

Longitudinal MR Microscopy at 7T of Human Derived Osteosarcoma Mouse Models – An Early Assessment of Drug Response

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Introduction: Osteosarcoma is the most common type of malignant bone cancer with a peak incidence in the second decade of life (1). Currently, the mainstay of sarcoma therapy is surgical removal of the malignant lesion and chemotherapy to treat micrometastatic disease, which has shown to be present in 80% of the patients at the time of diagnosis but is often not detectable. Although a combination of novel targeted drugs have been employed in the treatment of different sarcomas, the cure rates for metastatic and recurrent sarcomas have only been modestly improved in the last decade (2). In this study, magnetic resonance imaging (MRI) at 7 T was employed to evaluate the therapeutic effects of MK1775 (3) and gemcitabine in patient derived xenograft mouse models.

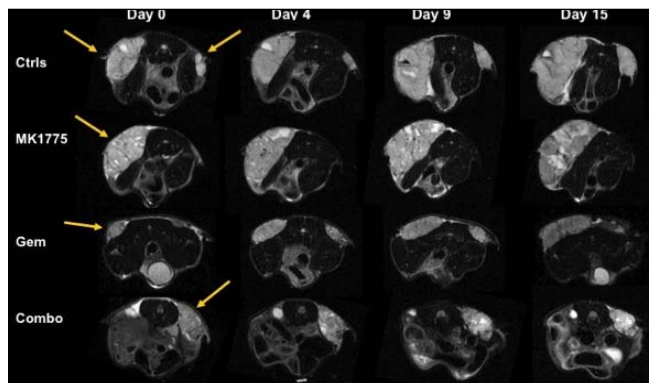


Fig 1. T₂-weighted FSE images (resolution=136 μm) showing tumor volumes at days 0, 4, 9 and 15 for demonstrative animals.

Methods: Patient derived tumor tissue was implanted subcutaneously into the flanks of 32 female SHO/SCID mice grouped as follows: (1) control; (2) MK1775 (30 mg/kg. p.o. twice daily on days 1, 3, 8 and 10); (3) gemcitabine (100 mg/kg, i.p. once daily on days 1, 3, 8, 10); (4) MK1775 and gemcitabine in equivalent doses. Animals were anesthetized using 2% isoflurane, restrained in a cradle and placed within the magnet while continuously receiving isoflurane. Physiological functions were monitored using the SAI system (Small Animal Instruments, Inc.). MR data were acquired using a 7-T horizontal magnet (Agilent Technologies) equipped with 205/120/HDS gradients and performed at baseline, 24 and 48 hours following each drug administration. Using a 72-mm birdcage coil (Agilent Techn.), axial T₂-weighted fast spin-echo (FSE) sequences were acquired (TE/TR= 60/1403 ms) with a resolution of 136 μm over 6 minutes. Applying the same slice plane, diffusion weighted (DW) FSE sequences using four b-values = 50, 500, 1000 and 2000 and TE/TR=36/1881 ms also were acquired in 12 minutes. Tumor volumes were obtained using VnmrJ (Agilent Techn.) and apparent diffusion coefficients (ADC) were determined using Matlab. Following MR acquisitions, animals were euthanized and tissue prepared for histological staining with hematoxylin and eosin (H&E), Cleaved Caspase 3 (CC3) and γH2AX.

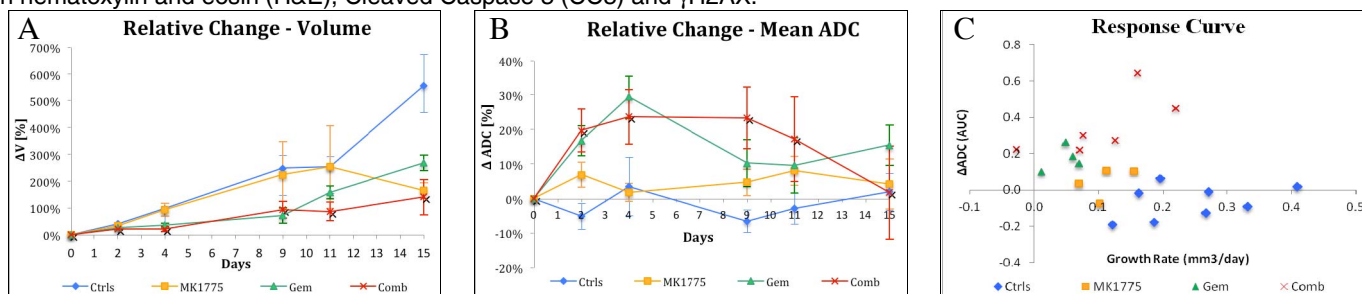


Fig 2. Percent change in tumor volume (A), mean ADC (B) and the area under the ADC curve and growth rates for each mouse (C).

Results & Discussion: Visually, Gem and Como demonstrated rather constant tumor volumes with treatment while MK1775 and controls displayed notably larger tumors at the later time points (Fig. 1). Similarly, quantitative analysis showed significantly larger tumors for the controls compared to the Gem and Comb group following the first treatment (Fig 2A). The MK1775 showed similar tumor growth to the controls until day 11 following which the MK1775 showed significantly smaller tumor volumes. Accordingly, significant increases in mean ADC for all treated animals, particularly the Gem and Comb groups, were observed immediately following the first treatment (Fig 2B). While the ADC of the MK1775 returned back to baseline by day four, the Gem and Comb ADC remained significantly higher throughout the final treatment at day 11. To compare across mice, the area under the ADC curve and growth rates were calculated for each mouse and plotted in Fig 2C. Not surprisingly, the Gem and Comb in particular, showed lower growth rates and larger areas under the ADC curve indicating response. In agreement, quantitative analysis of ADC skewness and kurtosis showed similar distribution prior to and following treatment for the controls, whereas the treated animals demonstrated significant alterations already after the first treatment. Histologically, while no differences were observed in amount of necrosis or in potential bone formation at day 4, the most overt difference by day 15 was in fact the osteoid formation with the controls demonstrating the lowest and the Comb group the highest amount (*i.e.* 10 and 65%). For both time points, significantly higher apoptosis and DNA damage was observed in the treated groups than in controls. Taken together, these findings verify that MK-1775 and Gem, particularly in combination, induce significant apoptosis and DNA damage. More importantly, while quantification of tumor volume correlates well with these insults, changes in ADC occur at earlier time points and may thereby serve as an early biomarker for treatment response.

References:

1. Joseph L *et al* (2008), TCT: 317-329.
2. Blanke CD *et al*. (2008), JCO 26: 626-632.
3. Krehling JM *et al*. (2012), MCT 11: 174-182.