Introduction: Inflammation plays an important role in tumor progression and anti-inflammatory drugs have therefore been proposed as anti-cancer agents. However, the adverse effects caused by the high doses required for tumor growth inhibition are a major limitation. Liposome incorporated glucocorticoids (GC), a prominent class of anti-inflammatory agents, cause effective tumor growth inhibition at much lower doses than free drug, with little side effects [1]. The aim of this study was to test the hypothesis that liposomal GC therapy involves angiogenesis inhibition, using a multi-parametric MRI protocol.

Materials and Methods: Prednisolone phosphate-loaded liposomes (PLP-L; ø100nm) were prepared as in [2]. C57BL/6 mice were inoculated s.c. with 10⁵ B16F10 cells. After pre-treatment MRI (day 0), mice received a single i.v. dose of either PLP-L (20mg PLP/kg; n=6) or saline (n=6). Post-treatment MRI was done on day 2, 4 and 6. Thereafter mice were sacrificed and tumors dissected for microscopy. MRI was done at 6.3T, using isoflurane anaesthesia, and included: (a) T₁-w spin-echo (TE/TR=35/2000ms; b-value=0 or 400s/mm²; NA=2); (c) T₂ mapping from spin-echo images with 16 different TEs from 9-144 ms (TR=2000ms; NA=2); (d) DCE-MRI, using T₁-w FLASH (TE/TR=3/80ms; α=50°; slices=8; NA=1) for 25min collecting 200 frames. A tail vein infusion line was used to deliver 0.3mmol gadoteridol/kg approx 50s after the start of DCE-MRI. For all scans: matrix=128×128; FOV=3×3cm²; slice=1mm. Tumors were segmented on DW-images and their volumes calculated. ADC maps were calculated from DW-scans. DCE-MRI data analysis was restricted to pixels with signal enhancement ≥5 times the noise. DCE-MRI data were converted to CA-time (C₁) curves with the muscle reference method [3], using a pre-contrast muscle T₁ of 1285ms, muscle endothelial transfer constant (Ktrans) of 0.11min⁻¹, fraction of extravascular extracellular space (ve) in muscle of 0.20 and gadoteridol relaxivity of 3.7mM⁻¹s⁻¹. C₁ curves were fitted with the two-compartment Tofts model [4], using a golden section search. Pixels with ve<0.95 were discarded. The descriptive parameters time-to-peak (Tpeak) and initial slope (Slope) were derived from the C₁ curves. Microscopy was done on tumors dissected 6 days after therapy. 5-µm-thick sections were stained for endothelial cells (CD31) and cell nuclei (DAPI). Images were acquired at 200×magnification. MicrovesSEL density (MVD) was estimated from the number of vessels in 5 vascular regions per tumor. Vessel normalization was probed by anti-actin staining, followed by CD31 co-staining. Data are presented as mean±SD. DCE-MRI indices were analyzed on the basis of median values, determined from parameter histograms. Paired t-tests were used to evaluate time effects of treatment compared to the baseline. Between-group comparison was done using repeated measures one-way Anova.

Results & Discussion: The growth-inhibiting effects of PLP-L were significant at day 6 (Fig 1). The median values of DCE-MRI (Fig 2) derived parameters showed large variations (Fig 3), indicative of substantial inter-tumor differences in vascular function. Therefore, baseline measurements were used to assess potential changes in vascular status. Median Ktrans was significantly reduced from day 2 to 6 in the treatment group. Tpeak was prolonged by therapy from day 2, while Slope was significantly lowered only on day 6. In the control group, the above three DCE-derived indices were the same at day 0 to 4. At day 6, median Ktrans and Slope were significantly reduced compared to day 0, while Tpeak was unchanged. ve remained constant throughout, in both groups. The comparison between longitudinal data for both groups revealed no significant PLP-L treatment effect on any vascular index. ADC and T₁ mapping were used to study the effects of PLP-L therapy on tissue status. Neither parameter was significantly altered, indicating that PLP-L caused no gross necrosis. However, the control group showed a significant reduction in ADC from day 2, suggesting that normal tumor growth was accompanied by an increase in cell density. PLP-L had no significant influence on ADC or T₁ compared to the control group. Microscopy of day 6 samples showed that average MVDs were 34 and 58 vessels/mm² in PLP-L treated and control tumors, resp, however, no significant difference was found (p=0.05). Microscopy provided no evidence for PLP-L induced vascular maturation.

Conclusions: The present study yields a complex picture of the effects of liposomal PLP on tumor vascular status. No significant differences in DCE-MRI indices and MVD values were observed between treated and control tumors. Nevertheless, PLP-L caused a significant reduction in Ktrans from day 2 post-therapy and prevented the ADC reduction seen in nontreated tumors. More specific indices of PLP-L treatment are desired to identify its mechanism-of-action. Potential candidates are tumor-associated inflammatory markers.