Diffusion-weighted Magnetic Resonance Imaging of Response to PI3-Kinase/mTOR Inhibition Studied in Human Ovarian Cancer Xenografts

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Target audience: This work is targeted to preclinical and clinical researchers investigating imaging biomarkers of tumor therapy response.

Purpose: The PI3-kinase pathway is frequently upregulated in cancer and is a promising target for anti-cancer therapies[1]. As a result, various drugs inhibiting this pathway have been developed and are currently undergoing clinical trials. Not all cancer patients respond to treatment (tx), and therefore it is important to have biomarkers of response that can be non-invasively and repeatedly assessed during the course of tx. It has been shown that diffusion weighted (DW) magnetic resonance imaging (MRI) can predict tumor response to conventional chemotherapy in ovarian[2] and other cancers[3]. In this study, the aim was to evaluate the predictive value of DW-MRI for tumor response to PI3-Kinase/mTOR inhibition in ovarian cancer xenografts.

Methods: All experimental procedures involving animals were approved by the institutional ethics committee and were in accordance with national, regional and institutional guidelines. Two groups of athymic nude mice were inoculated subcutaneously on the hind limb with 5×10^6 cells of the human ovarian cancer cell lines TOV-21G (n= 12) and TOV-112D (n=9). Experiments were performed when the tumors had reached an average volume of (539 ± 224) mm^3. The PI3-Kinase/mTOR inhibitor BEZ-235 was given orally on three consecutive days at a dose of 65mg/kg to eight mice with TOV-21G xenografts and five mice with TOV-112D xenografts. The remaining four mice per group served as non-tx controls (ctrl). MRI was performed one day before the first drug administration and again two to six hours after the last drug administration. The images were acquired using a 7T Bruker Biospec with a 72mm volume resonator for RF transmission and a quadrature mouse brain surface coil for reception. Five sagittal slices were acquired with FOV=23x23mm^2, slice thickness=0.7mm, interslice distance=1mm. T2-weighted (T2w) anatomical images were acquired using a RARE spin echo sequence with the following parameters: TE=12ms, TR=2000ms, rare factor=8, NEX=4, Matrix size=256x256. An EPI sequence was used to acquire DW images using TE=28ms, TR=3000ms, 4 segments, NEX=4, Matrix size=64x64, b-values =100, 300, 600, 1000 s/mm^2 and three orthogonal gradient orientations. Pre-and post-tx images were acquired in the same tumor region. Maps of the apparent diffusion coefficient (ADC) were calculated voxelwise from a monoexponential fit of the signal intensity versus b-values. Tumor regions of interest were drawn using the T2w images as anatomical guides. Tumor volumes were computed by approximating the tumor shapes as ellipsoids with semi-axes measured from the MRI data.

Results: Both tumor volume data and ADC data indicate that TOV-21G xenografts responded to treatment whereas the TOV112D xenografts did not. More specifically, **Fig. 1** shows that both tx and ctrl TOV-112D xenografts tumors showed a significant increase in tumor volume throughout the study. No change in ADC and no significant differences between ctrl and tx groups could be found for these xenografts. The TOV-21G xenografts showed a significant difference in tumor size and median ADC values between ctrl and tx groups. While the volume of ctrl tumors increased, the tx tumors decreased in size. The median ADC values did not change in the ctrl group while they increased significantly for the tx group. Histogram analysis was performed for the ADC values of the tumors. For the TOV-21G xenografts we found a significant decrease in kurtosis and skewness after tx (p=0.002 paired sample t-test). Visually, the change in ADC can be described by a right shift of the histogram and a more symmetrical shape after treatment (**Fig.2b**). No significant changes were found in the TOV-21G ctrl group and in the TOV-112D tumors (**Fig. 2a,c,d**). **Fig.3** illustrates the tx response of a representative TOV-21G xenograft. The decrease in tumor size after treatment is apparent. The tumor shows low ADC values with an area of high diffusivity in the center. The ADC values increase throughout the tumor after treatment (shift from dark blue to light blue colors). The tumor can be well distinguished from the surrounding muscle and connective tissue with high ADC values (yellow to red).

