A Phenotypically Distinct Population of CLL Cells with Lower CD20 Expression is Present Within Abdominal Subcutaneous Adipose Tissue of Patients with CLL

Rebecca Oliver[1,2], Frankie F Brown[1,3], Adam J Causer[1], Annabelle Emery[1], Harrison D Collier-Bain[1], Francoise Koumanov[1], James E Turner[4], James Murray[5], John P Campbell[1,6]

[1]Clinical Rehabilitation and Exercise Medicine, Department for Health, University of Bath, United Kingdom; [2]Department for Haematology, Royal United Hospitals Bath NHS Foundation Trust, United Kingdom; [3]School of Applied Sciences, Edinburgh Napier University, Edinburgh, United Kingdom; [4]School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, United Kingdom; [5] Department for Haematology, University Hospital of Wales, United Kingdom

Correspondance to rebecca.oliver@nhs.net

INTRODUCTION

- In individuals with CLL, a high BMI is associated with a higher rate of CLL-related morbidity and mortality, reduced progression-free survival and a poorer response to baseline therapy.[1,2]
- Except for smoking, increased BMI is the only known modifiable characteristic associated with poorer outcomes in CLL.
- Adipocytes play an active role in the microenvironment of many cancers, including leukaemias[3]. *In vitro* studies have shown that fatty acid provision by adipocytes can contribute to chemoresistance in leukaemias[4,5]
- To-date there have been no studies investigating whether CLL cells are present within lipid-rich subcutaneous adipose tissue, or whether CLL cells interact with adipocytes within that microenvironment.

AIMS

- To establish whether clonal cells are present within the subcutaneous adipose tissue (AT) stromal vascular fraction (SVF) of patients with CLL
- To investigate whether interaction between adipocytes and CLL cells *in vitro* affects CLL cell survival and migration

METHODS

- Paired abdominal subcutaneous AT biopsies and peripheral blood (PB) samples were obtained from patients with stable, treatment-naïve CLL, Binet stages A and B (n=15). See table 1 for patient characteristics.
- Flow cytometry was used to characterise leucocyte subsets and CD5+/CD19+ kappa/lambda restricted B-CLL cells
- *In vitro* studies were performed to investigate the effect of human adipocytes on CLL cell survival and migration
- Primary CLL cells were isolated from the PB of patients with treatment-naïve CLL
- Isolated CLL cells were cultured alone and in the presence of human mature adipocytes (HAd cells) for 48 hours. Viability was assessed using flow cytometry
- A 3µm pore membrane Transwell migration assay was used to assess the migratory capacity of CLL cells in the presence and absence of adipocyte-conditioned media (ACM). Prior to the assay, CLL cells were cultured alone or in the presence of the CXCR4 antagonist AMD3100.

Table 1: Patient Characteristics

Variable	Treatment naïve
Total participants (n)	15
Male:female participants (n:n)	11:4
Age (years) (mean[range])	62.9 (51-82)
Height (cm)	177.2 (170-185.1)
Body mass (kg)	82.5 (62.9-98.3)
Body mass index (kg.m^{-2})	25.9 (21.7-32.2)
Body fat (%)	29.65 (18.9-46.7)
Haemoglobin (g/L)	133 (114-146)
Leukocytes (×10^9/L)	35.3 (8.9-81.7)
Lymphocytes (×10^9/L)	30.4 (6.6-74.1)
Neutrophils (×10^9/L)	3.1 (0.93-5.41)
Monocytes (×10^9/L)	0.62 (0.04-1.2)

RESULTS

1 CD5+/CD19+ kappa/lambda restricted B cells are present within the subcutaneous adipose tissue SVF of patients with treatment naïve CLL

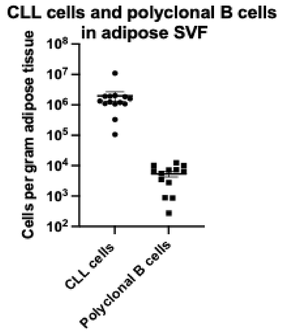

Figure 1: Dot plot showing the number of CLL cells and polyclonal B cells identified in adipose tissue SVF. The number of light chain restricted CD5+/CD19+ CLL cells and polyclonal B cells per gram of adipose tissue isolated from the adipose SVF of the 14 participants with treatment naïve CLL. Individual values and mean plus standard error of the mean (SEM) displayed.

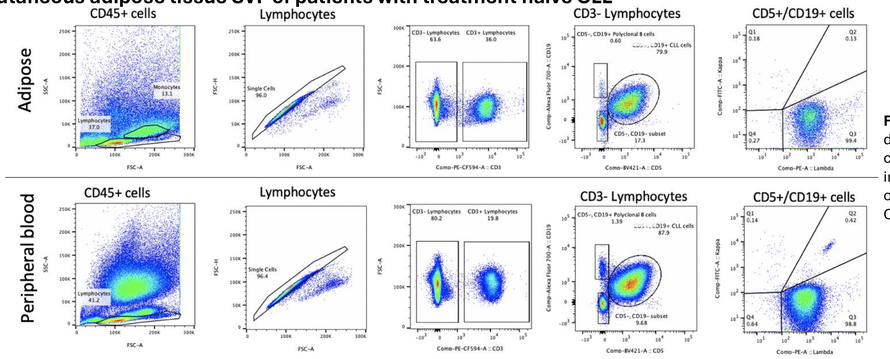

Figure 2. Flow cytometry plots demonstrating a population of light chain restricted CD5+/CD19+ CLL cells in the peripheral blood and adipose SVF of a single participant with untreated CLL.

