Detection of Acute Response to Proteasome Inhibitor Treatment in Mouse Colorectal Tumour Models Using Amide Proton Transfer (APT) Magnetic Resonance Imaging

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Introduction: Amide proton transfer magnetic resonance imaging (APT MRI) allows the non-invasive imaging of tumour protein and pH[1]. APT MRI is based on chemical exchange-dependent saturation transfer (CEST), in which MRI contrast is produced from the exchange of saturated amide protons in endogenous immobile proteins with those in bulk tissue water[2]. APT MRI is sensitive to the accumulation of cellular protein and pH changes in tumours[3], and as such provides a means for quantitative assessment of protein-targeting anti-cancer therapy. Proteasome inhibitors (PIs) are a class of anti-cancer drug that block the breakdown of unwanted or malfunctioned proteins in tumours. It is thought that PIs result in a perturbation in protein homeostasis, leading to tumour cell death. Currently, the pharmacodynamic response of PIs is evaluated in fixed or frozen tumour tissues, by performing Western blot or immunochemistry on tumour samples. This study investigates whether APT MRI could non-invasively detect an acute effect of PIs on mouse colorectal models after a single dose of the PI treatment, in conjunction with diffusion, T1, and T2 MRI and ^{31}P magnetic resonance spectroscopy (MRS).

Methods and Materials: *Animal Models*: Nine female MF1 nu/nu mice were subcutaneously inoculated on the lower right flank with $5x10^6$ SW1222 colorectal tumour cells. Five mice were treated with 11mg/kg of investigational PI ixazomib (MLN2238, *Takeda Pharmaceutical International Corporation*) and four control mice were administered with the drug vehicle i.v. 16 days post-implantation. PI treated mice were maintained in a warmed cage using a thermostatic heating pad at 24-26 °C.

In vivo measurements: MRI was performed a day before treatment, 24 hrs and 72 hrs post-treatment, using a 9.4T Agilent VNMRS scanner with either a 39 mm birdcage coil (Rapid MR International, Columbus, Ohio) or a dual-tuned 1 cm ^{1}H/^{31}P surface coil (for ^{31}P MRS) The mice were anaesthetized using isoflurane in O_2 (2.5% for induction, 1.5% for maintenance) and the core body temperature was maintained 37°C using a heating tube. The tumours were covered with dental paste to minimize motion.

Imaging Parameters: Single-slice gradient echo CEST imaging was used for APT data acquisition (matrix=64x64, FOV=30mmx30mm, flip angle=20°). A full z-spectrum was acquired with an unsaturated image for control (S_0). Three other MRI parameters were estimated for comparison: i) ADC (diffusion weighted fast spin echo, matrix =128x128, TR=1500ms, TE=7.86ms ms, ETL=4, b-value=7-1069ms), ii) T1 (Look-Locker Segmented Inversion Recovery, 50 inversion points, TR=110ms, TE=1.18ms) and iii) T2 (multi-spin echo, TR=1500ms, TE=16-712ms, 12 echoes). ^{31}P MRS data were acquired for pH quantification, using the chemical shift difference between phosphocreatine and inorganic phosphate peaks. The APT effect was quantified by measuring the area under the asymmetric magnetization transfer ratio (MTR_{asym}) curve between 1.5 and 4.5ppm from water. Two-way ANOVA (GraphPad Prism 6) was used for statistical analysis.

Results and Discussion: Tumour volume calculated from both caliper measurement and diffusion data indicated that treated mice showed an inhibited tumour growth rate, compared with control mice, after a single dose of ixazomib or vehicle after 72hrs ($P<0.001$, Fig.1a).

The median MTR_{asym} in the treated group significantly decreased by 80% from 24 hrs to 72 hrs post-treatment ($P<0.01$, Fig.1b, c). This decrease in MTR_{asym} could be due to changes in protein concentration or pH due to proteasome inhibition by the drug.

This was accompanied by a significant increase in ADC (Fig.1d, e), which could be due to an increase in necrosis. This interpretation is currently being evaluated using histology and Western blotting.

There was a significant difference in median T1 (Fig.1f) between the control and treated group at 24 and 72 hrs. The cause of this difference could be due to changes in water content caused by changes in protein concentration, but this also requires histological verification.

No significant changes in T2 were observed.

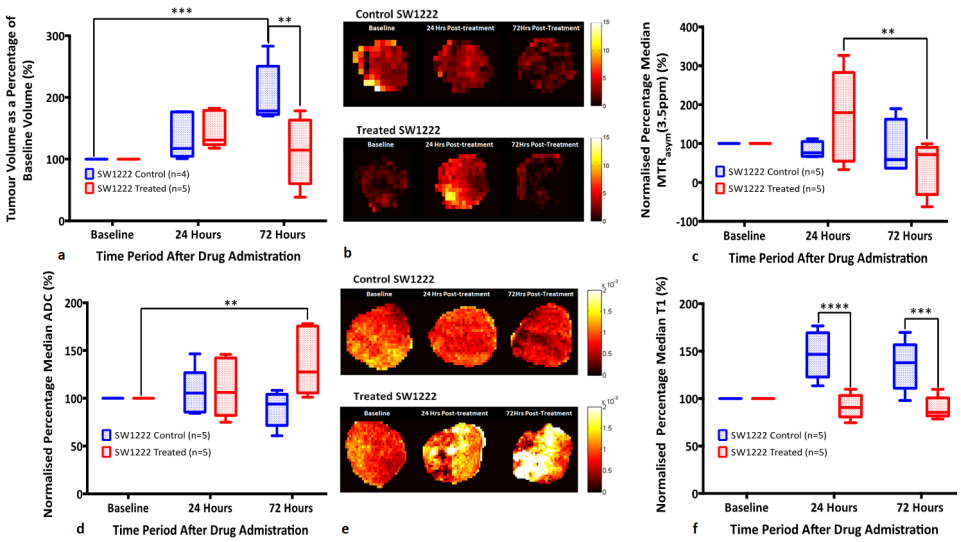

Figure 1: *Relative changes in imaging parameters, in a group of SW1222 colorectal tumour xenograft models, at 24 and 72 hrs following a single dose of the proteasome inhibitor, ixazomib.*
a. *Tumour volume;* ***b.*** *Representative examples of area under MTR_{asym} map from control and treated group;* ***c.*** *Median area under MTR_{asym};* ***d.*** *Median ADC;* ***e.*** *Representative examples of ADC map from control and treated group;* ***f.*** *median T1.*
**P<0.05, **P<0.01, ***P<0.001.*

Conclusion: We observed early changes in APT, ADC, and T1 imaging following PI treatment with ixazomib, which may be due to protein alterations. Further in vivo, in vitro and histological studies will be carried out to identify the factors associated with the changes in the imaging parameters.

References: 1.van Zijl P and Yadav N. *Chemical Exchange Saturation Transfer (CEST): What is in a Name and What Isn't?* Magnetic Resonance in Medicine. 2011; **65**(4): 927-948. 2. Zhou J. *Amide Proton Transfer Imaging of the Human Brain.* Magnetic Resonance Neuroimaging: Methods and Protocols. 2011; **711**: 227-237. 3. Salhotra A, et al. *Amide proton transfer imaging of 9L gliosarcoma and human glioblastoma xenografts.* NMR Biomed. 2008; **21**(5): 489-497