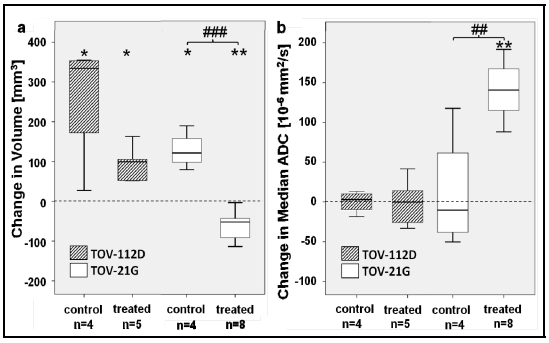

Fig. 1: Box plots of change in (a) tumor volume and (b) median ADC after three-day tx. * p<0.05, ** p< 0.005 paired sample t-test pre vs post tx. ## p<0.005, ### p<0.001 two sample t-test of change in ctrl vs tx.

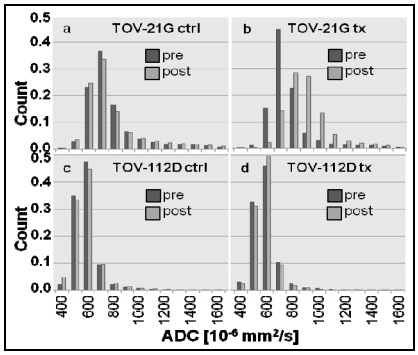

Fig. 2: Histograms averaged over group showing the distribution of ADC values.

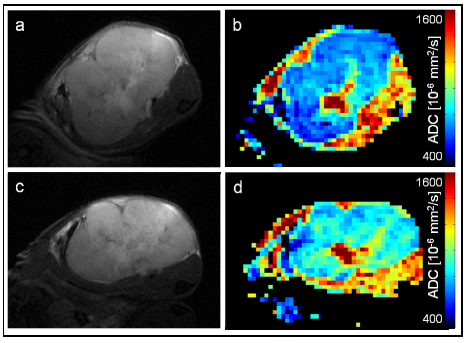

Fig.3: T2w images (left) and ADC maps (right) of one representative TOV-21G xenograft pre-tx (a,b) and the corresponding slice post-tx (c,d).

Discussion: Previous studies have demonstrated that TOV21G cells have low expression of the tumor suppressor gene PTEN and high expression of pAkt, indicating high PI3K signalling activity. In contrast, TOV112D cells have a low pAkt expression and high PTEN expression, indicating that PI3K signalling is of little importance for the growth of these cells. In this study we demonstrated that TOV-21G, but not TOV112D xenografts responded to PI3-kinase/mTOR inhibition. This is consistent with the high PI3K signalling activity in the TOV21G xenografts. The changes in ADC histograms after treatment are in accordance with results obtained from studies using conventional chemotherapy[2]. The increase in ADC after treatment may be caused by apoptosis of cancer cells, leading to less restrictive water movement due to a breakdown of cell walls. This hypothesis will have to be verified by histology.

Conclusion: DW-MRI is a clinically available imaging modality. Our results indicate that median ADC as well as kurtosis and skewness of ADC histograms are promising candidates for clinical biomarkers of response to PI3-Kinase/mTOR inhibition.

References: 1. Chalhoub N, Baker SJ. PTEN and the PI3-kinase pathway in cancer. *Annu Rev Pathol.* 2009;4:127-50. **2.** Kyriazi S, Collins DJ, Messiou C, et al. Metastatic Ovarian and Primary Peritoneal Cancer: Assessing Chemotherapy Response with Diffusion-weighted MR Imaging – Value of Histogram Analysis of Apparent Diffusion Coefficients. *Radiology*. 2011;261(1):182-92. **3.** Padhani AR, Liu G, Koh DM, et al. Diffusion-weighted magnetic resonance imaging as a cancer biomarker: consensus and recommendations. *Neoplasia*. 2009;11(2):102-25.