculates the haemodynamic maps derived from the DSC perfusion sequence (rCBV, relative cerebral blood flow (rCBF), mean transit time, and $K_2$ permeability. All perfusion maps are normalized against contralateral unaffected white matter volume to achieve consistency and comparability across patients and cohorts. The normalization is performed automatically by a convolutional neural network trained with about 100 cases, which detects the contralateral unaffected white matter region with about 90% accuracy. To ensure a correct perfusion quantification and to avoid under- and over-estimation of perfusion marker, DSC perfusion quantification includes correction for contrast agent leakage effects. The HTS method implements the Boxerman leakage-correction method for $T_1$- and $T_2$-leakage effects, as well as gamma-variate fitting to remove the extravasation phase and second pass of the contrast bolus.

d. **HTS**. In this phase, an automated unsupervised segmentation algorithm performs the detection of the four vascular habitats each with its specific haemodynamic behaviour: the HAT habitat, the LAT habitat, the IPE habitat, and the VPE habitat. HTS habitats were delineated using a DCA-SVFMM structured clustering of rCBV and rCBF maps. The clustering includes two stages: (I) a two-class clustering of the whole enhancing tumour and oedema regions and (II) a two-class clustering performed by using only the rCBV and rCBF data within the regions obtained in the first stage. To ensure the reproducibility of the HTS, both stages were initialized with a deterministic seed method.

A more detailed definition of the methodology is included in Reference 12. The robustness of the HTS method, used to process the MR images and to calculate the rCBV from different hospitals and with different acquisition parameters, was demonstrated in the previous multicentre study. The results obtained in this study, which included 184 patients from seven different international centres, demonstrated that the
HTS standardization and quantification tools are robust to changes in MRI acquisition protocols, scanners, and MR techniques. In this sense, we employ this technology to ensure a robust analysis of the MRI, mitigating any potential bias introduced by the different acquisition protocols.

The vascular biomarker used in our study was the mean relative cerebral blood volume (rCBV_{mean})^{23-25} calculated in the HAT habitat, which was shown to be a relevant prognostic marker in previous studies.\textsuperscript{12,13}

### 2.4 Statistical analyses

We described the main demographic, clinical, and molecular variables for the LTS and STS groups and for the entire cohort. Possible differences in the distributions of these variables for the LTS and STS groups were assessed using Mann-Whitney and Fisher exact tests in MATLAB R2017b (MathWorks, Natick, MA). The significance level used in all the statistical analyses was 0.05.

To analyse the time-dependent influence of the vascular biomarker (rCBV_{mean} at HAT) on patient survival, we used Aalen’s additive regression model included in the library “survival” for R software. This model allows plotting of time-varying effects of covariates on patient survival.\textsuperscript{26}

To analyse the association between rCBV_{mean} at HAT and patient survival, we used both uniparametric and multiparametric Cox proportional hazard regression analyses with the entire cohort, and independently with LTS and STS groups. The proportional hazard ratios (HRs) with a 95% confidence interval (CI), as well as the associated \textit{p}-values, are reported. The multiparametric analysis includes MGMT methylation status as a covariable, as this factor could influence patient survival and affect the results of the uniparametric test.

In addition, the stratification capability of the rCBV_{mean} at HAT was evaluated with the Kaplan-Meier test. The analyses were performed with the entire cohort, as well as independently with the LTS and STS groups. For all tests, we evaluated the capability of the rCBV_{mean} at HAT to stratify the population into moderate vascular and high vascular groups, and we analysed if these two vascular groups presented different survival rates. We define moderate and high vascular as the two groups of patients generated by dividing a population using the optimum cutoff threshold according to the vascular marker. We calculated the optimum vascular cutoff threshold using the rCBV_{mean} at HAT and determined by the C-index method previously used\textsuperscript{13} which consists in analysing the effectiveness of each possible threshold by maximizing the C-index or area under the curve (AUC). The moderate vascular group included patients with an rCBV_{mean} lower than the calculated cutoff, and the high vascular group included patients with an rCBV_{mean} higher than the cutoff.

The log-rank test was used to determine any statistical difference between the estimated survival functions of the vascular groups. The optimal threshold, the number of patients included in each vascular group, the median OS rates of each group, the estimated C-index, and the \textit{p}-value are reported.

