Micro-vascular effects of photodynamic therapy in tumors evaluated with dynamic contrast-enhanced MRI

Tom Schreurs[1,2], Stefanie Hectors[1], Igor Jacobs[1], Holger Grüll[1,3], Gustav Strijkers[1,2], and Klaas Nicolay[1]

[1]*Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands,* [2]*Biomedical Engineering and Physics, Academic Medical Center, Amsterdam, Netherlands,* [3]*Oncology Solutions, Philips Research, Eindhoven, Netherlands*

Target audience Researchers interested in dynamic contrast enhanced MRI for image guidance of cancer therapy

Purpose Photodynamic therapy (PDT) is an emerging cancer therapy. PDT is a local treatment which aims to induce tumor cell death by activation of a photosensitizer that produces reactive oxygen species under (laser) light irradiation. Apart from direct cell death, PDT treatment also induces vascular occlusion and immune response activation. Although successful results have been obtained with PDT[1,2], there is an unmet need to predict treatment outcome shortly after therapy. Therefore, the **aim** of this study was to use dynamic contrast-enhanced (DCE) MRI to study early vascular tumor effects of PDT. Besides, 4 types of endogenous MR contrast parameters were measured quantitatively, to detect structural tissue changes. We hypothesized that this information can be used to distinguish successfully treated (non-viable) tumor tissue from residual or recurrent (viable) tumor tissue.

Methods CT26 colon carcinoma cells were injected ~10 days before PDT subcutaneously into the hind limb of Balb/c mice. The PDT protocol consisted of an i.v. injection of 6.45 mL/kg body weight of the photosensitizer Bremachlorin (0.35% solution), activated 6 h later by 10 min illumination with 655 nm laser light, which was aimed onto the skin (120 J/cm^2) covering the tumor (n=5 animals). A control group (n=4) received no photosensitizer and no light treatment. Tumor size was monitored every 2-3 days until the animals were sacrificed 14 days after PDT. MRI was performed on a 7T Bruker BioSpec one day before, 3 h after, and 24 h after PDT. The protocol consisted of T2-weighted multi-slice spin echo scans, used as anatomical reference, followed by quantitative measurements of T1, T2, magnetization transfer ratio (MTR) and the apparent diffusion coefficient (ADC), all using 128^2 pixels multi-slice EPI readout acquisitions covering the whole tumor. Next, 3D FLASH scans were used for B1 mapping (FA = 145, 180, 215°), for quantitative measurements of pre-contrast T1 using a variable flip angle approach, and for the actual DCE scan. The latter had a scan time of 3.6 s, repeated for 15 min. After 2 min, an i.v. injection of contrast agent (4 mL/kg b.w. 75 mM Dotarem) was performed in 5 s using an infusion pump. From the DCE scans, contrast agent concentration curves were obtained for each pixel, which were used to calculate the transfer constant K_{trans} and fractional volume of the extravascular extracellular space v_e, using the standard Tofts-Kermode model.

Results Response to PDT was similar in all animals in the first 2-3 days after treatment: the whole or a large part of the tumor became necrotic. The necrotic mass subsequently shrunk and turned into a dry scab. In 2 mice, only a small scab was left 14 days after PDT. In the other mice, part of the tumor remained viable leading to recurrent tumor growth with a delay of approx 10 days as compared to controls. No significant treatment effects were observed in any of the endogenous parameter maps, except at the tumor surface (dry scab), and around the tumor (edema), see Fig 1. DCE-MRI analysis indicated that tumors were initially almost completely enhanced (Fig 1). In contrast, large tumor parts were non-enhanced right after treatment, which was even more pronounced after one day. The non-enhanced tumor fraction, averaged over all treated mice, increased from 1.9±1.5% before PDT, to 20.3±9.2 right after PDT, and 73.0±14.7 % after 1 day, whereas no significant increase was observed in controls. Parts of the tumor that still showed contrast agent uptake at 24h after PDT coincided with positions where tumor nodules regrew after some days (Fig 2), for the 3 mice with tumor recurrence. Pharmacokinetic modeling revealed that the average K_{trans} over all treated animals decreased from 0.25±0.07 before PDT, to 0.09±0.03 right after PDT. Non-decreased K_{trans} values at 1h after PDT spatially correlated with non-decreased K_{trans} at 24h after PDT.

