Supplementary discussion

Perfusion MRI methodology

Perfusion analysis in the present study is based on DCE-MRI. The pharmacokinetic model used to derive perfusion parameters is the adiabatic approximation to the tissue homogeneity model (ATH), implemented according to (1). Local arterial input functions were extracted from the temporal muscle adjacent to the tumor using a blind deconvolution approach (2). Finally, absolute perfusion parameters values were obtained by scaling the parameters to a known value of the extravascular extracellular space, \( V_e \), in the muscle, as described in (3). This method provides advantages over DCE-MRI methods based on the (extended) Tofts model, and over the DSC-MRI methods which are commonly used in the clinic.

With the extended Tofts model in DCE-MRI, only three independent parameters can be calculated (\( V_b, V_e \) and \( K_{\text{trans}} \)) from which others can be derived, but no separation between F and PS is possible. With the ATH model four independent parameters can be calculated (\( V_b, V_e, PS \) and F) from which others such as \( K_{\text{trans}} \) can be derived, providing the needed separation between blood flow and vessel permeability parameters.

Dynamic Susceptibility MRI (DSC-MRI) is based on T2 or T2* weighted images. These images are converted to R2 or R2* relaxation rate which is proportional to the contrast agent concentration through the proportionality factor r2 or r2* (T2- or T2*-relaxivity). This factor depends on tissue and on the actual contrast agent concentrations in the intra- and extra-vascular space (contrast agent compartmentalization). This makes absolute quantification of the contrast agent and hence perfusion parameters a challenge (4, 5). It has been shown in many studies that cerebral blood flow (CBF) and volume (CBV) can be quantified only relatively using DSC-MRI (6, 7). Only the mean transit time (MTT) can be absolutely quantified in case of non-leaky capillaries. This is not
a problem with DCE-MRI which is based on T1 weighted images. DCE-MRI provides estimates of absolute contrast agent concentration because the relaxivity $r_1$ is not tissue dependent and is not affected by contrast agent compartmentalization.

DSC-MRI mostly assumes no extravasation of the contrast agent. Although techniques exist to partially compensate for this contrast agent leakage, the method is inherently not suited to evaluate the permeability of blood vessels independently from the blood flow.

**Robustness of the pharmacokinetic model**

The robustness of a related pharmacokinetic model (distributed capillary adiabatic tissue homogeneity model – DCATH) has been analysed in (8). In this paper, the accuracy and precision of the perfusion parameters are given as confidence intervals calculated using two different approaches. Confidence intervals ranging from 15% to 38% have for example been calculated for the parameters used in our study in the case that corresponds to high vascular permeability (see Table 3 & 4, $F_p/PS/K^{\text{trans}}/v_p$, percentile 50, Tumor II signals). In comparison to this reference study, our study has the benefits of a simpler model (ATH model with 4 perfusion parameters versus DCATH with 5 perfusion parameters). The reduction of the number of parameters to estimate can be expected to lead to better accuracy and precision. Furthermore, our temporal sampling period (0.7 s) is substantially lower than in (8) (1.5 s) which also leads to a better accuracy and precision achievable with our data.

The SNR calculated in our recordings is however somewhat lower than in this reference study, reaching 10 dB in the control animals and less in the bevacizumab treated animals (SNR definition according to (8)). How this exactly affects the accuracy and precision of the parameters has not been evaluated. We could have improved the SNR by temporally and spatially grouping measurement points. We instead chose to favour spatial and temporal resolution
(to account for heterogeneity in the histogram analysis) and were still able to provide robust mean parameters values over the whole tumors. This resulted in the realistic parameters maps reported in Fig 2, 3 & 4. Furthermore, in our study we report results of statistical analysis of a large set of voxels which, to some extent, compensates the suboptimal precision of single-voxel perfusion parameter estimates.

Another evaluation of the accuracy and precision achievable with the ATH model can be found in (9), in which covariance of calculated parameters are shown as 95% confidence intervals for simulated data of two different recordings corresponding to different vascular permeabilities. As in our model, the bolus arrival time is estimated as one of the perfusion parameters (although not using the Fourier domain as in our approach). Compared to our data, the temporal sampling interval in (9) is much higher (3.5-5.1s) and the length of the acquisition is shorter (7-10min), meaning that a better accuracy and precision can be expected for the processing of our recordings.

The effect of a residual contrast agent is negligible as the half-life for blood elimination of low molecular weight contrast agents (as used in the present study) is about 1.5 hrs for clinical examinations and about 15 min for rats (10). We typically allowed a minimum delay of 48 hrs (that is about 200x the half-life) between 2 consecutive scans of the same animals so we can safely assume that all the contrast agent was cleared from the blood by then. Baseline signals (acquired prior to contrast agent injection) for the different curves remain similar for all time points and were subtracted from the raw data as a part of conversion to contrast agent concentration. This provides an additional guarantee that residual contrast agent in tissue (if any) did not bias the analysis.