## 3 RESULTS

### 3.1 Description of the entire cohort, and the LTS and STS groups

A total of 99 IDH1/2 wt GB patients complied with the inclusion criteria and formed the entire cohort of the study. This population was divided into (i) the LTS group, which includes 45 patients (seven removed), and (ii) the STS group, which includes 54 patients (eight removed). The information related to the entire cohort and the LTS and STS groups is summarized in Figure 2.

Table 2 summarizes the most relevant demographic, clinical, and molecular features of the entire cohort and patients included in the LTS and STS groups.

No significant differences (\textit{p} > 0.05) in gender, age, resection status, chemoradiation, MGMT methylation status, or rCBV_{mean} at HAT were observed between the LTS and STS groups (Mann-Whitney and Fisher exact tests).

### 3.2 Differences between LTSs and STSs and the effect of rCBV on patient survival

The studied variables of the LTS and STS groups displayed similar distributions (Table 2), as did the mean rCBVs of the two groups (Table 3). However, we found a different effect of rCBV_{mean} at HAT on the patients’ OS in the LTS group compared with the STS group.

A significant negative association between the rCBV_{mean} level and OS was found for the LTS group (\textit{p} = 0.0140) but not for the STS group (\textit{p} = 0.3543). Results of the uniparametric Cox analysis are shown in Table 3. The highest HR (1.19) found for the LTS group implies an increase of one unit in the rCBV_{mean} at HAT, which will be equivalent to a 19% higher risk of death. This result suggests that, for the LTS group, patients with lower rCBV_{mean} in the HAT habitat had significantly longer survival. For the entire cohort, the beneficial effect of having moderate vascularity in the HAT habitat was close to statistical significance at \textit{p} = 0.06.
Table 4 depicts the results of the multiparametric Cox analyses, including the MGMT methylation status as a covariable. We did not have the MGMT methylation status information of 41 patients, so for those cases we used a mean imputation method. Collectively, combining MGMT methylation status and rCBVmean at HAT was significantly associated with OS for the entire cohort. Patients with lower rCBVmean at HAT and methylated MGMT had longer OS. Again, the influence of the combination of these two variables on OS is higher for the LTS group compared with the entire population (HR: 1.22 versus 1.10 for rCBVmean; 2.68 versus 1.80 for MGMT methylation status). Additionally, the statistical power

**TABLE 2** Demographic, clinical, and molecular features of the patients included in the study (whole cohort) and for each group (LTSs and STSs). p-values resulting from the Mann-Whitney and Fisher exact tests are also included. N, number of patients; RT-CT, radiochemotherapy

| Variables                  | LTSs (n = 45pts) | STSs (n = 54pts) | Entire cohort (n = 99pts) |
|----------------------------|------------------|------------------|--------------------------|
| N (%)                      | 45 (45.5)        | 54 (54.5)        | 99 (100)                 |
| % females                  | 31.1%            | 35.2%            | 33.3%                    |
| Mean age at diagnosis      | 59               | 61               | 60                       |
| Type of resection (N)      |                  |                  |                          |
| -Total                     | 22               | 18               | 40                       |
| -Partial                   | 17               | 25               | 42                       |
| -Biopsy                    | 6                | 10               | 16                       |
| -Unknown                   | 0                | 1                | 1                        |
| RT-CT (N)                  |                  |                  |                          |
| -Complete                  | 24               | 23               | 47                       |
| -Incomplete                | 2                | 6                | 8                        |
| -Unknown                   | 19               | 25               | 44                       |
| MGMT methylation status (N)|                  |                  |                          |
| -Methylated                | 14               | 11               | 25                       |
| -Unmethylated              | 14               | 19               | 33                       |
| -Unknown                   | 17               | 24               | 41                       |

**TABLE 3** Uniparametric Cox analysis of the association between the vascular marker (rCBV\textsubscript{mean} at HAT) and the survival for the entire cohort and for the LTS and STS groups

| Variables                  | LTSs (n = 45pts) | STSs (n = 54pts) | Entire cohort (n = 99pts) |
|----------------------------|------------------|------------------|--------------------------|
| Mean HAT rCBV \pm standard deviation | 7.49 \pm 2.39 | 7.48 \pm 2.41 | 7.45 \pm 2.39 |
| HR [95% CI]                | 1.19 [1.04, 1.38] | 1.06 [0.94, 1.20] | 1.09 [1.00, 1.20] |
| p-value                    | 0.0140*          | 0.3543           | 0.0601                   |

