# PERIPHERAL BLOOD CXCR4$^{HI}$CD5$^{HI}$ CHRONIC LYMPHOCYTIC LEUKEMIA CELLS ARE PRIMED FOR RETURNING TO THE LYMPH NODES FOR FURTHER ACTIVATION AND PROLIFERATION

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## INTRODUCTION

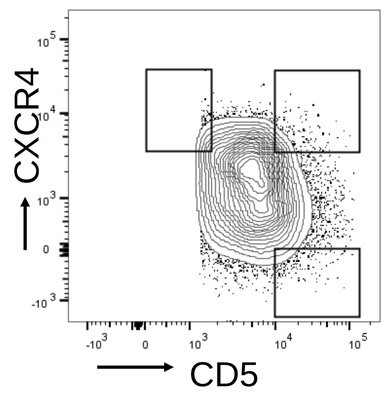
- Chronic Lymphocytic Leukemia (CLL) cells proliferate in lymph nodes with turnover rates ranging from 0.1-1% of the entire clone per day[1].
- In peripheral blood (PB), CXCR4$^{lo}$CD5$^{hi}$ cells are described as proliferating cells that have recently egressed from the lymph node (LN) with quiescent cells found in the CXCR4$^{hi}$CD5$^{lo}$ fraction[2].
- However, we poorly understand the intraclonal variation and dynamics of CLL cells

## AIM

To functionally characterise subfractions of PB CLL cells.

## METHODS

- Ki67 and DAPI were used to identify proliferating CLL cells in Eμ-TCL1 transgenic mice PB, LN and Spleen compartments.
- Cryopreserved PBMCs of 22 human CLL; 12 with unmutated *IGHV* genes (U-CLL), and 11 with mutated *IGHV* genes (M-CLL) were stained for CD19, CD5 and CXCR4 along with markers involved in proliferation, activation and migration and analysed using flow cytometry.
- CXCR4$^{lo}$CD5$^{hi}$, CXCR4$^{hi}$CD5$^{lo}$ and CXCR4$^{hi}$CD5$^{hi}$ cell fractions were defined as containing ~2% of the total CD19$^{+}$CD5$^{+}$ population. Representative example of gating:




- To mimic the lymph node environment *in vitro*, human U-CLL cells (n=8) were seeded on irradiated CD40L-expressing 3T3 fibroblasts and cultured in the presence of IL-4 and IL-21 for 9 days.

## RESULTS

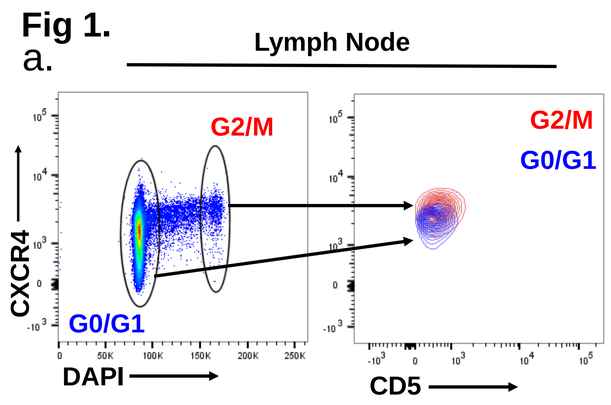



**Fig 1. The CXCR4$^{hi}$CD5$^{hi}$ fraction contains the most proliferative leukemia cells in the Eμ-TCL1-tg mouse.** (a) Representative plots of CXCR4 and CD5 levels on dividing leukemic cells in G2/M phase, as identified by DAPI staining. (b) CLL cells from PB, LNs and Spleens (SPs) were stained for intracellular Ki67 and Ki67+ cells quantified. Bars show mean±SEM. *p <0.05, **p <0.01, (one-way ANOVA with Tukey's multiple comparisons). n=8

