Assessment of the anti-vascular activity of MN-029 in Calu-6 human lung tumors using DCE-MRI and FLOOD MRI

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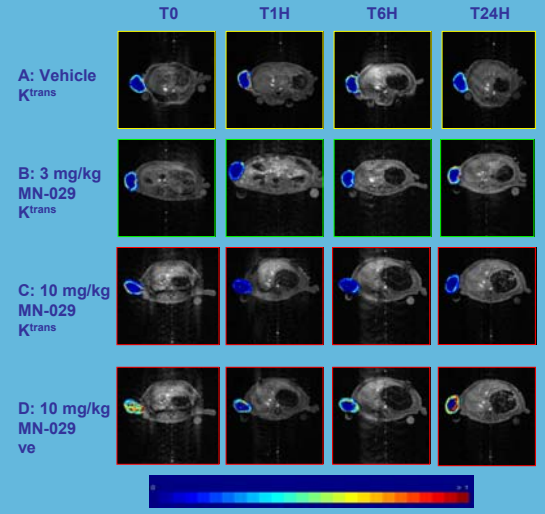
Introduction: Anti-vascular and anti-angiogenic drugs are currently of great interest in the treatment of cancer (1-2). These drugs selectively disrupt tumor blood vessels or inhibit their formation. MediciNova is developing a novel vascular disrupting agent (MN-029) that reversibly disrupts the tubulin cytoskeleton of proliferating tumor endothelial cells and shuts down tumor blood flow. For clinical trials and the further development of anti-vascular therapeutics, methods for evaluating the vascular response of tumors to treatment are urgently required. Dynamic Contrast-Enhanced MR imaging (DCE-MRI) and T2* techniques were used with success to assess the tumor response to anti-vascular treatments non-invasively showing the usefulness of biomarkers such as Ktrans (3). In this study, we characterized the blood flow changes induced by MN-029 in Calu-6 human lung tumors using DCE-MRI and T2* techniques.

Methods: 10^7 human Calu-6 cells were injected subcutaneously in the right flank of 70 female Nude rats. When tumors reached a volume of 400 to 600 mm^3, the rats were randomized into 3 groups of 12 rats. One group of rats received an intravenous (IV) infusion of vehicle, one group received an IV infusion of 3 mg/kg of MN-029 and one group received an IV infusion of 10 mg/kg of MN-029. 3 rats per group were imaged before treatment and at 1H, 6H and 24H post-treatment, while 3 other rats per group underwent MRI before treatment and at 3H and 24H post-treatment. The other 12 rats per group were sacrificed at the same time points (3 rats per time point) after injection of Hoechst and pimonidazole for histological assessments. All of the MR acquisitions were performed in a 4.7T magnet (Bruker, Wissembourg). Multi-slice T2-weighted MR images covering the entire tumor were followed by multi-gradient echo T2* MR acquisitions (TR/TE/α=500ms/5,10,15,20,25ms/45°; FOV=70x70mm; Matrix=256x256, slice thickness=2mm). DCE-MRI was performed using a FLASH-2D sequence with a temporal resolution of 12.8s per image (TR/TE/α=100ms/3.3ms/70°; FOV=70x70mm, Matrix=128x128, slice thickness=2mm). T1-weighted pre-contrast images were acquired 1-minute prior to an IV bolus injection of 0.3 mmol/kg Gd-DOTA (Guerbet, France). 115 post-contrast images were acquired during 20 minutes. Images were processed under IDL 6.1 using in-house written software. Region of interest (ROI) analysis and pixel by pixel analysis were performed to determine R2* values and Ktrans and ve values were determined using the Tofts and Kermode pharmacokinetic model.

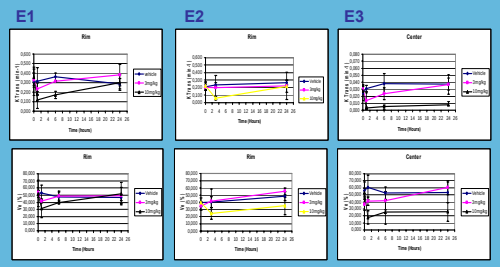
Results: The results showed a significant drop in tumor perfusion at 1H and 3H post-MN-029 treatment in the 3 and 10 mg/kg treatment groups with Ktrans values in the tumor rim dropping by 28% at 1H post-treatment in the 3 mg/kg group and by 67% and 72.6% at 1H and 3H post-treatment, respectively, in the 10 mg/kg group compared to pre-treatment values. In the tumor center, Ktrans dropped by 53% at 1H in the 3 mg/kg group and by 90% and 73% at 1H and 3H post-treatment, respectively, in the 10 mg/kg group compared to pre-treatment values. Reduced perfusion was still apparent in the 10 mg/kg group at 24H post-treatment. Significant differences were also noted compared to the vehicle group. R2* quantification showed a decrease in R2* at 1H for the 10 mg/kg group corresponding to vascular collapse and necrosis which reduced the overall amount of deoxyhaemoglobin concentration. Immunohistochemistry findings confirmed these results showing decreased perfusion of Hoechst dye in the 10 mg/kg group at 1H and 3H post-treatment and increased necrosis at 24H post-treatment.