Table 4 depicts the results of the multiparametric Cox analyses, including the MGMT methylation status as a covariable. We did not have the MGMT methylation status information of 41 patients, so for those cases we used a mean imputation method. Collectively, combining MGMT methylation status and rCBV\textsubscript{mean} at HAT was significantly associated with OS for the entire cohort. Patients with lower rCBV\textsubscript{mean} at HAT and methylated MGMT had longer OS. Again, the influence of the combination of these two variables on OS is higher for the LTS group compared with the entire population (HR: 1.22 versus 1.10 for rCBV\textsubscript{mean}; 2.68 versus 1.80 for MGMT methylation status). Additionally, the statistical power
of the results for the LTSs was the highest (with the lowest \( p \)-values). No significant result was found for the STS group when analysing the association between the HAT rCBV_{mean} and the MGMT methylation status with the OS.

The effect of the vascularity on OS is also shown using Aalen’s additive regression model. Figure 3 shows the marked incremental effect of both MGMT methylation status and rCBV_{mean} at HAT on OS from 400 days after diagnosis. Again, the influence of the rCBV_{mean} at HAT on OS is revealed for the LTS group. In addition, the patient baseline conditions (represented by the intercept) start to be relevant from 400 days after diagnosis, and relate to a beneficial effect on survival.

The results of the Kaplan-Meier analysis are summarized in Table 5, including estimated optimal cutoff thresholds (used to define the different groups—moderate and high vascular), number of patients per vascular group, estimated C-index, median OS calculated per each group, and log-rank test results (\( p \)-values).

We found a significant stratification capacity of the rCBV_{mean} when analysing the entire cohort (\( n = 99 \) patients) and when analysing the LTS group (\( n = 45 \) patients). However, the stratification in vascular groups related to survival was more robust when we analysed the LTS group, which yielded the highest C-index or AUC (0.690). Additionally, for the LTS group, we found the greatest difference in OS, 2.9 months, between the moderate vascular group (patients with an rCBV_{mean} at HAT lower than 8.97) and the high vascular group (patients with an rCBV_{mean} at HAT higher than 8.97). For the entire cohort, the test yielded a difference of only 1.7 months between these vascular groups. For the STS group, the OS was similar for the moderate and the high vascular groups and the log rank test did not yield significant results. Figure 4 shows an example of rCBV map and HTS habitats for a patient of each group (LTS—low vascular; LTS—high vascular; STS—low vascular, STS—high vascular).

These differences between the LTS and the STS groups in the efficacy of the vascular marker, when using the optimal cutoff threshold calculated for the entire cohort (6.30), are also illustrated with the Kaplan-Meier curves for both groups (LTS and STS) in Figure 5. For the STS group, the survival curves of the high vascular and moderate vascular groups are overlapping, indicating no apparent differences in survival time for

| Variables                  | LTSs (\( N = 45 \) patients) | STSs (\( N = 54 \) patients) | Entire cohort (\( N = 99 \) patients) |
|----------------------------|-------------------------------|-------------------------------|---------------------------------------|
|                            | HR [95% CI] \( p \)-value     | HR [95% CI] \( p \)-value     | HR [95% CI] \( p \)-value             |
| HAT rCBV_{mean}            | 1.22 [1.05, 1.42] *0.0085      | 1.05 [0.92, 1.19] \*0.4777     | 1.10 [1.00, 1.21] *0.0468             |
| MGMT methylation status    | 2.68 [1.15, 6.26] *0.0230      | 0.45 [0.18, 1.13] \*0.0898     | 1.80 [1.01, 3.22] *0.0471             |

**TABLE 4** Multiparametric Cox analysis of the association between the vascular marker (rCBV_{mean} at HAT) and the MGMT methylation status with survival for the entire cohort, the LTS group and the STS group

**FIGURE 3** Curves of Aalen’s additive regression model that illustrate the incremental effect of the variables MGMT methylation status, rCBV_{mean}, and intercept over time
patients included in the STS group, independently of their rCBVmean at HAT. However, for the LTS group, the vascular marker is capable of stratifying survival according to the level of rCBVmean in the HAT habitat.

