Developing a novel lipid-based imaging tool for the surveillance of chronic lymphocytic leukemia.

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Introduction

The reliance of B-CLL cells on non-glucose-based metabolic pathways (1) largely explains why [^{18}F] fluoro-2-deoxyglucose positron emission tomography (FDG-PET) provides limited diagnostic value in patients who have not undergone Richter's transformation (2). We have previously shown primary CLL cells and CLL cell lines prefer long-chain saturated fatty acids such as palmitic acid (C16:0) over glucose, thereby highlighting a clear preference for lipids (3).

Hypothesis

We hypothesise a lipid-based imaging tool would be more informative than the current FDG-PET tracer for the diagnosis and surveillance of CLL.

Aims

1. Synthesise lipid derivatives to assess uptake and localisation *in vitro*.
2. Confirm uptake of the lipid probes in CLL and healthy cell lines using flow cytometry and gas chromatography mass spectrometry (GCMS).
3. Investigate localisation of the lipid probes in CLL cell lines.

Methods

1. Synthesise C16:0 lipid derivatives

Fluorescently labelled

Unlabelled

2. Incubate cell lines with fluorescent or unlabelled lipids (< 48 hours)

- OSU-CLL (CLL)
- FH-9 (healthy B-cells)
- C2C12 (skeletal muscle, mouse)

3a. Flow cytometry

- Lipid uptake (fluorescent lipids)
- Viability
- Proliferation

3b. Gas chromatography mass spectrometry

- Lipid uptake (unlabelled lipids)

*Investigate if fluorescent tag impacts uptake

3c. Fluorescence microscopy

- Localisation in CLL cells
  - Nucleus
  - Mitochondria

Results

1. CLL cells had significantly higher uptake of the fluorescent methyl ester derivative.

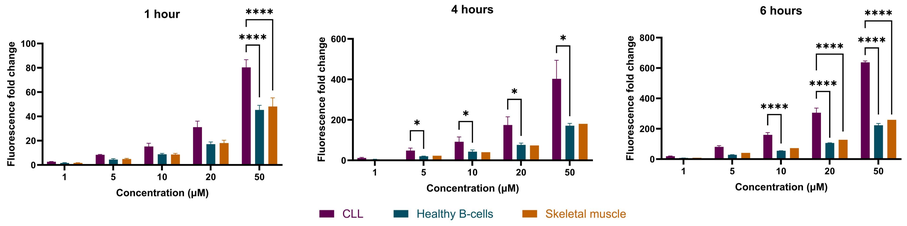

Figure 1: CLL cells had higher uptake of the fluorescent methyl ester derivative. Over a period of six hours, uptake of the fluorescent methyl ester derivative was quantified in CLL cells (OSU-CLL), a healthy B-cell line (FH-9) and skeletal muscle (C2C12) using flow cytometry. Uptake was determined by calculating the fold change in mean fluorescence intensity normalised to the respective 0 μM control. Statistical significance was determined using a two-way ANOVA with a *post hoc* Tukey's test (*, p<0.05, **, p<0.01, ***, p<0.001, ****, p<000.1). n = 3.

2. CLL cells had lower uptake of the fluorescent carboxylic acid derivative.

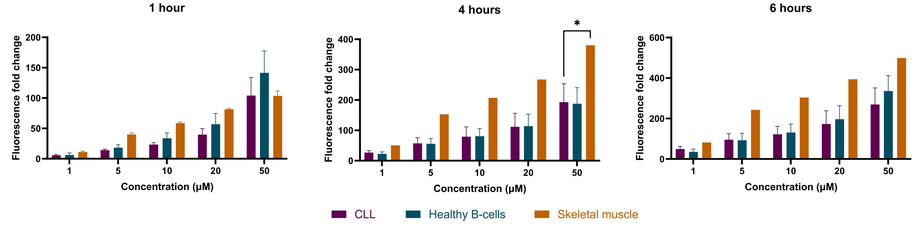

Figure 2: CLL cells had lower uptake of the fluorescent carboxylic acid derivative. Over a period of six hours, uptake of the fluorescent carboxylic acid derivative was quantified in CLL cells (OSU-CLL), a healthy B-cell line (FH-9) and skeletal muscle (C2C12) using flow cytometry. Uptake was determined by calculating the fold change in mean fluorescence intensity normalised to the respective 0 μM control. Statistical significance was determined using a two-way ANOVA with a *post hoc* Tukey's test (*, p<0.05, **, p<0.01, ***, p<0.001, ****, p<000.1). n = 3.