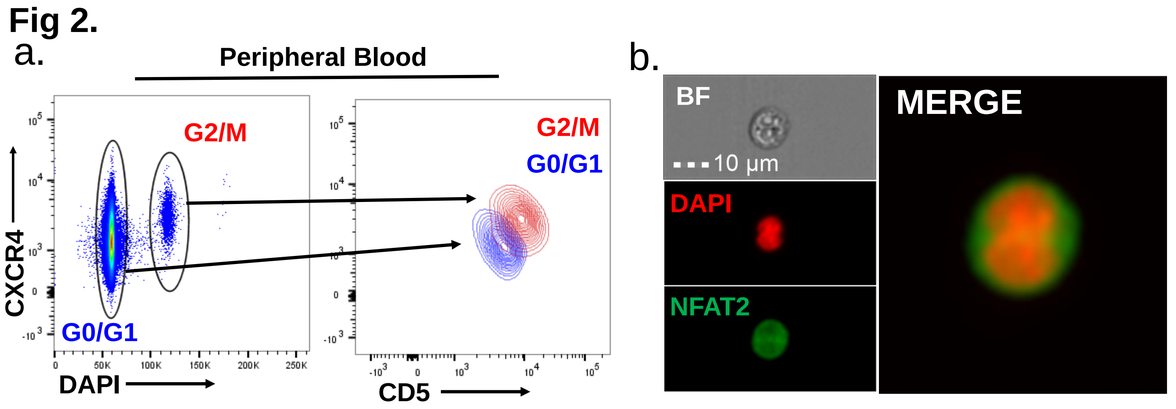



**Fig 2. The CXCR4$^{hi}$CD5$^{hi}$ fraction is enriched for dividing leukemia cells in human peripheral blood.** (a) Representative plots of CXCR4 and CD5 levels on dividing leukemic cells in G2/M phase, as identified by DAPI staining. (b) Representative images of a dividing PB CLL cell in G2/M phase acquired using imaging flow cytometry. Fluorescent antibodies against NFAT2 were used to mark the cytoplasmic compartment.