The results of the Kaplan-Meier and log-rank tests derived from the analysis of the rCBVmean calculated at the LAT and IPE habitats are included in the Supporting Information (Tables S1 and S2, Figures S1 and S2).

**TABLE 5** Kaplan-Meier and log-rank test results for the vascular marker (rCBVmean at HAT) and the OS for the entire cohort and the LTS and STS groups

| Group           | rCBVmean HAT | Patients per group | Median OS per group | ΔOS (months) | p-value (log-rank test) |
|-----------------|--------------|--------------------|---------------------|--------------|------------------------|
|                 | rCBV threshold |                  |                     |              |                        |
|                 | [moderate, high] | AUC (C-index)      | [moderate, high]    |              |                        |
| Entire cohort   | 6.30         | 0.605              | [13.8, 12.1]        | 1.7          | 0.0275*                |
| LTS             | 8.97         | 0.690              | [18.7, 15.8]        | 2.9          | 0.0343*                |
| STS             | 6.32         | 0.415              | [10.6, 9.6]         | 1.0          | 0.5149                 |

**FIGURE 4** Example of rCBV maps and HTS habitats for each group

This study of the differential effect of vascularity in LTSs and STSs with IDH1/2 wt GB is based on the data from multicentre clinical trial NCT0343933213 and included 99 patients with IDH1/2 wt GB. We found that the beneficial effect of having a moderate rCBVmean can only be observed in patients surviving more than 400 days, ie those included in the LTS group. In addition, we also found significant stratification capacity of rCBVmean for the entire cohort, although the difference in OS between vascular groups was higher for the LTS (1.7 versus 2.9 months). We did not find an association between the rCBVmean biomarker and patient OS for the STS group. These results are compatible with previous studies in which the effect of vascularity was evaluated.8,12,13 In our study we went a step further to analyse whether the beneficial effect of having moderate vascularity in the HAT habitat is constant over time and if it is present in both long- and short-survival groups.

**DISCUSSION**

This study of the differential effect of vascularity in LTSs and STSs with IDH1/2 wt GB is based on the data from multicentre clinical trial NCT0343933213 and included 99 patients with IDH1/2 wt GB. We found that the beneficial effect of having a moderate rCBVmean can only be observed in patients surviving more than 400 days, i.e. those included in the LTS group. In addition, we also found significant stratification capacity of rCBVmean for the entire cohort, although the difference in OS between vascular groups was higher for the LTS (1.7 versus 2.9 months). We did not find an association between the rCBVmean biomarker and patient OS for the STS group. These results are compatible with previous studies in which the effect of vascularity was evaluated.8,12,13 In our study we went a step further to analyse whether the beneficial effect of having moderate vascularity in the HAT habitat is constant over time and if it is present in both long- and short-survival groups.
A possible explanation for the association of vascularity with survival in LTSs may rely on how the influence of vascularity increases significantly after approximately 400 days from diagnosis (Figure 3). This implies that, from that time, LTS patients with a moderate vascular signature will have longer survival than those patients with a high vascular signature. By contrast, in the STS group there are unknown factors that lead to poor survival, and tumour vascularity does not represent a variable that influences survival.

For the LTSs, vascularity marks the tumour behaviour, thus the rCBV\textsubscript{mean} image marker can be used as prognostic biomarker at an advanced stage, allowing a more accurate decision making from this time. In fact, we found a much higher HR than the one reported in our previous study, which included both wt and mutant IDH1/2 GB (HR: 1.19 versus 1.05, respectively).\textsuperscript{13} This may suggest that the prognostic value of this vascular biomarker is more accurate and clinically relevant for the IDH1/2 wt LTS group, rather than the entire population of GB patients.

Moreover, the different influence of tissue vascularity on survival between the LTS and STS groups may be also influenced by several clinical factors.\textsuperscript{27,28} Comorbid conditions, such as cardiovascular or pulmonary diseases,\textsuperscript{27} or early deaths caused by treatment complications, could hide the effect of vascularity on OS in the STS group, whereas for the LTS group the significant effect of the vascularity becomes apparent, revealing a strong association with patient OS. Comparing the distributions of main demographics (age and gender) and clinical characteristics (type of resection, completeness of standard treatment), we did not find significant differences between the groups.