3. CLL cells have higher uptake of the unlabelled methyl ester derivative.

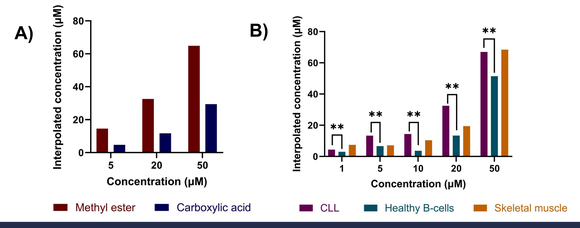

Figure 3: CLL cells have higher uptake of unlabelled lipids than healthy cell lines. A) The OSU-CLL cell line had increased uptake of the unlabelled methyl ester derivative compared to the unlabelled carboxylic acid. Uptake was normalised to an internal standard. **B)** Uptake of the unlabelled methyl ester derivative was significantly higher in CLL cells (OSU-CLL) compared to healthy B-cells (FH-9). For GCMS experiments, cells were treated with unlabelled lipid for 1 hour prior to lipid extraction using a Folch extraction. Lipid extracts were analysed on an Agilent 7890A GCMS with a 5975C MSD. Statistical significance was determined using a two-way ANOVA (**, p<0.01).

4. Cell proliferation and viability were stable over 48-hour treatment with the fluorescent methyl ester derivative.

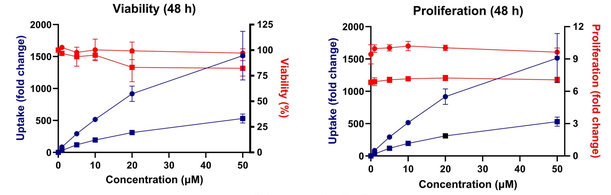

Figure 4: Cell proliferation and viability were stable over 48-hour treatment with the fluorescent methyl ester derivative. Viability was calculated with DiIC$_1$(5) using flow cytometry and was normalised to 0 μM control. Proliferation was calculated with CellTrace™ Far Red (Thermo Fisher) using flow cytometry and was normalised to 0 h fluorescence signal. n = 3.

5. Methyl ester derivative partially localises to mitochondria.

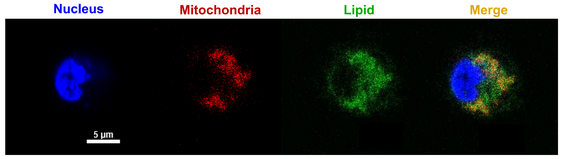

Figure 5: Methyl ester derivative partially localises to mitochondria. The OSU-CLL cell line was treated with 10 μM fluorescent methyl ester derivative for 1 hour. Cells were stained with 1 μM Hoechst 33342 and 100 nM MitoTracker™ Deep Red (Thermo Fisher) for 30 mins at 37°C. Images acquired with a Zeis LSM 880 Confocal Microscope.

Conclusions

- CLL cells have higher uptake of the methyl ester derivatives than healthy B-cells and skeletal muscle.
- GCMS results indicate the fluorescent label is not driving uptake in CLL and healthy cell lines.
- Uptake of the methyl ester derivative does not impact cell proliferation or viability in CLL or healthy B-cells.
- The methyl ester derivative partially localises to the mitochondria in CLL cells.

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References
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2. Nabih, S.O., et al., *18F-FDG positron emission tomography-computed tomography and Richter transformation: A retrospective study with a cohort of 12 consecutive patients.* ARNM, 2024. 2(1).
3. Thurgood, L.A., et al., *Lipid uptake in chronic lymphocytic leukemia.* Exp. Hematol, 2022. 106: p. 58-67.
Methods figure created with BioRender.com.

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