Evaluating epigenetic markers for naïve, memory and overall B cells for disease diagnosis and prognosis in patients with B-CLL

Abstract# 7591

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Introduction

Chronic lymphocytic leukemia (CLL) is a hematological cancer classified as low-malignancy non-Hodgkin lymphoma that accounts for approximately a quarter of all leukemias.

Since only some patients will develop aggressive cancer and require treatment, accurate disease prognosis is crucial.

A wide range of parameters with prognostic value have been described, including age, clinical stage, mutational status, protein markers, and flow cytometry levels of CD19+CD38+ B cells above 20%.

Objective

Here, we investigated epigenetic markers for B cell types including a marker for overall B cells, naïve B cells, memory B cells and IgM positive B cells in a cohort of more than 40 patients with B-CLL.

When comparing to a cohort of more than 100 healthy donors, the epigenetic B cell marker identified all patients with 100% sensitivity and specificity. In addition, the epigenetic B cell markers for naïve and memory B cells identified with high sensitivity and excellent specificity a subgroup of B-CLL patients with CD19+CD38+ B cell levels above 20% which is associated with an unfavorable prognosis.

The results suggest that the epigenetic analysis of B cells in capillary blood may be a promising tool for B-CLL diagnosis and disease prognosis.

Summary and Conclusion

Flow cytometry and epigenetic immunophenotyping showed excellent concordance when measuring CD19+ B cells. Both methods identified all patients with B-CLL, when comparing with a cohort of healthy donors (Fig. 3).

A subgroup of B-CLL patients showed high levels of an epigenetic marker for naive B cells and low levels of a marker for memory B cells (Fig. 4a, blue), in spite of showing the reverse when analyzed by flow cytometry (Fig. 4b, blue).

All patients in this B-CLL subgroup except one had CD19+CD38+ frequencies close to or above 20%, a marker for unfavorable prognosis (Fig. 5a, blue).

This suggests that high ratios of an epigenetically active marker for naive B cells compared to memory B cells, when present in patients with B-CLL, might be an independent indicator of unfavorable prognosis (Fig. 6).

Method

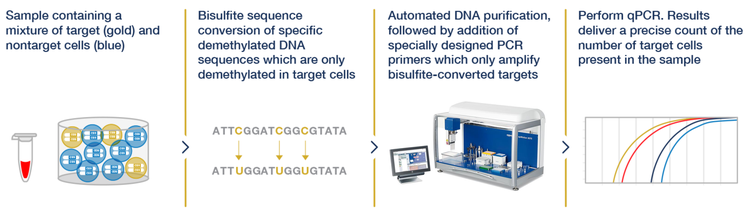

Fig. 1: Bisulfite sequencing of genomic regions used to identify B cells, memory B cells, and naïve B cells in blood cells sorted by FACS.

Blood samples are lysed and treated with bisulfite, converting unmethlyted cytosines into uracil. Subsequent amplification by quantitative PCR using methylation-specific primers quantitates only gene copies from cells with previously unmethylated target sequences. Parallel quantification of the gene copy number of the GAPDH gene, which is unmethylated in all cells, allows the determation of immune cells by DNA-based immunophenotyping.

Results – Epigenetic Marker Identification

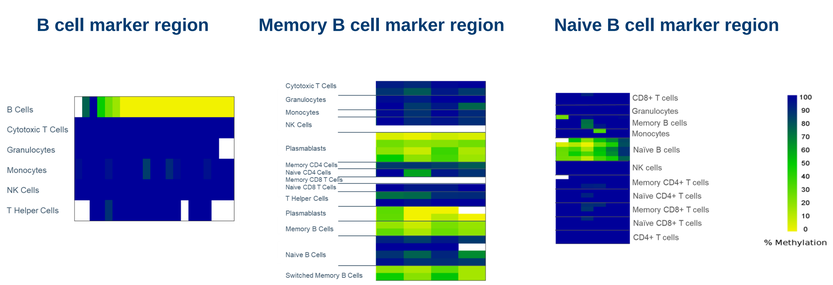

Fig. 2: Bisulfite sequencing of genomic regions used to identify B cells, memory B cells, and naïve B cells.

Cell popluations were sorted by FACS and bisulfite sequencing was performed. Methylated and unmethylated CpG regions are depicted as blue and yellow, respectively. Epigenetically active marker regions are shown for B cells (Fig. 5a, LRP5 gene), memory B cells (Fig. 5b, CBX5 gene) and naïve B cells (Fig. 5c, C7orf50 region).

Results – Comparing Epiontis ID and Flow

Analysis of B cells by flow cytometry and by the epigenetic assay Epiontis ID

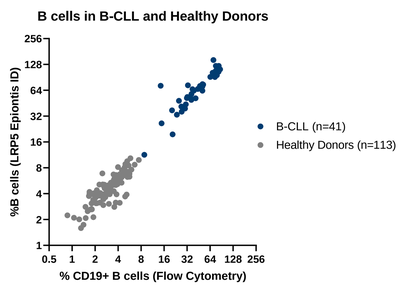

Fig. 3: Concordance of flow cytometry and epigenetic assay results for B cells.

