^{13}C-labeled malate as a treatment response marker in a murine lymphoma model *in vivo*

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Introduction:

Early measurements of tumor responses to therapy have been shown to help predict the subsequent treatment outcome in many tumor cell lines. Previously it has been shown that the flux of hyperpolarized ^{13}C label between pyruvate and lactate in a murine lymphoma model can be used as an early marker of cell death following chemotherapy both *in vitro* and *in vivo* [1]. This study show that a promising new tumor biomarker - hyperpolarized [1,4-$^{13}C_2$]fumarate - can be used in the same model to image the effects of chemotherapy.

Methods:

[1,4-$^{13}C_2$]fumarate was polarized to (30±3)% in the liquid state using Dynamic Nuclear Polarisation (DNP) and a method similar to that previously described [2].

Tumors were grown by implanting 5 million EL4 cells subcutaneously into female c57BL/6 mice. On day 9 after implantation, the EL4 tumor was imaged. The *in vivo* MR experiments were performed at 2.35T (Bruker Biospec Avance II) and ^{13}C spectra were acquired with a 20 mm surface coil placed around the tumor. Mice were anaesthetized with 2% isoflurane in 1:1 mixture of N_20 and O_2. Breathing rate and temperature was monitored and controlled (SA instruments, USA). The hyperpolarized [1,4-$^{13}C_2$]fumarate was injected i.v. (175 µl of 20 mM injected over 6 s) and a slice selective FID was acquired with a 10° RF flip angle, TR = 3 s, and 60 repetitions. On day 10, the mice were injected with 2 mg etoposide i.p. (300 µl of Eposin® 20 mg/ml diluted 1:2 in saline, PCH Pharmachemie BV). On day 11, 22-26 hours after etoposide injection, the MR experiments were repeated by another injection of hyperpolarized [1,4-$^{13}C_2$]fumarate (n=5). Spectra were analyzed in the time domain using jMRUI. Signal amplitudes for both malate and fumarate were normalized to the maximum fumarate signal and were measured as a function of time.

Results and discussion:

After the injection of [1,4-$^{13}C_2$]fumarate, [1-^{13}C]malate and [4-^{13}C]malate signals could be clearly observed in all the slice-selective spectra - both in the untreated as well as the treated EL4 tumors. The etoposide-treated tumors showed a 65% higher malate signal compared to the untreated tumours (Fig. 1). It has been previously demonstrated that the Lactate Dehydrogenase (LDH) catalysed flux of hyperpolarized ^{13}C between [1-^{13}C]lactate and [1-^{13}C]pyruvate decreases in the same model following treatment [1]. Here we show an increase in the hyperpolarized malate signal following fumarate injection in treated tumors. This offers the possibility of a positive contrast agent to detect tumor response.

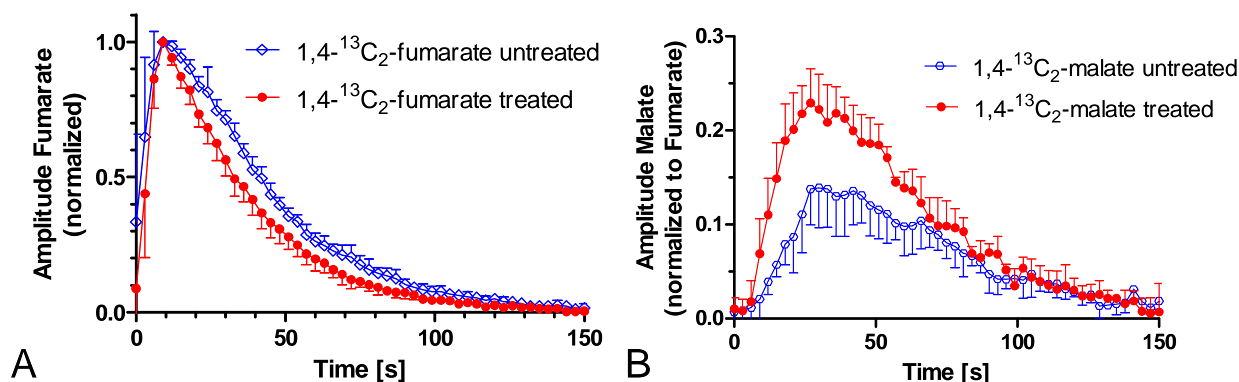

Figure 1. The amplitude of [1,4-$^{13}C_2$]fumarate (A) and ^{13}C malate (B) as a function of time in the etoposide treated EL4 lymphoma tumors and the untreated controls (n=5).

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References: [1] Day *et al*., Nature Med, 2007, [2] Ardenkjaer-Larsen *et al*., PNAS, 2003.