Typical examples of raw signal curves obtained in our study are shown in the supplementary figure S4. The tumor signal curves for the control animal (Suppl. Fig. S4A) shows the typical shape of leaky vasculature and a signal level notably
weaker than the one in the muscle on Day 1 but of a similar level at the other
time points. The tumor signal curves for the animal treated with bevacizumab
(Suppl. Fig. S4B) still shows the shape of leaky vasculature (despite the
morphological normalisation) and a weaker signal than in the muscle for every
time point.

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Supplementary figures

**Figure S1**: Animal models

**Figure S1**: Morphological and histological characteristics of P3 and P13. T1 gadolinium-enhanced MRI shows a decrease in CE in the bevacizumab treated animals (A). H&E sections show necrotic areas in both models, highly pronounced in the P13 model (B) (Scale bars = 100 μm). Vessel staining performed with anti-human vWF, shows decreased angiogenesis in the bevacizumab treated group in both models (C) (Scale bars = 300 μm). Nestin staining performed to assess invasion into the cortex and the collateral hemisphere, indicates an increased invasion in the treated group in both P3 and P13 (D). Hypoxia staining with pimonidazole indicates that the treated tumors are more hypoxic in both models (E) (Scale bars = 100 μm).
Figure S2: Study design

Figure S2: Nude rats were implanted with P3 or P13, screened by MRI after 3 weeks, and split into treatment groups (controls, bevacizumab 10mg/kg, bevacizumab 5mg/kg). Treatment was given twice a week. Animals were scanned longitudinally for up to two weeks by MRI or PET, after which they were sacrificed and tissue was collected for histology.
Figure S3: Tumoral $K_{\text{trans}}$ evolution

(A) Illustrative maps of $K_{\text{trans}}$ evolution for animals implanted with the highly angiogenic P13 tumor model, showing one control (top line) and two animals treated with respectively high and low doses of bevacizumab (middle and bottom lines). (B) Quantification of tumors mean $K_{\text{trans}}$ for P13 animals (top line) and histogram analysis of tumoral voxels $K_{\text{trans}}$ distribution (bottom line). In comparison to controls, animals treated with bevacizumab high or low doses, show a reduced tumor mean $K_{\text{trans}}$ and a reduced fraction of high $K_{\text{trans}}$ voxels shortly after the start of the treatment, again suggesting a normalisation of blood vessel morphology. For the less angiogenic, more infiltrative P3 model (C-D), the normalisation windows extent throughout the whole observation period. $K_{\text{trans}}$ expressed in absolute values of ml/min/100ml of tissue. Thresholds for the high, medium and low values (dark, hatched and light blue/red) were defined by the 75% and 25% percentiles of the whole voxels population for the given tumor model. Animals per group: P13 Controls-5, P13 Bev high-5, P13 Bev low-5, P3 Controls-7, P3 Bev high-7. Bev high: 10mg/kg, Bev low: 5mg/kg.
Figure S4: Examples of DCE-MRI raw data and signal curves for a P13 control rat (A) and a P13 animal treated with high doses of bevacizumab (B). Top rows: examples of images of the DCE-MRI sequences at various time points - time frame at 1 min which corresponds to the peak of the tissue curves. Bottom rows: corresponding mean signal intensity time curves in the muscle (green), from which arterial input functions were extracted, tumor (red) and contralateral brain (blue), with outlines shown in the DCE-MRI images above. Dots - raw signal, line - median filtered signal (window length: 21 samples). For better visibility, the signals of the contralateral brain regions were shifted upwards by adding a constant (0.3x10^5). The tumor signal curves for the control animal (A) shows the typical shape of leaky vasculature and a signal level notably weaker than the one in the muscle on Day 1 but of a similar level at the other time points. The tumor signal curves for the animal treated with bevacizumab (B) stills shows the shape of leaky vasculature (despite the morphological normalisation) and a weaker signal than in the muscle for every time point. S.I. [a.u]: Signal Intensity in arbitrary unit.
### Supplementary tables

