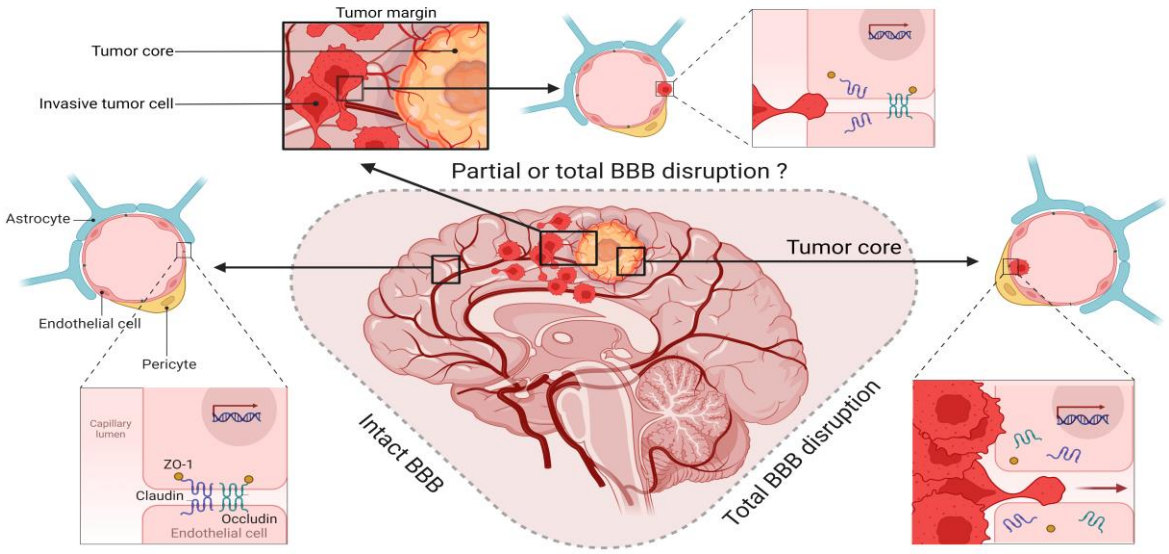
Developing more representative laboratory models of the blood-tumour barrier to improve the drug development pipeline for patients with glioblastoma.

AIMS

The project aimed to characterise the blood-brain barrier (BBB) in marginal regions of glioblastoma, examining its structure and composition, and to determine how these features influence the ability of small molecule drugs to penetrate different regions of glioblastoma and the healthy brain. Our objectives were to:

- Define the structural, cellular and molecular properties of the BBB in tumour margin blood vessels and establish how they differ from the intact BBB of normal brain vessels and the extensively disrupted BBB found within tumour core regions.
- Evaluate how alterations in the BBB affect the ability of small-molecule drugs to penetrate infiltrative tumour margin regions.



KEY FINDINGS

- We developed a new analytical pipeline to characterise the structure and composition of blood vessels within tumour margin regions of glioblastoma.
- This pipeline enabled us to demonstrate for the first time that blood vessels in tumour margin regions are distinct from those in the tumour core and the normal brain. We observed that vessels in tumour core regions were more abundant and displayed greater variability in size and structure. Margin regions contained smaller numbers of blood vessels density, some of which were unique to the region and others similar to tumour core or normal brain vessels.
- Using state of the art microdialysis techniques we demonstrated different distributions of two different PARP inhibitors in GBM core and margin regions and the normal brain.

WHAT DID THE STUDY INVOLVE?

This study was conducted by a multidsciplinary team of laboratory scientists and clinicians and used human tumour samples along with studies in mice whose brain had been implanted with GBM tumours. These mice underwent advanced MRI scans to measure blood flow and blood vessel leakage, and had tiny needles inserted in different regions of their brains to measure drug levels at different times after dosing.

Human tissue analysis:

Tumour core, tumour margin and adjacent normal brain regions were identified on surplus tissue sections previously obtained from patients with GBM. Tissue sections were stained with antibodies detecting different components of blood vessels and the BBB. These procedures generated massive amounts of data from hundreds of blood vessels. To analyse this data we developed a new computational image analysis pipeline and used statistical methods to identify the structural and molecular features that differed between blood vessles in the tumour core, tumour margin, and normal brain.

