

Monitoring of glioblastoma response to a dual PI3K/mTOR inhibitor using hyperpolarized ¹³C MRSI and ¹H MRS

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Introduction Glioblastoma (GBM) is one of the most aggressive brain tumor types, and is associated with very poor prognosis. Cellular and molecular heterogeneity of GBM frequently lead to resistance to traditional therapies. PI3K/Akt/mTOR, one of the major signaling pathways, is activated in ~88% of GBM [1] and different steps in this pathway can serve as alternative therapeutic targets. We have previously shown that treatment with either PI3K or mTOR inhibitors leads to decreased production of ¹³C MRSI-detectable hyperpolarized (HP) lactate from pyruvate, associated with decreased lactate dehydrogenase (LDH) expression and activity downstream of PI3K/mTOR signaling [2, 3]. The purpose of this study was to monitor the metabolic response to XL765 (also known as SAR245409), a novel dual inhibitor of PI3K and mTOR, which has shown efficacy in tumor models [4] and is currently in clinical trials.

Materials and Methods *Animal model:* All procedures were performed according UCSF IACUC approval. 6 weeks old athymic nu/nu mice were injected intracranially with 3x10⁵ GBM6 cells [3]. Once tumors reached a diameter of 2-3mm, animals were treated p.o. twice daily with either XL765 (SAR245409 courtesy Sanofi at 30mg/kg, 4ml/kg) or vehicle (10mM HCl, 4 ml/kg). All animals were imaged as described below 2-3 times a week. At the end of the study, tumors and normal brain were excised and analyzed by immunohistochemistry. *MR in vivo:* MRI studies were performed using a vertical wide bore Agilent 600MHz scanner. Axial images were recorded using a spin echo sequence (TE/TR=20/1200ms, FOV=30x30mm, 256x256, ST=1.8mm, NA=2). [¹⁻¹³C]-pyruvic acid containing 15mM trityl radical OX063 was hyperpolarized using a HyperSense DNP polarizer, followed by dissolution to a 100mM solution in buffer and injection of 300μl through an i.v. tail-vein catheter over 12s. ¹³C MRSI spectra were recorded 37s after injection using 2D CSI (TE/TR=0.58/66ms, frequency dimension=256, phase dimension=16x16, SW=4223Hz, FOV=32x32mm). Data were processed using the Sivic software [5]. Peak integrals were normalized to noise and normal brain and Lac/Pyru ratios were normalized to pre-treatment values. ¹H MRS spectra were recorded using PRESS sequence (TE/TR=10/4000ms, voxel=2x2x4mm, 256-512, 4096 points, SW=10000Hz) and analyzed using jMRUI.

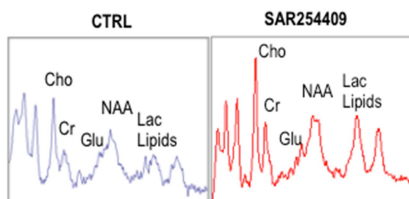


Fig. 3. *In vivo* ¹H MRS spectra.

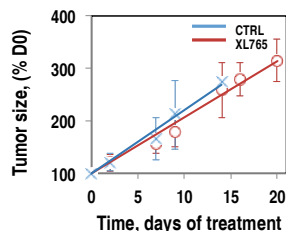


Fig. 4. Evaluation of tumor size during treatment.

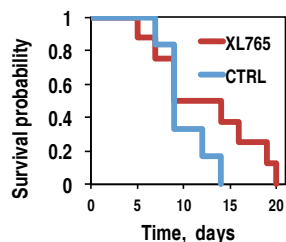


Fig. 5. Kaplan-Meier survival curve.

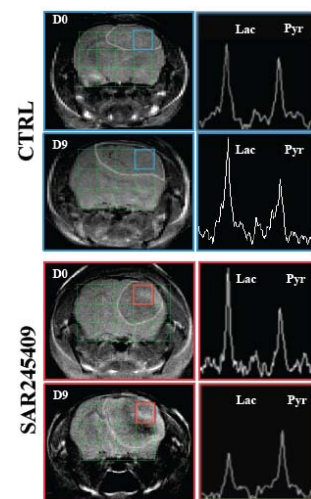


Fig. 1. The left column corresponds to anatomical axial images, the right column to ¹³C MRSI spectra.

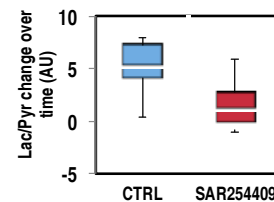


Fig. 2. Box and whisker plot of temporal evolution of Lac/Pyru.

Results and discussions Fig. 1 presents anatomical axial images and overlaid HP ¹³C MRSI grid (left column) and HP ¹³C MRSI spectra (right column) acquired from the tumor voxels in one control and one treated animal at day zero (D0) and day nine (D9). Fig. 2 shows the box and whisker plot of changes in Lac/Pyru ratios during the treatment period ($p=0.04$, $n=5$ and $n=6$ for control and treated animals respectively). *In vivo* ¹H MRS spectra (Fig. 3) indicate a small but insignificant increase in total choline peak (tCho; $p=0.08$, $n=4$ for control and $n=5$ for treated animals) studies are underway to further assess these findings. Fig. 4 shows the evolution of tumor size over time and Fig. 5 illustrates the survival curve of control and XL765/SAR245409-treated mice. Collectively, our data show that the temporal evolution of the Lac/Pyru ratio differs significantly between control and XL765/SAR245409 treated mice and is associated with a significantly longer survival (logrank<0.00003) of the treated animals. Importantly, this effect occurs prior to a detectable change in tumor size and is associated with inhibition of PI3K/Akt/mTOR signaling and LDH-A expression (data not shown). Upon clinical translation, this approach could assist the treatment decision-making process based on individual response to drug at early time points when tumor size is still unchanged.

References: 1. CGARN, *Nature* **2008**, 455(7216). 2. Ward C.S. et al. *Cancer Res.*, **2010**, 70(4). 3. Chaumeil M.M. et al. *Neuroimage*, **2012**, 59(1). 4. Prasad G. et al., *Neuro-Oncology*, **2011**, 13(4). 5. Crane J.C. et al *Inter. J. Biomed. Imag.* **2013**, Article ID 169526.

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