**Table S1:** Summary of perfusion parameters for P13

|       | Vb (ml/100ml tissue) | PS (ml/min/100ml tissue) | Ktrans (ml/min/100ml tissue) | F (ml/min/100ml tissue) |
|-------|----------------------|--------------------------|------------------------------|-------------------------|
|       | Nobs | Mean  | STD  | Ratio | p    | Mean | STD  | Ratio | p    | Mean | STD  | Ratio | p    |
| Controls |      |       |      |       |      |       |      |       |      |       |      |       |      |      |
| Day1   | 514  | 0.9   | 0.96 | 0.8    | 0.66 | 1.02 | 0.77 | 14.57 | 17.74 |
| Day3   | 776  | 0.84  | 1.03 | 0.91   | 1.1  | 1.1  | 1.23 | 10.62 | 13.63 |
| Day5/6 | 980  | 1.12  | 1.22 | 1.25   | 1.62 | 1.48 | 1.6  | 15.39 | 18.04 |
| Day7/8 | 1165 | 1.83  | 1.68 | 1.91   | 1.99 | 2.21 | 2.05 | 19.65 | 19.42 |
| Bev high |      |       |      |       |      |       |      |       |      |       |      |       |      |      |
| Day1   | 935  | 0.9   | 0.89 | 0.8    | 0.9  | 1.02 | 1.05 | 14.57 | 15.26 |
| Day3   | 832  | 0.73  | 0.95 | 0.875  | **  | 0.75 | 0.79 | 0.82   | **  | 0.93 | 1.65 | 0.840 | **  |
| Day5/6 | 1298 | 0.69  | 0.84 | 0.614  | *** | 0.6  | 1.19 | 0.483  | *** | 0.75 | 1.09 | 0.506 | *** |
| Day7/8 | 1999 | 1.32  | 1.61 | 0.723  | *** | 1.29 | 1.44 | 0.677  | *** | 1.6  | 1.61 | 0.727 | *** |
| Bev low |      |       |      |       |      |       |      |       |      |       |      |       |      |      |
| Day1   | 562  | 0.9   | 0.73 | 0.8    | 2.32 | 1.02 | 1.42 | 14.57 | 13.11 |
| Day3   | 703  | 0.85  | 0.74 | 1.018  | **  | 0.92 | 1.19 | 1.012  | **  | 1.24 | 1.24 | 1.119 | **  |
| Day5/6 | 1539 | 0.6   | 0.67 | 0.536  | *** | 0.88 | 5.56 | 0.703  | **  | 1.01 | 6.56 | 0.685 | **  |
| Day7/8 | 911  | 1.12  | 1.25 | 0.615  | *** | 1.54 | 5.12 | 0.810  | **  | 1.77 | 4.82 | 0.803 | **  |

**Table S1:** Summary of perfusion parameters at all time points for the P13 tumor model. Nobs: Number of observations (voxels) in a given group and time point; Mean: Mean parameter value for all voxels; STD: Standard deviation for all voxels; Ratio: Ratio of mean for parameters in a treatment group relative to controls at the same time point; p: p-value results from a Student t-test comparing the mean values in treatment groups to those in the controls group at the same time point. */**/*** indicate p-values lower than 0.1/0.05/0.001 respectively. Vb: Blood volume; PS: Permeability surface area product; Ktrans: Blood-to-tissue transfer constant; F: Blood flow.
**Table S2**: Summary of perfusion parameters for P3

| Nobs | Mean | STD | Ratio | p-value | Mean | STD | Ratio | p-value | Mean | STD | Ratio | p-value | Mean | STD | Ratio | p-value |
|------|------|-----|-------|---------|------|-----|-------|---------|------|-----|-------|---------|------|-----|-------|---------|
| Controls |     |     |       |         |      |     |       |         |      |     |       |         |      |     |       |         |
| Day1  | 1627 | 0.96| 2.45  | 0.95    | 3.55 | 1   | 2.17  | 12.02   | 19.05 |
| Day3  | 5124 | 2.04| 3.68  | 1.74    | 2.52 | 2.11| 2.49  | 18.91   | 17.08 |
| Day5/6| 7430 | 1.74| 2.31  | 2.31    | 2.46 | 2.63| 6.61  | 19.32   | 20.42 |
| Day7/8| 7213 | 2.44| 5.34  | 3.47    | 29.11| 3.23| 14.59 | 21.39   | 33.74 |
| Bev high |    |     |       |         |      |     |       |         |      |     |       |         |      |     |       |         |
| Day1  | 2859 | 0.96| 1.86  | 0.95    | 12.94| 1   | 2.52  | 12.02   | 13.51 |
| Day3  | 5545 | 0.69| 1.35  | 0.339   | 0.45 | 1.1 | 0.250 | 0.78    | 0.372 |
| Day5/6| 7032 | 0.54| 1.31  | 0.243   | 0.41 | 2.29| 0.177 | 0.7     | 0.268 |
| Day7/8| 7586 | 0.44| 0.88  | 0.179   | 0.40 | 3.22| 0.133 | 0.81    | 0.251 |

**Table S2**: Summary of perfusion parameters at all time points for the P3 tumor model. Nobs: Number of observations (voxels) in a given group and time point; Mean: Mean parameter value for all voxels; STD: Standard deviation for all voxels; Ratio: Ratio of mean for parameters in a treatment group relative to controls at the same time point; p: p-value results from a Student t-test comparing the mean values in treatment groups to those in the controls group at the same time point. */**/*** indicate p-values lower than 0.1/0.05/0.001 respectively. Vb: Blood volume; PS: Permeability surface area product; Ktrans: Blood-to-tissue transfer constant; F: Blood flow.