MRI studies in mice (WP2):

To study differences in BBB function in different regions of the brain in living mice, we implanted GBM tumours in mice and undertook specialised MRI scans. These scans provided information on tumour infiltration, blood flow and and blood vessel leakiness in the different regions. We used a particular mouse model of GBM called G7 that captures most of the features of the human disease.

Microdialysis studies (WP3):

Tiny needles called microdialysis probes were implanted into tumour core, tumour margin and healthy brain regions of mice with implanted GBM tumours. Mice received oral doses of two different drugs (olaparib or pamiparib), and samples of fluid from the different brain regions were collected at several different times after dosing. We then measured drug levels in these samples and compared these levels between the different brain regions.

Public involvement:

Public and patient involvement was embedded throughout the project through a longstanding relationship with Helen Bulbeck, Director of Services and Policy at the UK brain tumour charity brainstrust. Helen has extensive experience as both a patient and caregiver, as well as a very good understanding of how to involve patients and their families in brain tumour research. Helen attended and contributed to our monthly study meetings to ensure that the research addressed issues important to patients, and invited on of her PPI colleagues (Christine Littler) to join the team. Christine had not previously beein involved in any scientific research and provided useful feedback to the research team about how to make our discussions understandable to non-scientists.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

1. Blood vessels in the tumour margin are much smaller than vessels in tumour core regions and are also less variable in size and shape than tumour core vessels. They share some similarities with vessels in the normal brain in terms of shape and size but are more variable. There are many fewer blood vessels in tumour margin regions than in the tumour core (see Figure 1).

2. The composition of blood vessels in tumour core regions is very different from vessels in normal brain – they have very high levels of certain BBB proteins but these are expressed in a very disorganised fashion. The composition of blood vessels in tumour margin regions is more similar to those in the healthy brain but there are some important differences and some of the vessels show the disorganised features seen in tumour core regions, although to a lesser extent (see Figure 2).

These results confirm that the BBB is very different in tumour margin regions than either normal brain or tumour core and has specific characteristics that should be taken into consideration when designing new drugs for GBM.

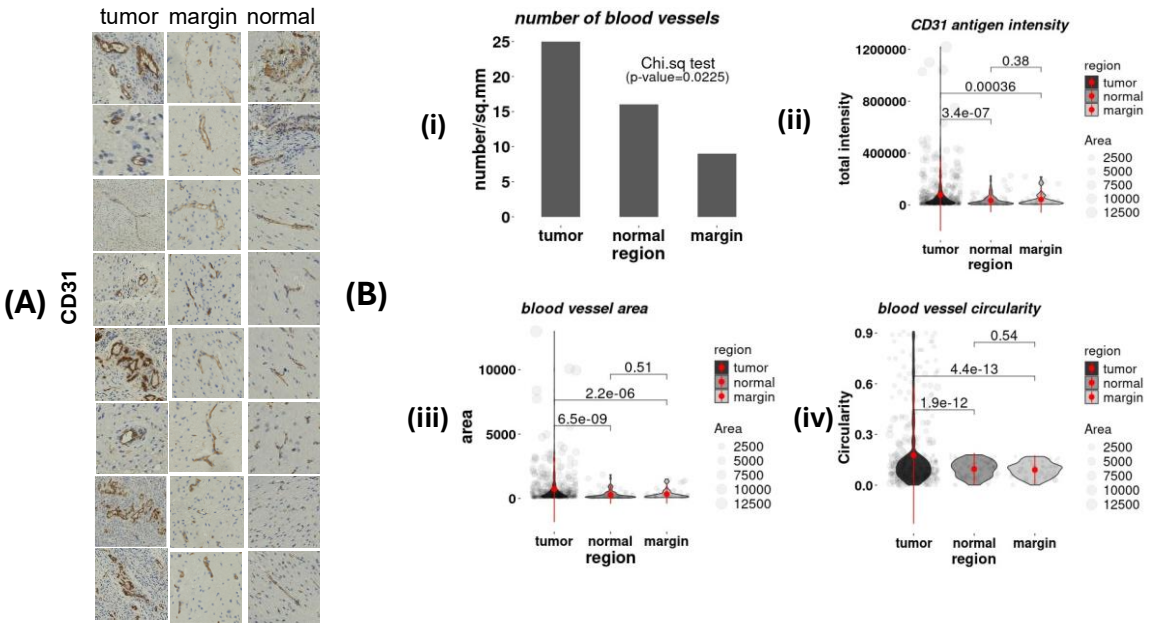


Figure 1. (A) Blood vessels outlined by CD31 staining of tissue from tumor core, margin and normal brain regions of patients with GBM. (Bi) The number of blood vessels in each region. (Bii) Intensity of staining for CD31 (found in the lining of small blood vessels) in each region. (Biii) The size of blood vessels in each region. (Biv) The circularity of the CD31 stained blood vessels in each region.

