BRD4 maintains the surface phenotype of CLL cells

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INTRODUCTION

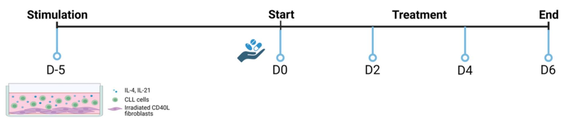
- Epigenetic dysregulation is a hallmark of cancer, including Chronic Lymphocytic Leukemia (CLL).
- The bromodomain and extra terminal (BET) family proteins (BRD2, BRD3, BRD4 and BRDT) are epigenetic regulators that play a critical role in mediating gene transcription.
- BRD4 is overexpressed in CLL and is enriched in super enhancer regions that regulate key pathways and genes known to contribute to the development and progression of CLL (e.g. B cell receptor signalling)[1].
- Inhibiting BRD4 has shown promising results in arresting proliferation in primary CLL cells[1-3].
- BRD4 is involved in differentiation and maintaining cell identity in various cell types, including embryonic stem cells and CD8 T cells. The role of BRD4 in maintaining CLL cell identity has not been investigated yet.

AIM

Assess the effect of BRD4 inhibition on CLL cell identity.

METHODS

Peripheral blood human CLL cells and murine CLL splenocytes were treated with the BRD4 inhibitor JQ1 (0.5μM) or Ibrutinib (1μM), followed by assessment of phenotypes by flow cytometry. Proliferation was induced in human primary CLL cells by stimulation with irradiated CD40L-transfected fibroblasts, IL-4 and IL-21 for five days, followed by JQ1 or ibrutinib treatment for 6 days.



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RESULTS

Figure 1: BRD4 inhibition suppresses CLL cell proliferation.

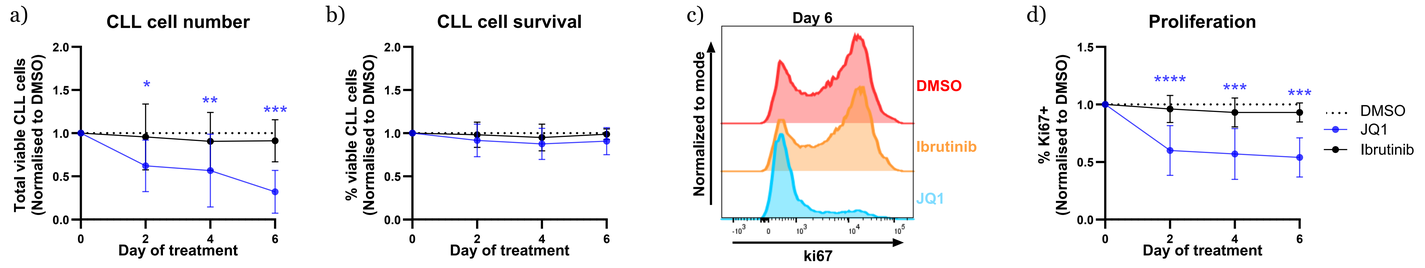

Figure1: (a) Live CLL cell counts over six days of treatment with JQ1 or Ibrutinib. **(b)** Quantification of viable CLL cells treated with JQ1 or Ibrutinib for six days. **(c)** Ki67 histograms of JQ1-treated vs Ibrutinib-treated vs DMSO control CLL cells on Day 6. **(d)** Quantification of Ki67+ CLL cells treated with JQ1 or Ibrutinib over six days in culture. Data were normalised to DMSO control. Data represent mean±SD. *p<0.05, **p<0.01, ***p <0.001, ****p <0.0001 (one-way ANOVA with Tukey's multiple comparisons). (n=12).

Figure 2: BRD4 inhibition downregulates CD19.

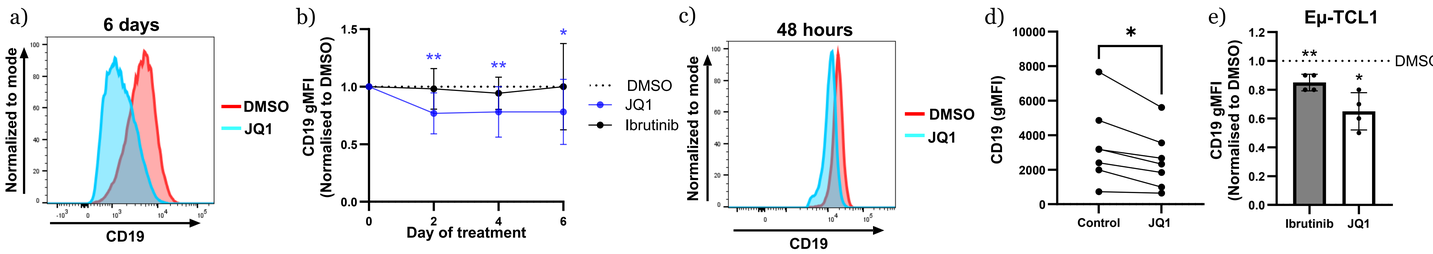

Figure 2: (a) CD19 expression after 6 days of JQ1 treatment. **(b)** Quantification of CD19 levels after 6 days of JQ1 or Ibrutinib treatment. *p<0.05, **p<0.01, (one-way ANOVA with Tukey's multiple comparisons) n=12. **(c)** CD19 expression after 48 hours of JQ1 treatment. **(d)** Quantification of CD19 levels after 48 hours of JQ1 treatment. *p<0.05 (Wilcoxon signed rank test). **(e)** CD19 levels on murine CLL cells after 48 hours of ibrutinib or JQ1 treatment *in vitro*. *p<0.05, **p<0.01, (one-way ANOVA with Tukey's multiple comparisons). Data normalised to DMSO control. Data represent mean±SD.