Discussion The lack of enhancement in large tumor parts after PDT clearly indicates vascular occlusion. Remaining non-enhanced regions after 24h coincided with regions of tumor recurrence. The drop in K_{trans} right after PDT suggests that a decrease in perfusion can already be observed within 3h after PDT, which possibly enables monitoring of treatment efficacy at this very early time point. However, no treatment effects were found in endogenous parameter maps, suggesting that little or no structural tissue changes occur in the first 24 h. It is important to note that treatment also induced vascular occlusion in adjacent muscle tissue. However, these effects may be reversible, because slight limping was only observed in some mice for at most 2 days after PDT.

Conclusion DCE-MRI could be used to visualize the drastic microvascular effects of PDT in our tumor model. Therefore, the technique may be of clinical value for monitoring of treatment efficacy. In future experiments, the DCE-MRI data will be spatially compared to a histological viability essay (NADH-diaphorase staining), to assess if observed vascular changes are indicative of changes in tissue viability.

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References 1) Dougherty *et al*., J. Natl. Cancer Inst. 55: 115-121, 1975; 2) Agostinis *et al*., CA Cancer J. Clin. 61: 250-281, 2011

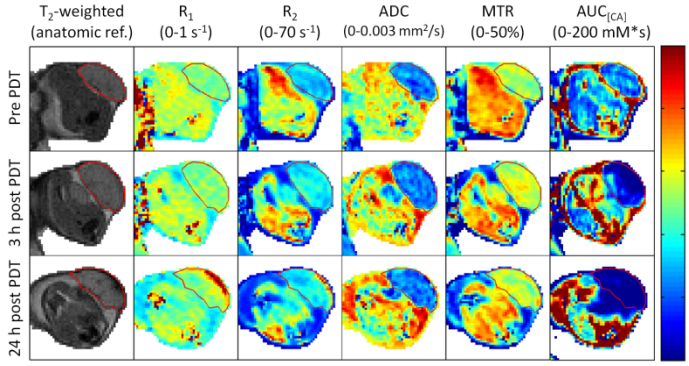

Figure 1: Parameter maps from a PDT-treated mouse in a cross-section of the hind limb. Column 1: T2-weighted anatomical reference images. The red outline indicates the tumor. Columns 2-5: endogenous parameter maps. Column 6: area under the time-curve (AUC) of contrast agent concentration, obtained by DCE-MRI. None of the endogenous parameters was strongly affected in the tumor core, but the tumor was surrounded by edema (high ADC, low R_2). According to the AUC maps, the tumor initially was completely perfused, whereas it was largely non-perfused 3h and 24h after PDT.

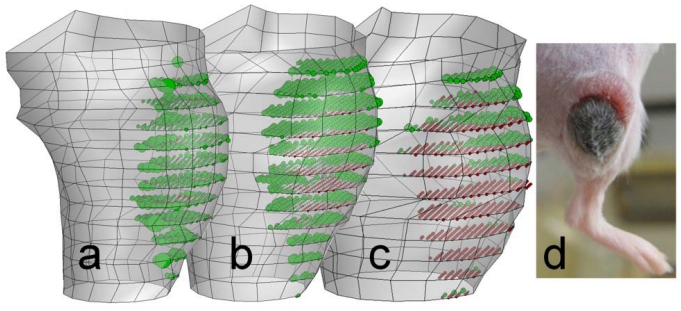

Figure 2: MRI-based 3D model of the hind limb before **(a)**, 3h post **(b)** and 24h post PDT **(c)**. Contrast-enhanced and non-enhanced tumor voxels are indicated in green and red, resp. After 3h, tumor perfusion was already shut down in the tissue close to the skin in the central slices. Only the upper part of the tumor was still enhanced after 24h, suggesting partially unsuccessful treatment. Indeed, tumor recurrence was seen in that part of the tumor after ~3 days. A photo of the tumor at day 7 is shown **(d)**, with a viable recurrence in the top.