A FRACTION OF LYMPH NODE CLL B CELLS EXHIBITS HIGH EXPRESSION OF T-BET

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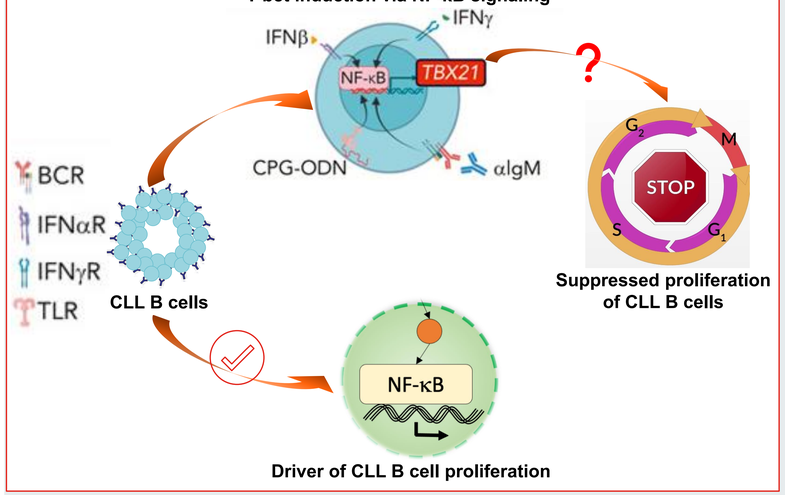
INTRODUCTION

T-box transcription factor TBX21 (T-bet) in CLL

- Expression: Found in CLL B cells from peripheral blood (PB)[1]
- Induction: Driven by inflammatory signals via **NF-κB**[1]
- Function: Acts as **a tumour suppressor** by enhancing interferon signalling and suppressing CLL B cell proliferation[1]
- Prognostic value: Positively correlated with longer overall survival in CLL patients[1]
- **Paradoxically,** NF-κB is a well-known driver of CLL B cell proliferation in lymphoid tissue[2,3]

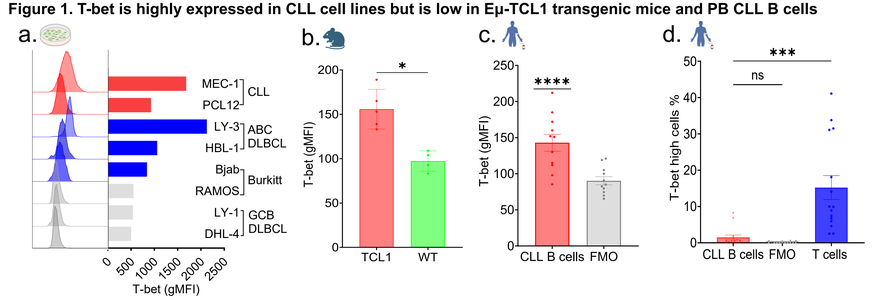
T-bet induction via NF-κB signaling



AIM

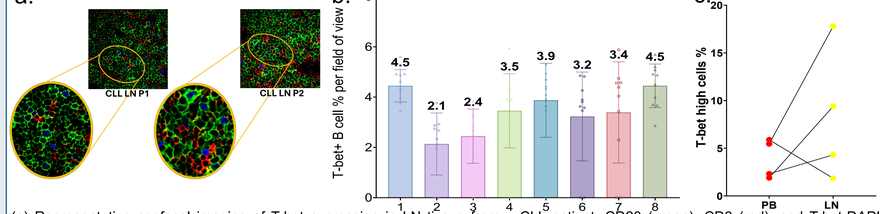
To ascertain compartment-specific T-bet levels in CLL and examine its relationship to microenvironmental signals and proliferative status

Figure 1. T-bet is highly expressed in CLL cell lines but is low in Eμ-TCL1 transgenic mice and PB CLL B cells



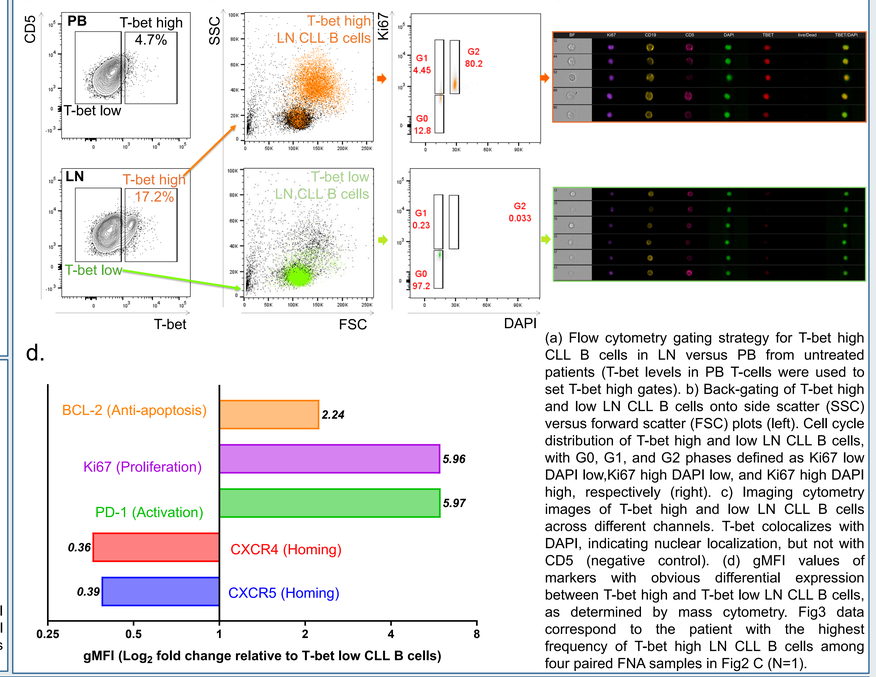
(a) Representative flow cytometry data quantifying T-bet expression in NHL cell lines. Blue: MYD88-mutated cell lines. Red: CLL cell lines. (b) Quantification of T-bet gMFI in CLL B cells ($CD5^+CD19^+$) from $TCL1^+$ transgenic mouse spleens (n=5) compared to B cells ($CD5^-CD19^+$) from WT mouse spleens (n=4). Data presented as mean ± SEM. **p<0.01 (one-way ANOVA). (c) T-bet expression levels in CLL B cells from cryopreserved PBMCs of CLL patients (n=15) show patient heterogeneity. Data presented as mean ± SEM. ****p<0.0001 (one-way ANOVA). (d) Frequency of T-bet$^+$ CLL B cells ($CD5^+CD19^+$) in cryopreserved PBMCs from CLL patients (n=15). Data presented as mean ± SEM. ***p<0.001 (two-way ANOVA).