Conclusions: The changes in Ktrans and ve in Calu-6 tumors after MN-029 treatment are reflective of tumor blood flow changes. Ktrans values for Gd-DOTA uptake into Calu-6 tumors could be a useful non-invasive marker of blood flow changes induced by vascular disrupting agents such as MN-029.

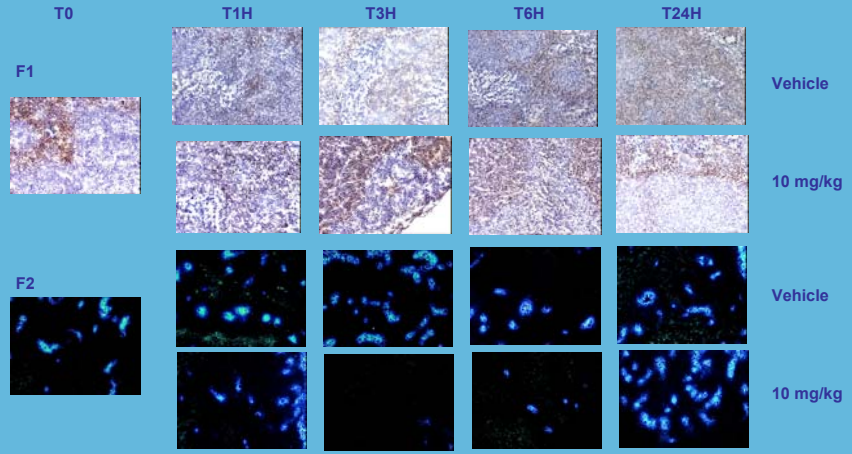
1. Micheletti G, Poli M, Borsotti P, Martinelli M, Imberti B, Taraboletti G, Giavazzi R. **Vascular-targeting activity of ZD6126, a novel tubulin-binding agent. Cancer Res 2003, 63(7):1534-7.**
2. Robinson SP, Kalber TL, Howe FA, McIntyre DJ, Griffiths JR, Blakey DC, Whittaker L, Ryan AJ, Waterton JC. **Acute tumor response to ZD6126 assessed by intrinsic susceptibility magnetic resonance imaging. Neoplasia 2005, 7(5):466-74.**
3. Robinson SP, McIntyre DJ, Checkley D, Tessier JJ, Howe FA, Griffiths JR, Ashton SE, Ryan AJ, Blakey DC, Waterton JC. **Tumour dose response to the antivascular agent ZD6126 assessed by magnetic resonance imaging. Br J Cancer 2003, 88(10):1592-7.**



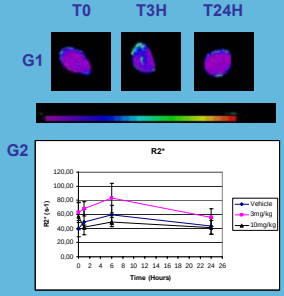
A-B-C: K^{trans} pixel by pixel maps overlaid over T1-weighted images at T0 before MN-029 injection and at T1H, T6H and T24H post-injection, respectively, for a vehicle-treated rat, a 3 mg/kg MN-029-treated rat and a 10 mg/kg MN-029-treated rat.
D: Ve pixel by pixel map for the 10 mg/kg MN-029-treated rat.



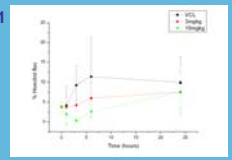
E: Mean K^{trans} and mean ve as a function of time in both the rim (E1,E2,E4,E5) and the center (E3,E6) of Calu-6 tumors for vehicle-, 3 mg/kg and 10 mg/kg MN-029-treated rats.

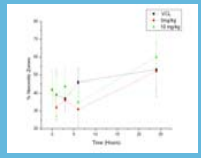


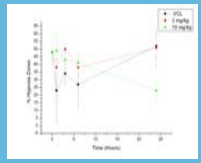
F1: Pimonidazole staining for vehicle-treated rats (1st row) and 10 mg/kg MN-029-treated rats (2nd row) at T0, T1H, T3H, T6H and T24H in subcutaneously xenografted Calu-6 human lung tumors.
F2: Hoechst staining for vehicle-treated rats (1st row) and 10 mg/kg MN-029-treated rats (2nd row) at T0, T1H, T3H, T6H and T24H in subcutaneously xenografted Calu-6 human lung tumors (same rats as pimonidazole).



G1: R2* pixel by pixel map in a subcutaneously xenografted Calu-6 lung tumor for a10 mg/kg MN-029-treated rat before treatment (T0) and at post-treatment timepoints T3H and T24H.
G2: R2* evolution as a function of time in vehicle-, 3 mg/kg and 10 mg/kg MN-029-treated rats.







H1: Hoechst fluorescence (%) evolution as a function of time for each group of rats prior to and after MN-029 treatment.
H2, H3: Proportions of necrotic and hypoxic zones in Calu-6 tumors (%) as determined from pimonidazole slides as a function of time for each group of rats prior to and after MN-029 treatment.