# Imaging Fluoropyrimidine-based Cancer Chemotherapy using $^{13}$C Hyperpolarised MR Technology

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## Introduction

Fluoropyrimidines are anti-metabolites used routinely in the treatment of several types of cancer. 5-FluoroUracil (5-FU) was the prototype fluoropyrimidine compound designed to target thymidylate synthase (TS), an enzyme essential for DNA synthesis and repair. In order to overcome the side effects associated with the systemic delivery of 5-FU, several strategies involving prodrugs of 5-FU such as Capacetabine, Gemcitabine have been developed. Other targeted strategies, such as gene-directed enzyme prodrug therapy (GDEPT) (1) employed the yeast cytosine deaminase (CD) to selectively activate the nontoxic prodrug 5-FluoroCytosine (5-FC) into 5-FU in tumors (Fig 1). $^{19}$F MRS has been elegantly used to monitor the pharmacokinetics and metabolism of fluoropyrimidines *in vivo* (1) as well as the CD-mediated activation of 5-FC into 5-FU (2). However $^{19}$F MRS remains a relatively insensitive method especially at clinical magnetic field strength, leading to very low SNR.

This study investigates the use of dynamic nuclear polarization (DNP) and $^{13}$C MRS to monitor fluoropyrimidine metabolism and the potential to monitor the CD-mediated activation of 5-FC into 5FU. We especially demonstrate that both 5-FC and 5-FU are readily hyperpolarizable, demonstrate long $T_1$ > 20s and a appreciable $^{13}$C chemical shift difference of ~ 5ppm. The translation of such strategy *in vivo* could lead to the generation of high spatial resolution map of CD-mediated activation of 5-FU as well as new imaging strategy to monitor fluoropyrimidine-based therapies.

## Materials and Methods

- ***Dynamic Nuclear Polarization:*** 5-FC and 5-FU (natural abundance $^{13}$C 1%) were dissolved (2 mg) in solutions (100 µl) containing 3mg of free radical OX63 with a glassing agent composed of 2:1 DMSO:$H_2O$ and polarized for 3 hours in a HyperSense® DNP at low temperature (1.4 K) with microwave irradiation at 94 GHz. The polarized samples were rapidly dissolved in 4 ml PBS and transferred into a 11.7T spectrometer. A series of $^{13}$C NMR spectra were subsequently acquired every 2s using a 10° pulse-and-acquire sequence (1 transient, 32k time domain points, a 19 kHz spectral width, acquisition time 2s, T = 37°C).

- ***Natural abundance $^{13}$C MRS:*** A solution of 5-FC (5 mg) and 5-FU (10 mg) in 2 ml PBS, 10% $D_20$ (pH 7) was placed into a 11.7T Bruker Avance system spectrometer. A $^1$H decoupled-$^{13}$C NMR spectrum was acquired at 37°C using a 90° pulse-and-acquire sequence (2048 transients, 32k time domain points, a 19 kHz spectral width, acquisition time ~12h).

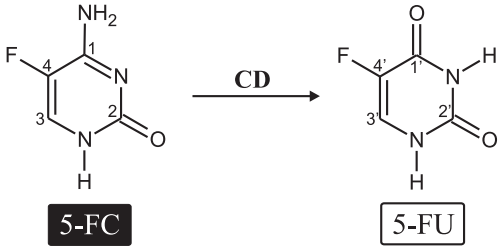


**Figure 1.** CD-mediated conversion of 5-FC into 5-FU

## Results

All the $^{13}$C resonances of both 5-FC and 5-FU were successfully hyperpolarized (Fig 2.**(a)** and **(b)**) with an estimated signal enhancement of over 10,000 compared with the thermal equilibrium spectrum (Fig 2.**(c)**).

The $^{13}$C $T_1$ values were calculated from the decay of the hyperpolarized signal over time as described in (4) and are reported in Table 1. The $^{13}C_2$ resonance is the most attractive to monitor the CD-mediated deamination as it would present the largest chemical shift difference (> 5 ppm) upon deamination, appears as a singlet (no $^{19}$F-$^{13}$C scalar coupling) and presents the longest $T_1$ ( ~29 s).

| 5-FC | | 5-FU | | ΔΩ (ppm) |
|---|---|---|---|---|
| $^{13}C_1$ | 16.7 ± 0.4s | $^{13}C_{1'}$ | 25.6 ± 0.5s | 1.6 |
| $^{13}C_2$ | 29.1 ± 0.5s | $^{13}C_{2'}$ | 23.2 ± 0.5s | -5.4 |
| $^{13}C_a$ | 9.3 ± 0.9s | $^{13}C_{a'}$ | 9.7 ± 1s | 3.4 |

**Table 1.** $^{13}$C $T_1$ values of 5-FC and 5-FU and observed chemical shift differences

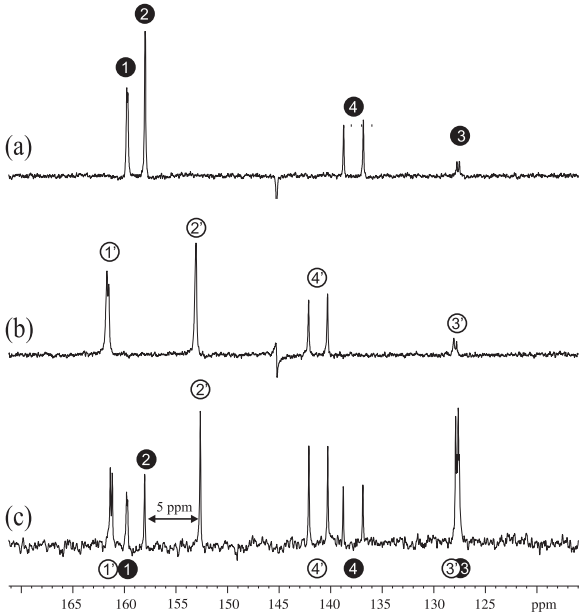


**Figure 2.** Hyperpolarised $^{13}$C MR spectrum of **(a)** 5-FC and,**(b)** 5-FU (~ 4mM, 1 transient). **(c)** Thermal equilibrium $^{13}$C MR spectrum of a solution containing both 5-FC (~20mM) and 5-FU (~ 40 mM) (2048 transients).

## Discussion and Conclusion

We report the successful hyperpolarization of 5-FC and 5-FU. 3,5- The long $T_1$ (> 20s at 11.7T) of $^{13}C_2$ of both 5-FC and 5-FU may provide a sufficient imaging window for detecting CD-mediated activation of 5-FU *in vivo* and encourage further investigations. Isotopic enrichment of the $C_2$ of 5-FC with $^{13}$C will provide a further 100-fold enhancement of the signal and the potential to generate high spatial resolution metabolic maps of CD-mediated activation of 5-FC into 5-FU. This study also encourages further investigation on the translation of this approach to monitor other fluoropyrimidine-based chemotherapies, especially on the use of hyperpolarized 5-FU or its other prodrugs: Capacetabine and Gemcitabine.

## Acknowledgements

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## References

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