The measurement of 41 samples from patients with B-CLL and 113 self-declared healthy donors with flow cytometry and an epigenetic assay for B cells shows Pearson and Spearman correlation coefficients of 0.95 and 0.89, respectively.

Epiontis ID results were on average 55% higher compared to flow cytometry.

Both analysis methods identified all 41 B-CLL patients when comparing with a cohort of healthy donors (100% sensitivity and specificity).

Results – Patient Subgroups in B-CLL

Samples from B-CLL Patients Show Distinct Epigenetic Patterns

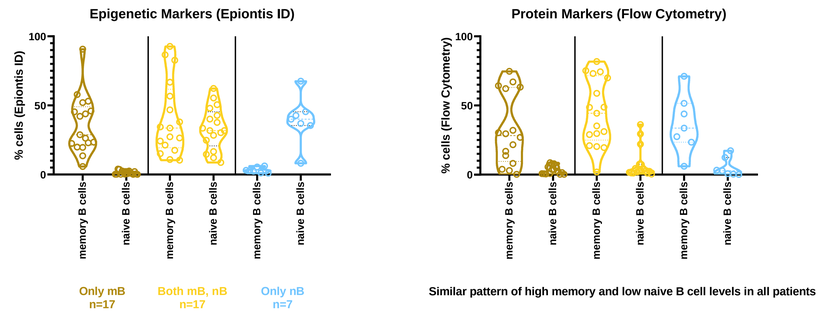

Fig. 4a: Epigenetic measurement of a marker for memory and naïve B cells in a cohort of n=40 B-CLL patients.

Three distinct patient subgroups were observed with i) memory B cell marker only, ii) both memory and naïve B cell markers, iii) naïve B cell marker only. Note: All patients had high levels of IgM positive B cells except n=2 in group i).

Fig: 4b: Flow cytometry measurements of the B-CLL sub-cohorts previously defined by epigenetic patterns.

All B-CLL patients show relatively high levels of memory B cells and low levels of naïve B cells when measured by flow cytometry.

The absence of an epigenetic marker for memory B cells coincides with the presence of protein markers for unfavorable disease prognosis

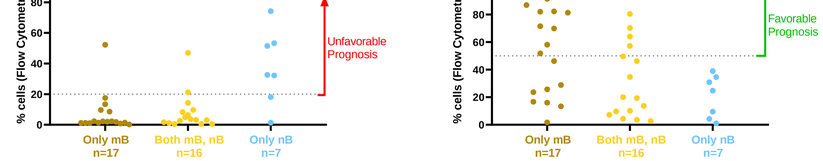

Fig 5a: Flow cytometry measurement of CD19+CD38+ cells, a prognostic factor for aggressive disease if above 20% (Ibrahim et al., Blood 2001).

Patients with an epigenetically active memory B cell marker (gold/yellow) rarely showed >20% CD19+CD38+ cells (3/33 patients = 9%).

In contrast, patients without an epigenetically active memory B cell marker (blue) frequently had high levels (5/7 = 71%).

Fig. 5b: Flow cytometry measurement of CD21lowCD38low cells, a favorable prognosis marker.

Patients with an epigenetically active memory B cell marker (gold/yellow) frequently showed high levels of CD21lowCD38 low B cells (13/33 = 39%).

In contrast, patients without an epigenetically active memory B cell marker (blue) did not show high levels (0/7 = 0%).

Samples from B-CLL Patients Show Distinct Epigenetic Patterns

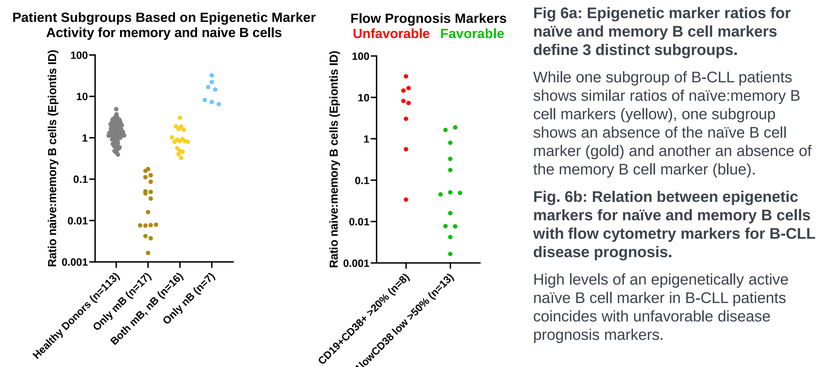

Fig 6a: Epigenetic marker ratios for naïve and memory B cell markers define 3 distinct subgroups.

While one subgroup of B-CLL patients shows similar ratios of naïve:memory B cell markers (yellow), one subgroup shows an absence of the memory B cell marker (gold) and another an absence of the memory B cell marker (blue).

Fig. 6b: Relation between epigenetic markers for naïve and memory B cells with flow cytometry markers for B-CLL disease prognosis.

High levels of an epigenetically active naïve B cell marker in B-CLL patients coincides with unfavorable disease prognosis markers.