Non-invasive molecular subtyping in breast cancer with non-contrast-enhanced multiparametric MRI hypoxia imaging

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Objective

Cancer development is driven by genomic instability and alternating selective pressures from the hypoxic tumor microenvironment (TME). Multiparametric functional MRI using a combination of BOLD MRI, depicting blood oxygen saturation, and IVIM imaging, depicting intracellular and intravascular diffusion, allows pivotal insights into the hypoxic TME, provides imaging biomarkers for tumor oxygenation and neovascularization simultaneously.
We hypothesize that the combined application of BOLD/IVIM imaging allows non-invasive, non-contrast-enhanced molecular subtyping of breast cancer (BC).

Purpose

- To test the capability of combined IVIM/BOLD MRI measurements to capture imaging biomarkers for hypoxia and the induced neovascularization.
- We assessed their performance to non-invasively diagnose molecular subtypes of BCs.

Material and Methods

Twenty-six nude mice were inoculated into the flank with low, intermediate, and highly aggressive BC cells (luminal A: MCF-7 (n=10), Her2+: SKBR-3 (n=8), basal-like: MDA-MB 231 (n=8)) and imaged using a 9.4T Bruker BioSpin system. For BOLD imaging, anesthesia was supplemented with four different levels of oxygen (21%, 50%, 80%, 100%). BOLD $\Delta R2^*$ relaxation rate, depending on the amount of erythrocyte-bound oxygen, was assessed using MATLAB code developed in-house (see **Fig. 1**).
IVIM measurements included 16 b-values. The perfusion coefficient (D^*) and IVIM fraction ($fIVIM$), capturing tissue microperfusion, as well as the diffusion coefficient (D) were assessed using MITK-Diffusion (version 2018.09.99, see **Fig. 2**).
All measurements were based on 2D segmentations of solid tumor regions and the statistical analysis was performed in Rstudio (version 3.6.2).