Moreover, it is well known that patients with the methylated MGMT promoter benefit most from treatment with temozolomide,\textsuperscript{29} an integral component of the standard treatment for GB patients.\textsuperscript{30} We analysed the distributions of patients with methylated and non-methylated MGMT in each group, as well as the completeness of the Stupp treatment. No significant differences were observed in this regard, suggesting that these variables do not impact the observed effect of vascularity on survival. Furthermore, the results of the combined analysis of the rCBV and the MGMT methylation status are consistent with the uniparametric analyses, so the MGMT methylation status does not seem to be affecting the differential influence of vascularity between LTSs and STSs. In addition, we have found that the MGMT methylation status, as for rCBV, seems to be relevant not at the beginning of the disease, but from 300-350 days onwards. Hence, it is consistent not to observe an influence of the state of methylation from the initiation, since the benefit produced by MGMT methylation is associated with treatment with temozolomide. However, the combination of these two factors (rCBV and MGMT methylation) improves their association with patient survival both for LTSs and for the entire cohort, and p-values were found to be lower than in the uniparametric analysis.

Other molecular factors, however, might also influence our results. The influence of telomerase reverse transcriptase (TERT) promoter mutation on survival of GB patients has been suggested by several authors. More than 90% of GB are IDH1/2 wt; amongst them, about 80% have mutations on the TERT promoter, which confers a worse prognosis. In two studies,\textsuperscript{31,32} the negative impact of TERT promoter mutations on survival of patients with IDH1/2 wt GB becomes visible only after approximately 400 days of evolution; before this point, survival curves overlapped. Thus, the distribution of TERT mutations in our patient series could help understand the different influence of vascular biomarkers on the LTS and STS groups. Of note, the molecular profiles of IDH1/2 wt and TERT mutation have been associated with the classical and mesenchymal subtypes,\textsuperscript{31,33} of which the latter is associated with active angiogenesis.\textsuperscript{33,34}

Our study has some limitations. First, more detailed clinical information on completion of treatment and the second line treatment type, comorbidities and treatment complications would have helped to explain our results, and would have allowed a more thorough discussion about this aspect. Moreover, MGMT promoter methylation status was unknown in 40% of patients, and other molecular features, such as TERT promoter
mutation status, were not recorded. The lack of extensive clinical and molecular information is due to the retrospective nature of the study. Thus, for future studies, we aim to analyse the association between the molecular profile of tumours and patients’ clinical factors with the imaging markers and the effect of vascularity on survival. The combined information may provide a better understanding of the influence of vascular biomarkers on the evolution of GB. Furthermore, despite having a considerably large total cohort (99 patients), the high rCBV-LTS group only includes 12 patients. Finally, the estimated thresholds calculated to define the different vascular groups are statistical values and not parameters that can be used in the clinic yet.

Related to further work, image-based vascular biomarkers have proven their ability to guide anti-angiogenic therapy for GB, but this therapy only benefits specific populations of glioma patients. Lui et al. used MRI features to define a subgroup of GB patients with higher angiogenic activity and better response to the antiangiogenic treatment. Based on the main results of our study, it would be worth studying whether only the group of LTSs with high values of rCBV_mean at HAT would be the best responders to antiangiogenic treatment.

In conclusion, in our study we found that a moderate rCBV_mean level in the HAT habitat is associated with prolonged survival in patients with GB, particularly in a cohort of 45 IDH1/2 wt GB patients that survive more than 400 days. For the long-surviving group, the vascular rCBV_mean image marker can differentiate patients with moderate vascularity and longer OS from patients with high vascularity and shorter OS. However, this association between vascularity and patient survival was not found for patients who did not survive more than 400 days. Therefore, we propose the use of rCBV_mean in HAT as a clinically relevant prognostic marker for LTSs with IDH1/2 wt GB. In addition, it could be used as a potential target for randomized clinical trials that focused on this group of patients.

DATA AVAILABILITY STATEMENT
Research data are not shared.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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