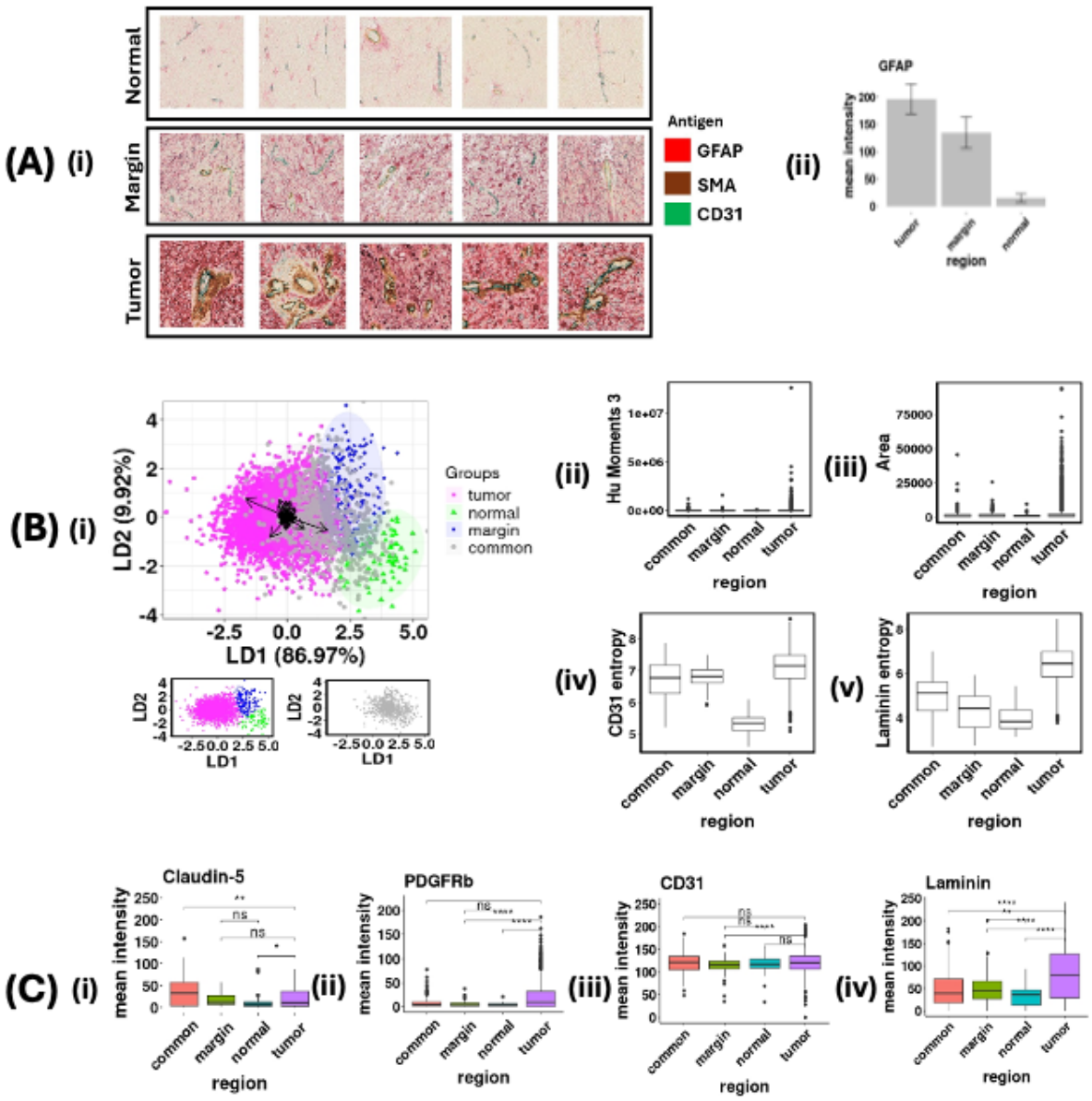


Figure 2. (Ai) Features and composition of blood vessels in each region after staining for three different proteins. (Aii) Intensity of staining GFAP which identifies tumour cells. (Bi) Our computational model was able to distinguish blood vessels from the different regions. (Bii-v) Some of the different image features that the model used to distinguish the different vessels (Ci-iv) Intensity of staining for different BBB proteins in the different regions.