Figure 2. CLL lymph node (LN) harbour a fraction of T-bet high B cells



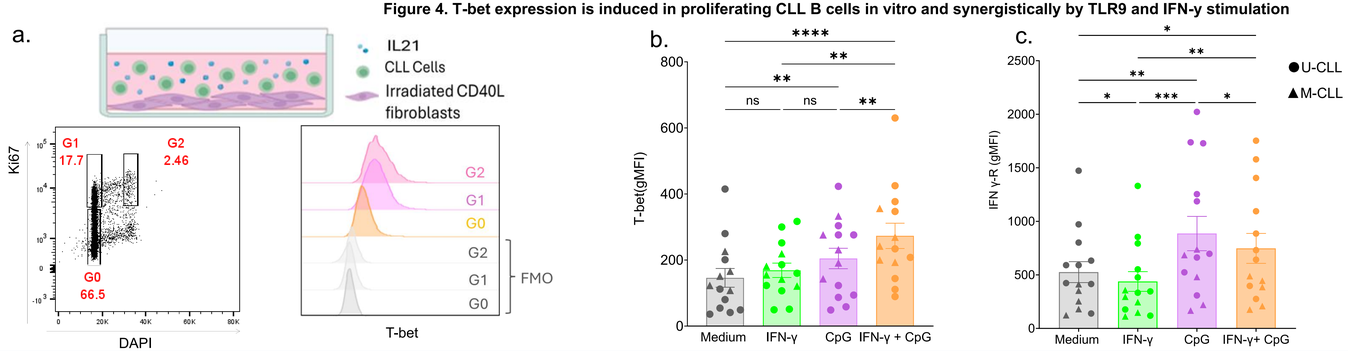
(a) Representative confocal imaging of T-bet expression in LN tissue from a CLL patient. CD20 (green), CD3 (red), and T-bet-DAPI colocalization (blue) at 60× magnification. b) Per field of view of T-bet high B Cells (CD20+CD3-DAPI+) frequency in LN B cells from all FFPE sections of 8 CLL patients. c) Comparison of T-bet high CLL B cells frequency between PB and LN in paired fine-needle aspirates (FNA) samples from 4 untreated CLL patients.

Figure 3. T-bet high CLL B cells from LN exhibit enlarged, granular blast-like morphology, G2-phase enrichment higher activation/proliferation marker expression, and LOWER homing receptor expression.



(a) Flow cytometry gating strategy for T-bet high CLL B cells in LN versus PB from untreated patients (T-bet levels in PB T-cells were used to set T-bet high gates). b) Back-gating of T-bet high and low LN CLL B cells onto side scatter (SSC) versus forward scatter (FSC) plots (left). Cell cycle distribution of T-bet high and low LN CLL B cells, with G0, G1, and G2 phases defined as Ki67 low DAPI low,Ki67 high DAPI low, and Ki67 high DAPI high, respectively (right). c) Imaging cytometry images of T-bet high and low LN CLL B cells across different channels. T-bet colocalizes with DAPI, indicating nuclear localization, but not with CD5 (negative control). (d) gMFI values of markers with obvious differential expression between T-bet high and T-bet low LN CLL B cells, as determined by mass cytometry. Fig3 data correspond to the patient with the highest frequency of T-bet high LN CLL B cells among four paired FNA samples in Fig2 C (N=1).

Figure 4. T-bet expression is induced in proliferating CLL B cells in vitro and synergistically by TLR9 and IFN-γ stimulation



(a) In vitro stimulation of CLL B cell proliferation using CD40L fibroblasts and IL-21 for 6 days to mimic the tumour microenvironment. Flow cytometry shows higher T-bet expression in G1/G2 phase CLL B cells compared to G0 phase. T-bet expression can be induced by 72 hours of stimulation with TLR9-ligand CpG and IFN-γ. b) Quantification of T-bet and c) IFN-γ receptor gMFI in CLL B cells. Data represent mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (one-way ANOVA, n=14; 9 U-CLL, 5 M-CLL).

REFERENCES

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CONCLUSIONS

1. T-bet expression in CLL is variable and dynamic, with relatively low levels in PB and higher levels at sites of known tumour proliferation.
2. T-bet can be induced in PB CLL cells under conditions that mimic the LN microenvironment, potentially via TLR9-driven enhancement of IFN-γ signalling.
3. Future studies will explore the relationship between T-bet and cellular proliferation, the mechanisms underlying its induction, and its potential as a therapeutic target.