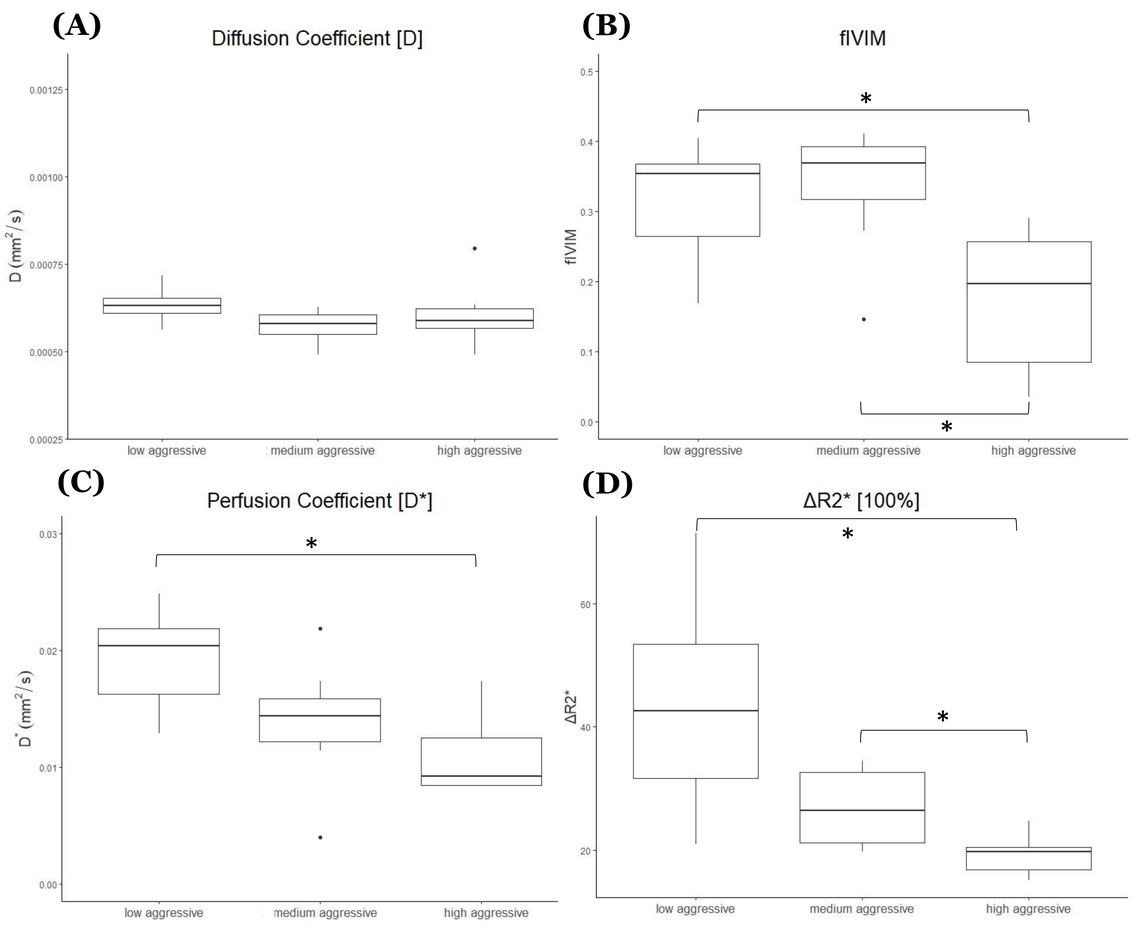

Figure 3: Boxplots for the analysis of three IVIM parameters and the BOLD parameter $\Delta R2^*$ for low, medium and high aggressive BC xenografts. A: *Boxplots for the diffusion coefficient D, resulting in no significant difference between low, medium and high aggressive breast cancer xenografts.* ***B:*** *Boxplots for the IVIM fraction (fIVIM), showing a clear difference between low/medium and high aggressive BCs (p=0.007).* ***C:*** *Boxplots for the perfusion coefficient D*, showing a significant difference between low and high aggressive BCs (p=0.035).* ***D:*** *Boxplots for $\Delta R2^*$ between differently aggressive breast cancer xenografts, showing a significant difference between low and high aggressive BCs (p=0.007), as well as medium and high aggressive BCs (p=0.045).*

Results

Among several IVIM-DWI parameters (see **Fig. 3**), $fIVIM$, capturing the relative amount of microvessels in a voxel, allows the most accurate differentiation of low and intermediate from highly aggressive BCs (p=0.007). D^*, capturing the diffusion rate within microvessels, allows differentiation between low and high aggressive BCs (p=0.036). The BOLD-related parameter $\Delta R2^*$ (see **Fig. 3**) showed differences between low and high aggressive BCs (p=0.007) as well as intermediate and high aggressive BCs (p=0.045), reflecting the improved ability of low aggressive BCs to deliver oxygen-rich hemoglobin to its tissue compared to more aggressive subtypes. This complements the finding that low aggressive tumors present a higher diffusion rate within microvessels (captured by the IVIM parameter D^*) compared to high aggressive BCs.

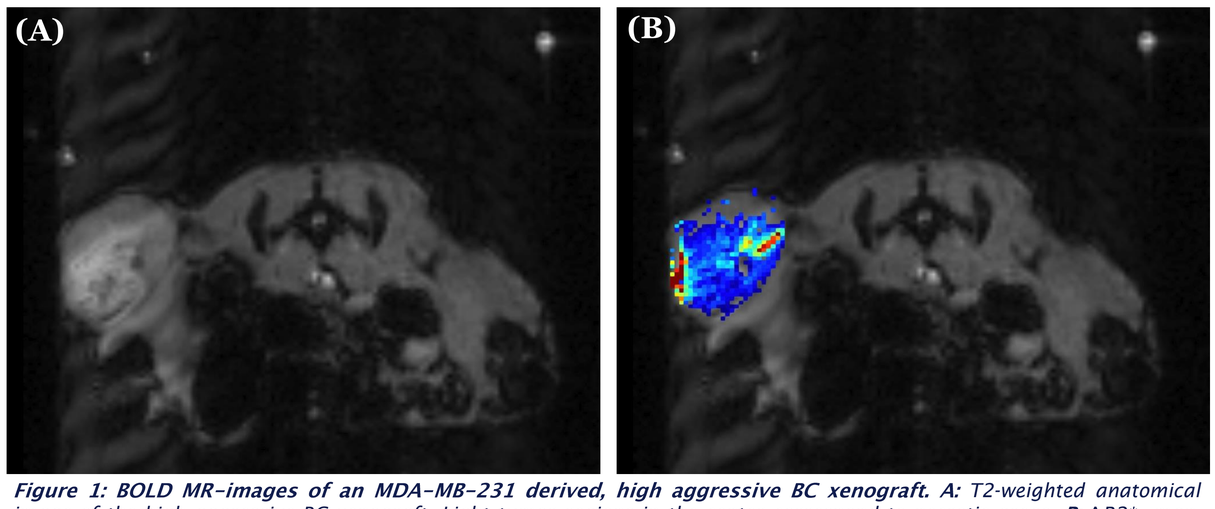

Figure 1: BOLD MR-images of an MDA-MB-231 derived, high aggressive BC xenograft. A: *T2-weighted anatomical image of the high aggressive BC xenograft. Light tumor regions in the center correspond to necrotic areas.* ***B:*** *$\Delta R2^*$ map, showing a pronounced response to the oxygen challenge in peripheral tumor regions (red), compared to necrotic tumor regions (blue).*