3. Advanced MRI scans can detect tumour cell infiltration in the margin regions of GBM and show that blood flow is lower in these regions than in the normal brain. This helped us understand the drug distribution information described in the next section and gives further useful information on how to design drugs that will penetrate the tumour margin regions.

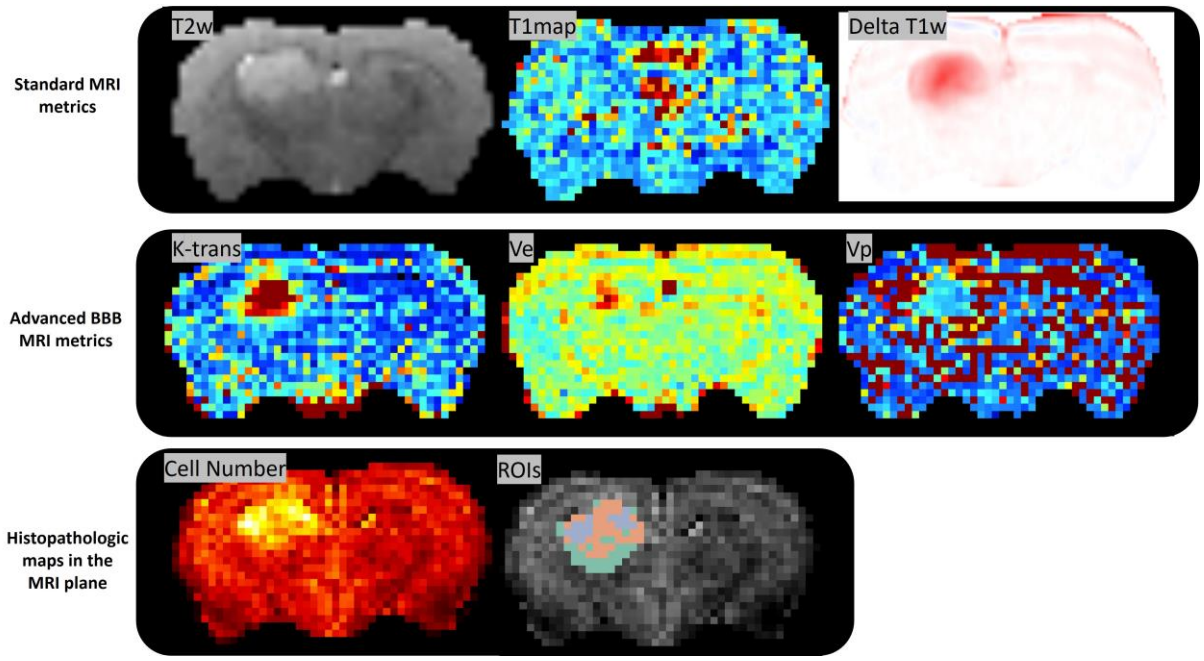


Figure 3. MRI images of mice with implanted GBM tumours. Each image shows a different MRI technique that gives specific information about tumour infiltration, blood flow and blood vessel leakiness. These images were registered with tissue sections obtained from the same mice (shown in the bottom row) – this enabled us to confirm what the MRI images were showing.

4. Our microdialysis drug sampling studies showed that different drugs cross the BBB to different extents in the different regions (tumour core, margin and normal brain), and that drug levels are influenced by blood flow as well as blood vessel leakiness (see Figure 4). These results confirm that features of the blood vessels in tumour margin regions have an impact on how well different drugs are able to penetrate those regions.

