**Hyperpolarized [2-$^{13}$C] pyruvate to [5-$^{13}$C] glutamate as biomarker of IDH1 mutant glioma response to temozolomide therapy**

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**Abstract: (500 words)**

Low-grade gliomas, driven by mutations in the cytosolic isocitrate dehydrogenase 1 (IDH1) gene, are less aggressive than primary glioblastoma, but nonetheless always recur and ultimately lead to patient death. The treatment of IDH1 mutant patients with Temozolomide (TMZ) improves survival, but there remains a need for complementary imaging methods to assess early response to therapy. The goal of this study was therefore to determine the value of magnetic resonance spectroscopy (MRS)-based biomarkers for detection of response to treatment.

Normal Human Astrocyte and U87 cells were genetically engineered to express mutant IDH1 and treated either with TMZ (100μM; N=5), or DMSO (0.2%; N=5) for 72 hours. Then, metabolites were extracted from cells using dual-phase extraction method. $^{1}$H and $^{13}$C spectra were acquired using 500 MHz Bruker Avance spectrometer. Data was analyzed using SIMCA, first using multivariate principal component analysis (PCA), followed by partial least squares discriminant analysis (PLS-DA) and Orthogonal-PLS-DA (OPLS-DA). Correlation values and variable importance in projection (VIP) scores were used to identify altered metabolites. Specific metabolites were also manually integrated for metabolic quantification, integrals normalized to TSP and to cell number and statistical significance of differences determined. For hyperpolarized $^{13}$C-MRS studies, cells were encapsulated in agarose and MRS studies performed in MR compatible cell perfusion system. Live cells were exposed to hyperpolarized 2-$^{13}$C-pyruvate and dynamic sets of $^{13}$C-MRS spectra recorded to monitor the production of hyperpolarized 5-$^{13}$C-glutamate. Hyperpolarized glutamate signal was then quantified and normalized to pyruvate signal and cell number.

When inspecting the $^{1}$H MRS spectra of the control and treated cells, twenty-nine metabolites could be identified using Human Metabolome DataBase and literature. PCA score plot showed separation of TMZ-treated cells from controls. Further, improved separation between the groups was obtained by PLS-DA and OPLS-DA. We then used this model, and the correlation values and VIP plot with threshold of ≥0.6 and ≥1, respectively to identify the most significant metabolites contributing to class separation. Following univariate analysis, several metabolites were found to be altered, most notably

an increase in glutamate and 2-HG observed following treatment. To further assess whether the increase in glutamate and 2-HG could be explained by an increase in TCA cycle flux, synthesis of glutamate and 2-HG from 1-$^{13}$C-glucose, as well as from 3-$^{13}$C-glutamine were probed. Consistent with the increase in total metabolite levels, both glucose- and glutamine-derived glutamate and 2-HG were increased in TMZ treated cells compared to controls, together explaining the increase in total pools. Furthermore, dynamically probing the metabolism of hyperpolarized 2-$^{13}$C-pyruvate revealed that build-up of 5-$^{13}$C-glutamate, which is associated with flux to the TCA cycle, was significantly higher in TMZ-treated cells compared with controls (Fig.1).

Our findings demonstrate that $^{1}$H MRS-detectable metabolomics combined with hyperpolarized 5-$^{13}$C-glutamate have the potential to serve as biomarkers of low-grade glioma response to TMZ therapy. Further studies are needed to confirm the generality of our findings in other mutant IDH1 models. Nonetheless, these findings may help in enhancing currently available imaging methods to improve the early detection of response to TMZ in low grade glioma.

**Highlights of abstract: (50 words)**

$^{1}$H and hyperpolarized $^{13}$C magnetic resonance spectroscopy-based metabolic profiling of cells genetically engineered to express mutant IDH1 and treated with TMZ showed significant alterations in metabolites majorly related to the TCA cycle, and identified hyperpolarized 5-$^{13}$C-glutamate production from 2-$^{13}$C-pyruvate as potentially translatable metabolic biomarkers of response to TMZ therapy.

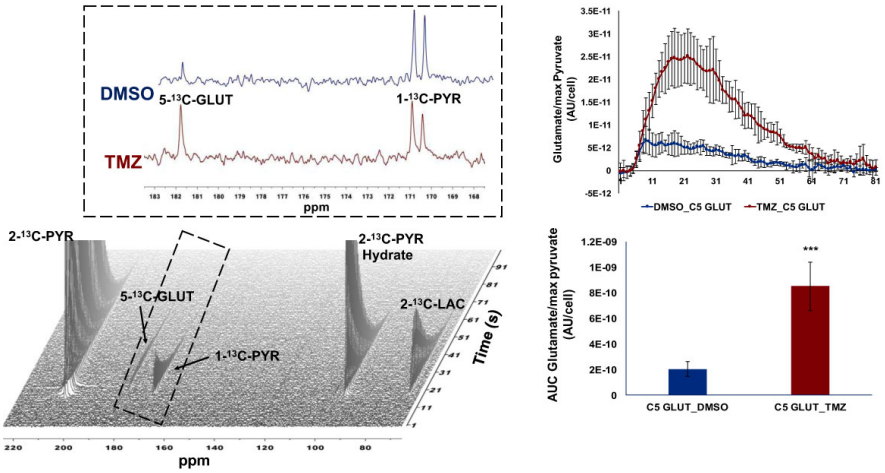


**Fig.1:** Dynamic $^{13}$C-MRS array showing metabolism of hyperpolarized 2-$^{13}$C-pyruvate to 5-$^{13}$C-glutamate in IDH1mut models treated with TMZ or DMSO for 72h. Inserts explain the sum of all spectra within dotted region of the array. 2-$^{13}$C-PYR: 2-$^{13}$C-pyruvate, 2-$^{13}$C-PYR Hydrate: 2-$^{13}$C-pyruvate hydrate, 1-$^{13}$C-PYR: 1-$^{13}$C-pyruvate, 5-$^{13}$C-GLUT: 5-$^{13}$C-glutamate. ***p<0.0001