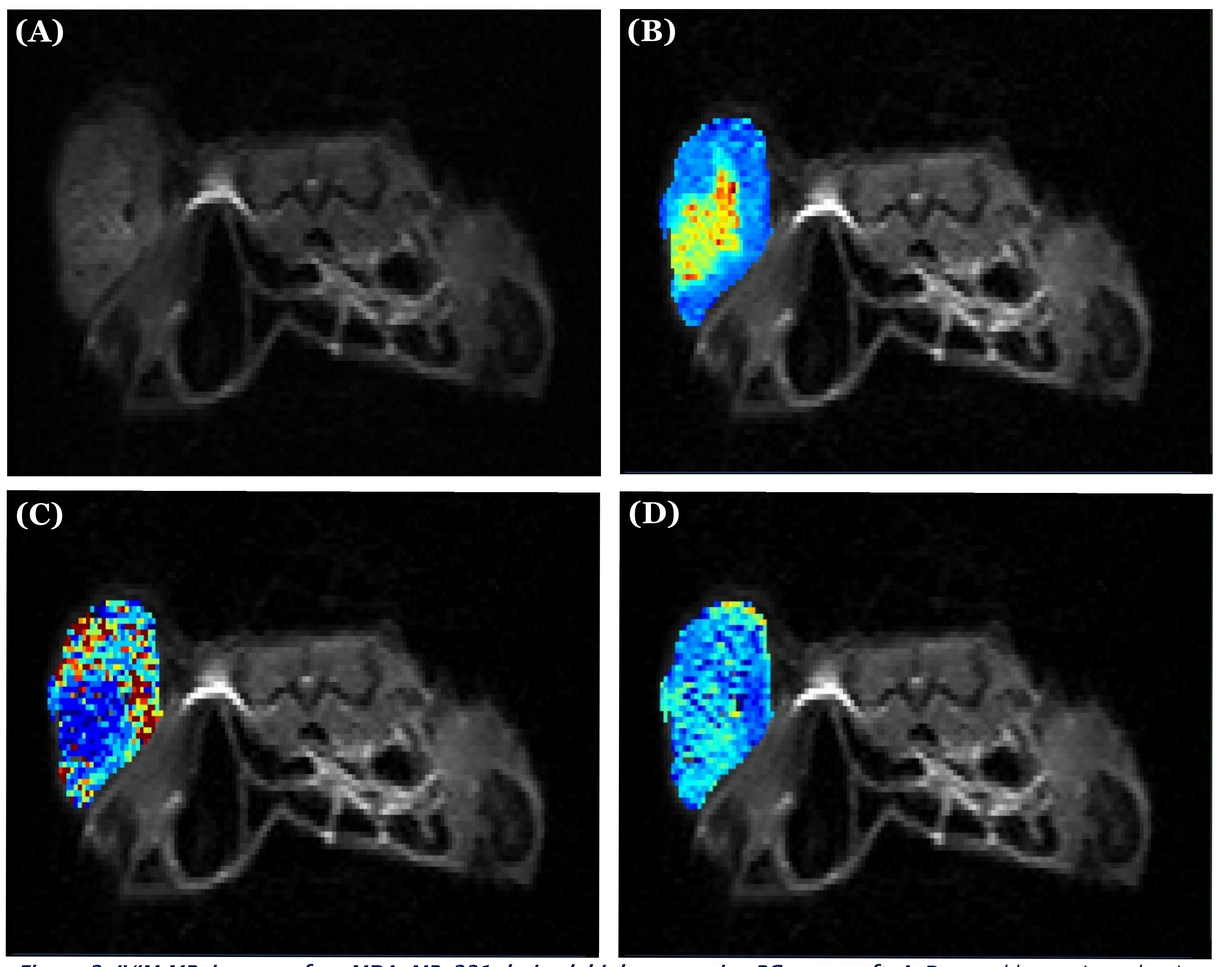

Figure 2: IVIM MR-images of an MDA-MB-231 derived, high aggressive BC xenograft. A: *D map, blue regions showing reduced diffusion of water protons in the periphery of the tumor, indicating actively proliferating BC cells, and the necrotic tumor core in yellow, characterized by increased diffusion due to cell membrane instability.* ***B:*** *fIVIM map, reflecting a lack of microperfusion in the tumor core, and a comparatively large fraction of microperfusion in peripheral tumor regions.* ***C:*** *D* map, showing increased diffusion in peripheral tumor regions due to the directional diffusion within tumor microvessels.*
Please note that, due to geometric distortion as a result of the EPI-readout of DWI sequences, the tumor appears larger compared to the T2-weighted images in Fig. 1.

Conclusion

- Multiparametric MRI using BOLD and IVIM imaging simultaneously captures information on tumor neovascularization and tumor oxygenation.
- Blood perfusion and oxygen delivery is restricted in high compared to low and intermediate aggressive BCs.
- BOLD and IVIM imaging provides non-invasive imaging biomarkers to decipher breast cancer heterogeneity driven by the hypoxic TME and enables molecular subtyping without the application of contrast agents.

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Contact information and reference

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