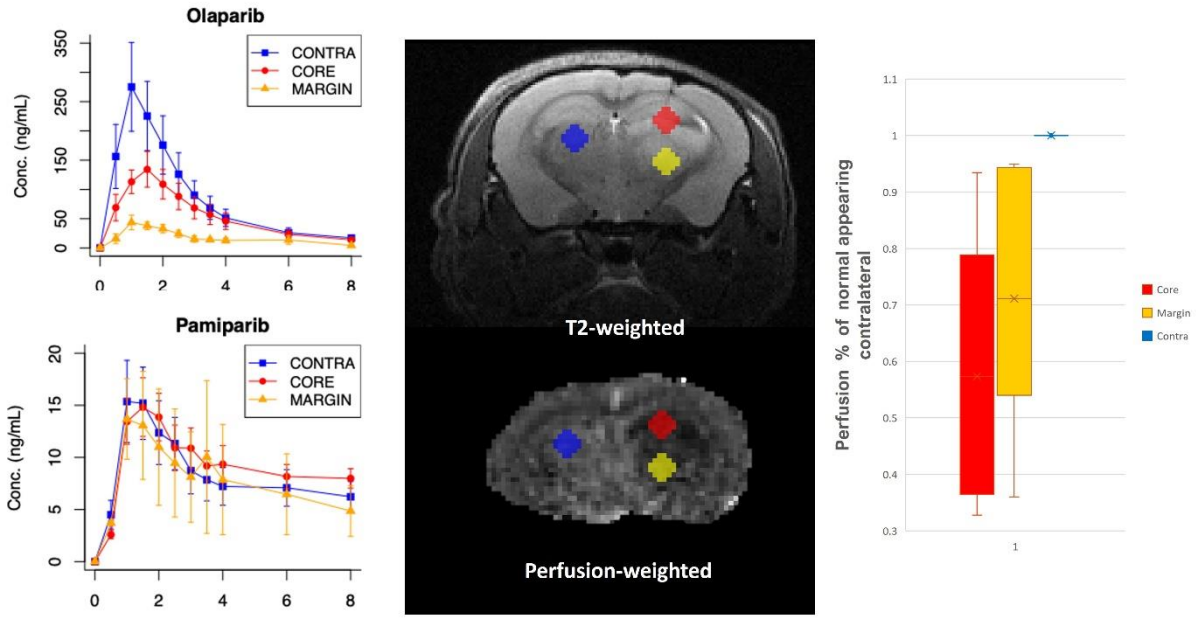


Figure 4. Concentrations of olaparib and pamiparib were measure in live mice using microdialysis catheters placed in tumour core, tumour margin and healthy brain regions represented by the different colours. The graph on the right shows the blood flow is lower in tumour core (red) and tumour margin (yellow) vessels than in the healthy brain (blue).

WHAT IMPACT COULD THE FINDINGS HAVE?

- Impact for patients: Current treatments for GBM are not effective and attempts to improve outcomes with new drugs have been unsuccessful. This shows that the current drug development pipeline is not working. Our results provide new information about the structure and function of blood vessels in tumour margin regions that will enable researchers to develop new drugs that are better able to penetrate this important region of GBM that has not previously been properly studied. This will increase the likelihood of developing more effective treatments for patients with this cancer.

- Impact on clinical practice: As described above, our findings may lead to the development of more effective treatments for patients with GBM.

HOW WILL THE OUTCOMES BE DISSEMINATED?

This work was presented as a poster at the 2025 Annual Meeting of the European Association of Neuro Oncology (EANO). Our new image analysis pipeline has been written up as a manuscript that will be submitted to Neuro Oncology Advances journal within the next four weeks. All the data generated by this pipeline, along with the MRI and microdialysis work, will be submitted to Neuro-Oncology journal within the next six months. This data will also be submitted as an abstract to the EANO Annual Meeting in 2027. Working with our PPI partners we will create a lay-friendly summary of our research findings for dissemination to patients, relatives and the general public.

CONCLUSION

We established a tissue staining protocol and image analysis pipeline capable of identifying blood vessels and characterising their structure and composition. This pipeline generated new data that improves our understanding of the blood vessels in tumour margin regions of GBM. Tumour margin vessels shared several features with vessels in the healthy brain but were less abundant and displayed greater variability in structure and composition. These findings were supplemented by MRI and microdialysis studies in mice implanted with GBM tumours, which provided additional new information about blood flow, blood vessel leakiness and the ability of different drugs to penetrate the different tumour margin regions of GBM. Altogether, our results significantly increase our understanding of the blood vessels in tumour margin regions, and this information will help researchers develop more effective drug treatments for GBM.

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