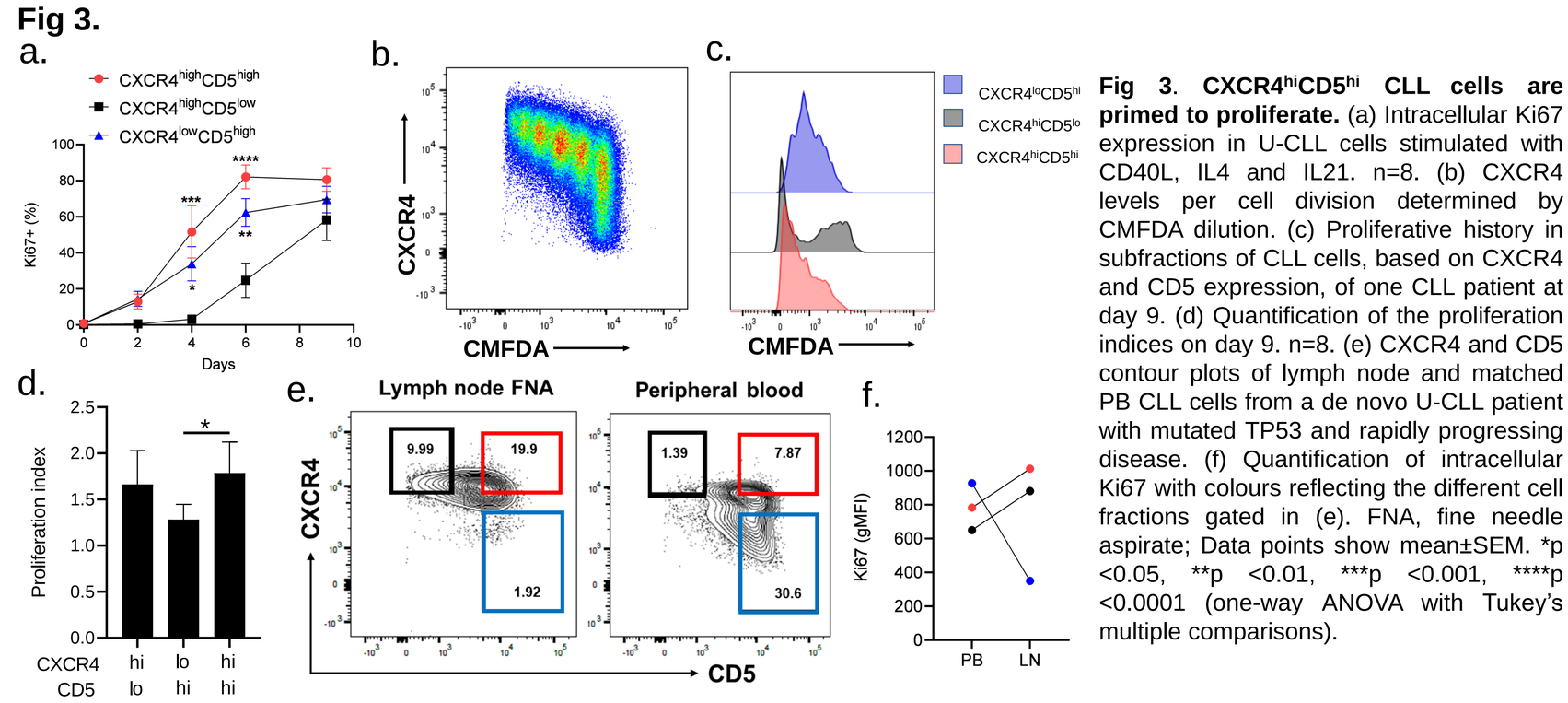



**Fig 3. CXCR4$^{hi}$CD5$^{hi}$ CLL cells are primed to proliferate.** (a) Intracellular Ki67 expression in U-CLL cells stimulated with CD40L, IL4 and IL21. n=8. (b) CXCR4 levels per cell division determined by CMFDA dilution. (c) Proliferative history in subfractions of CLL cells, based on CXCR4 and CD5 expression, of one CLL patient at day 9. (d) Quantification of the proliferation indices on day 9. n=8. (e) CXCR4 and CD5 contour plots of lymph node and matched PB CLL cells from a de novo U-CLL patient with mutated TP53 and rapidly progressing disease. (f) Quantification of intracellular Ki67 with colours reflecting the different cell fractions gated in (e). FNA, fine needle aspirate; Data points show mean±SEM. *p <0.05, **p <0.01, ***p <0.001, ****p <0.0001 (one-way ANOVA with Tukey's multiple comparisons).