2 CLL cells isolated from subcutaneous AT have significantly lower expression of CD20 and CD5, and higher levels of expression of CD38

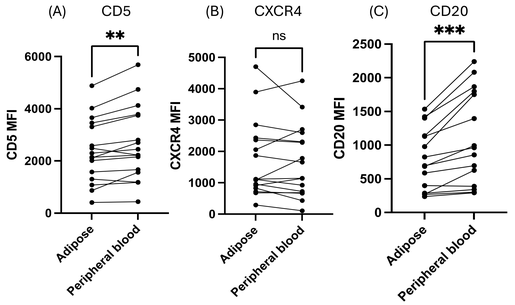

Figure 3. CD5, CXCR4 and CD20 expression in CLL cells isolated from adipose tissue and peripheral blood (PB). (A) Compared to PB-derived CLL cells, adipose SVF derived CLL cells have significantly lower expression of CD5 (p<0.005). (B) There is no difference in the CXCR4 MFI of adipose SVF-derived and peripheral blood-derived CLL cells. (C) CD20 MFI is significantly lower in CLL cells isolated from adipose SVF compared to the CD20 MFI of CLL cells isolated from paired peripheral blood samples (p<0.001).

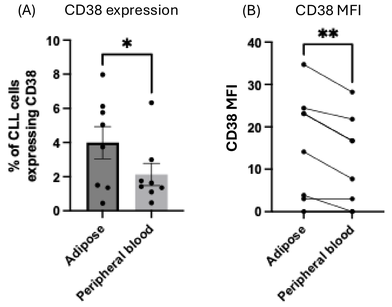

Figure 4. Comparison of CD38 percentage expression and MFI on adipose SVF and PB CLL cells in bi-modal CD38 expressors. (A) There is a significantly higher percentage of CLL cells expressing CD38 in adipose SVF compared to paired PB samples in participants with CLL with bi-modal expression of CD38 (n=8, paired students t test, p=0.0319). (B) There is a significantly higher CD38 MFI in CLL cells isolated from adipose SVF compared CLL cell isolated from paired PB samples in patients with bi-modal expression of CD38 (n=8, student's t test, p=0.0054)

3 Co-culture with human adipocytes (HAd) significantly increases the viability of CLL cells *in vitro*

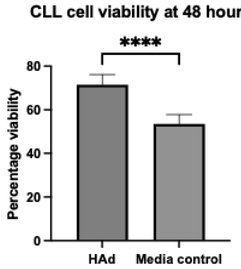

Figure 4. HAd cells protect CLL cells from spontaneous apoptosis. Percentage viability of CLL cells at 48 hours cultured in media alone (n=19), and in the presence of HAd cells (n=20). Viability was assessed at 0 hours and 48 hours and data is shown as percentage viability at 48hours, where viability at t=0 hours is 100%. Co-culture of CLL cells alongside HAd cells was associated with a significant increase in viability compared with culture in media alone (paired students t test, p<000.1). Data is presented as mean +/- standard deviation.

4 The presence of adipocyte-conditioned media significantly increases the migratory capacity of CLL cells

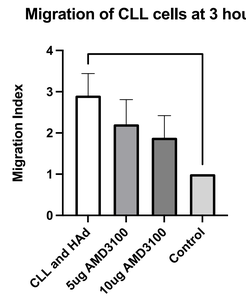

Figure 5. The presence of adipocyte conditioned media (ACM) increases the migration of primary CLL cells across a 3µm pore membrane (p<0.05) at 3 hours. There was no significant reduction in migration of CLL cells in the presence of ACM when incubated with 5ug or 10ug of the CXCR4 antagonist AMD3100, for 30 minutes prior to the Transwell assay.

CONCLUSIONS

- A phenotypically distinct population of clonal B cells with lower CD20 expression is present within subcutaneous AT of patients with treatment naïve CLL
- *In vitro* migration of CLL cells is enhanced in the presence of human adipocyte-conditioned media. This is not affected by blockade of the CXCR4-SDF1a pathway with CXCR4 antagonist AMD3100
- The presence of human adipocytes increases the viability of CLL cells *in vitro*
- Further studies are needed to establish whether this contributes to treatment resistance and relapse

REFERENCES

1. Yao, Y., et al., The global burden and attributable risk factors of chronic lymphocytic leukemia in 204 countries and territories from 1990 to 2019: analysis based on the global burden of disease study 2019. Biomed Eng Online, 2022. **21**(1): p. 4.
2. Furstenau, M., et al., Influence of obesity and gender on treatment outcomes in patients with CLL undergoing rituximab-based chemoimmunotherapy. Leukemia, 2020. **34**(4): p. 1177-1181.
3. Austin, MJ et al. Turning the spotlight on bone marrow adipocytes in haematological malignancy and non-malignant conditions. BJHaem, 2023. **201**(4)
4. Ye, H., et al., *Leukemic Stem Cells Evade Chemotherapy by Metabolic Adaptation to an Adipose Tissue Niche.* Cell Stem Cell, 2016. **19**(1): p. 23-37
5. Masoodi, M., et al., *A role for oleoylethanolamide in chronic lymphocytic leukemia*. Leukemia, 2014. **28**(7): p. 1381-7