Figure 3: BRD4 inhibition downregulates CD5.

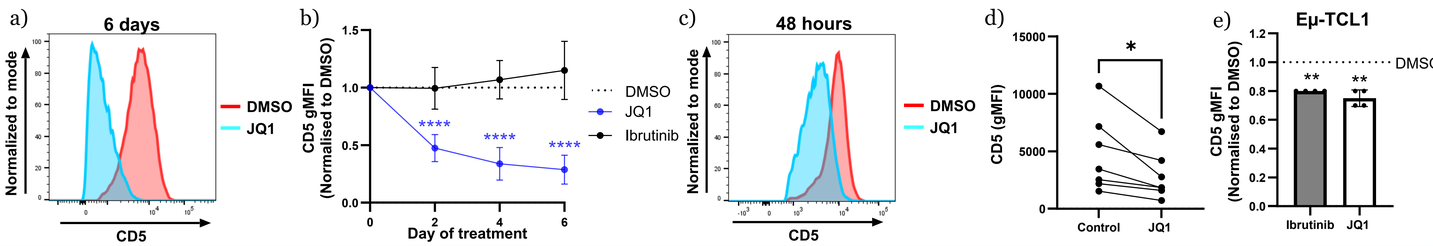

Figure3: (a) CD5 expression after 6 days of JQ1 treatment. **(b)** Quantification of CD5 levels after 6 days of JQ1 or Ibrutinib treatment. ****p<0.0001, (one-way ANOVA with Tukey's multiple comparisons) n=12. **(c)** CD5 expression after 48 hours of JQ1 treatment. **(d)** Quantification of CD5 levels after 48 hours of JQ1 treatment. *p<0.05 (Wilcoxon signed rank test). **e)** CD5 levels on murine CLL cells after 48 hours of ibrutinib or JQ1 treatment *in vitro*. **p<0.01, (one-way ANOVA with Tukey's multiple comparisons). Data normalised to DMSO. Data represent mean±SD.

Figure 4: BRD4 inhibition enhances CLL cell size and granularity.

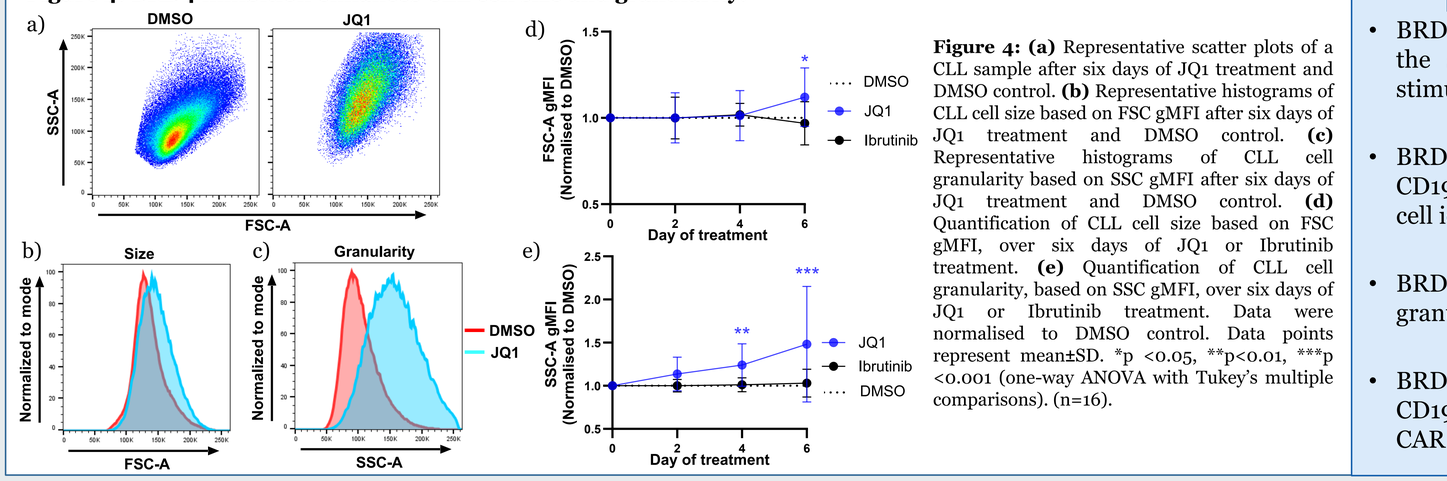

Figure 4: (a) Representative scatter plots of a CLL sample after six days of JQ1 treatment and DMSO control. **(b)** Representative histograms of CLL cell size based on FSC gMFI after six days of JQ1 treatment and DMSO control. **(c)** Representative histograms of CLL cell granularity based on SSC gMFI after six days of JQ1 treatment and DMSO control. **(d)** Quantification of CLL cell size based on FSC gMFI, over six days of JQ1 or Ibrutinib treatment. **(e)** Quantification of CLL cell granularity, based on SSC gMFI, over six days of JQ1 or Ibrutinib treatment. Data were normalised to DMSO control. Data points represent mean±SD. *p <0.05, **p<0.01, ***p <0.001 (one-way ANOVA with Tukey's multiple comparisons). (n=16).

CONCLUSIONS

- BRD4 inhibition reduces proliferation but not the survival of primary human CLL cells stimulated with CD40L, IL-4 and IL-21
- BRD4 inhibition reduces the expression of CD19 and CD5 in CLL cells, maintaining CLL cell identity
- BRD4 inhibition enhances CLL cell size and granularity in *in vitro* cultures
- BRD4 inhibition may affect the effectivity of CD19-directed immunotherapies (e.g. CD19 CAR T cells and CD19-BiTES)