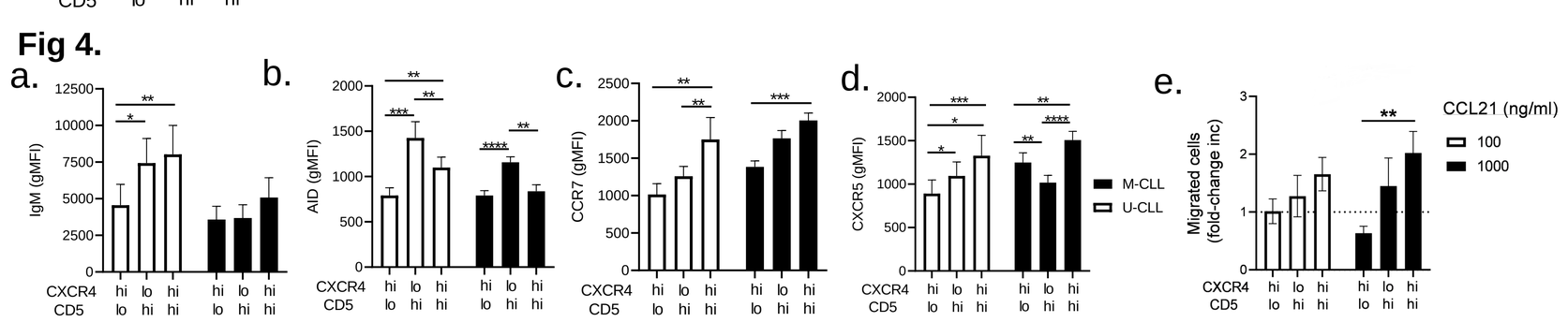



**Fig 4. Human CXCR4$^{hi}$CD5$^{hi}$ CLL cells are primed for migration to lymph nodes.** Expression of (a) IgM, (b) AID, (c) CCR7 and (d) CXCR5 was quantified on subfractions of human PB CLL cells by flow cytometry. n=12 U-CLL; n=11 M-CLL e) Migration towards CCL21 was quantified for subfractions of CLL cells. n =12 U-CLL. Bars show mean±SEM. *p <0.05, **p <0.01, ***p <0.001, ****p <0.0001 (one-way ANOVA with Tukey's multiple comparisons).

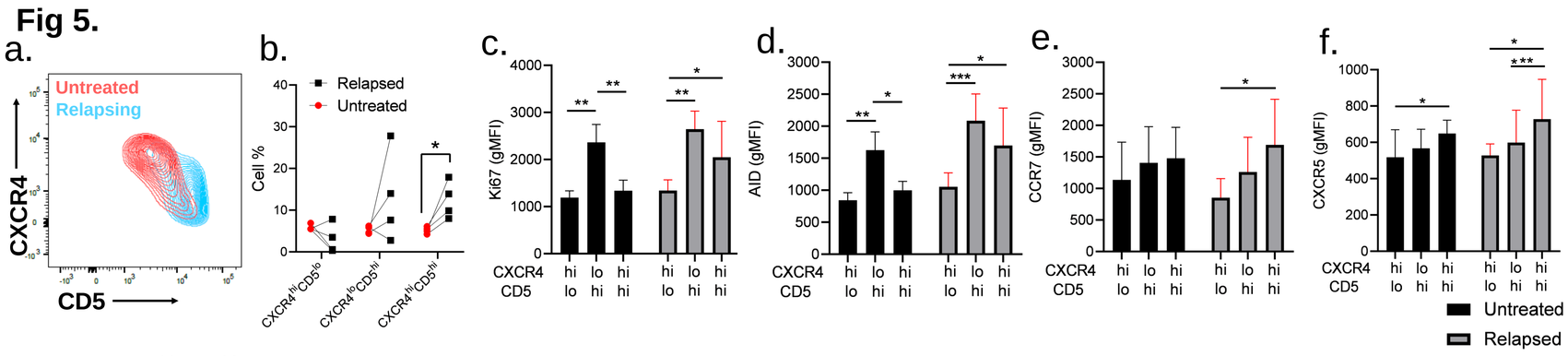



**Fig 5. CXCR4$^{hi}$CD5$^{hi}$ cells are expanded at relapse and have increased levels of Ki67, AID, CCR7 and CXCR5.** (a) CXCR4 and CD5 levels before treatment and at relapse. b) Frequencies of CXCR4$^{lo}$CD5$^{hi}$, CXCR4$^{hi}$CD5$^{lo}$ and CXCR4$^{hi}$CD5$^{hi}$ CLL cells before treatment and at relapse. Expression of (c) Ki67, (d) AID, (e) CCR7, and (f) CXCR5 was quantified by flow cytometry on subfractions of PB CLL cells before treatment (black) and at relapse (Grey). Bars show mean±SEM. *p <0.05, **p <0.01, ***p <0.001, (RM One-way ANOVA with Tukey's multiple comparisons). n=4.

## CONCLUSIONS

1. The CXCR4$^{hi}$CD5$^{hi}$ PB CLL cell subfraction contains dividing cells with high expression of CXCR4, CD5, IgM, CCR7 and CXCR5 open for both migration to tissue and reception of BCR signals.
2. CXCR4$^{hi}$CD5$^{hi}$ cells are primed to proliferate in response to LN environmental stimuli suggesting this fraction is a crucial link in the regeneration of newly dividing tissue CLL cells and plays a key role in the expansion and maintenance of CLL.

## ACKNOWLEDGEMENTS

- The King's College Hosiptal CLL patient cohort
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## REFERENCES

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