PRECISION for medicine®

CASE STUDY

# Using Epigenetic Immune Cell Profiling to Provide Insight into the Tumor Microenvironment in B-Cell Non-Hodgkin Lymphoma

## Situation

Non-Hodgkin lymphoma (NHL) is the most common hematologic malignancy worldwide, accounting for nearly 3% of global cancer diagnoses and deaths.[1] Early detection and differential diagnosis are essential for the initiation of appropriate therapy. However, with over 40 major subtypes and varied clinical manifestations, timely diagnosis and classification are difficult. Novel approaches are needed for stratifying patients to support accurate prognosis and treatment.

**Determining prognosis using biopsy-based subclassification is challenging, even with genetic insights. To improve patient prognosis accuracy, the sponsor aimed to use immune cell profiling in B-cell NHL.**

## Challenges

Determining prognosis according to current biopsy-based subclassification remains difficult, even when accompanied by identification of genetic aberrations or gene expression patterns. To support more accurate assessment of patient prognosis, the sponsor sought to use immune cell profiling to gain greater insight into the tumor microenvironment in B-cell NHL.

## Solution

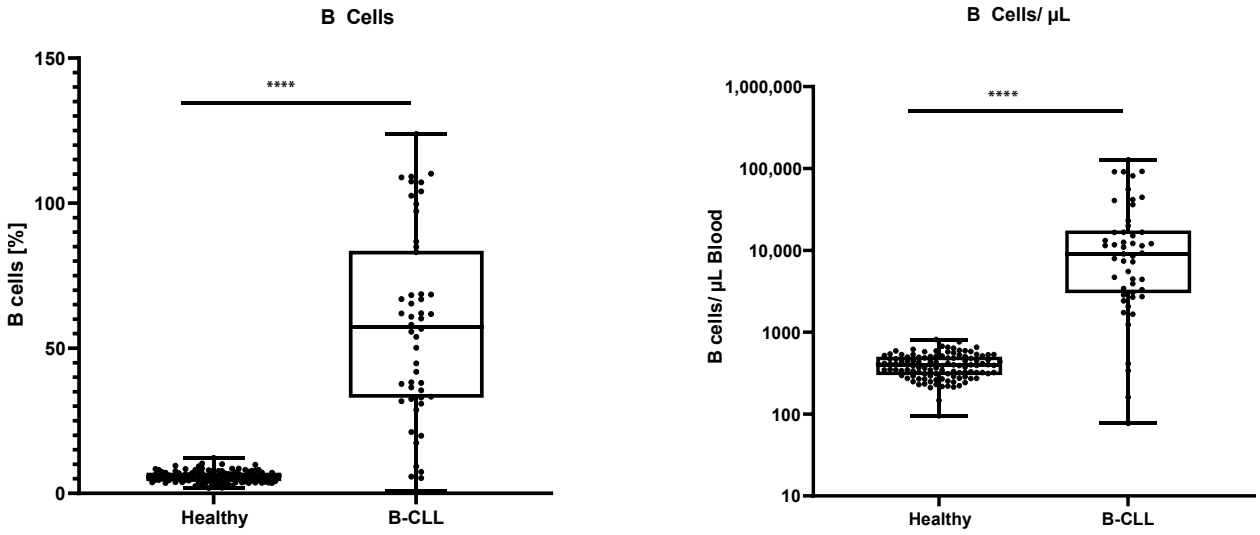
Precision for Medicine performed in-depth immunophenotyping of NHL biopsies using our proprietary immune monitoring technology, Epiontis ID. Epiontis ID measures cell type–specific epigenetic markers that identify uniquely demethylated regions on genomic DNA to quantify immune cell populations using quantitative polymerase chain reaction–based assays, which provide highly reproducible data. A significant advantage of epigenetic immune cell quantification is that it can be applied to fresh, frozen, or paper-spotted dried blood and other body fluids or tissues, eliminating the need for real-time assays and making it possible to perform additional testing at a later date.

Epiontis ID was used to analyze whole blood from patients with chronic lymphocytic leukemia (CLL) as well as tissue samples of patients with different B-cell NHL entities to determine if epigenetic immune cell quantification may be useful for characterizing and potentially assisting in the diagnosis or stratification of different B-cell lymphomas.

In addition, a secondary analysis was performed to determine whether epigenetic immune cell quantification could be used to distinguish between germinal center B-cell–like (GCB) and non–germinal center B-cell–like (non-GCB) diffuse large B-cell lymphoma (DLBCL) since the latter has a poorer prognosis.

[1]Thandra KC, Barsouk A, Saginala K, Padala SA, Barsouk A, Rawla P. Epidemiology of non-Hodgkin's lymphoma. Med Sci. 2021;9(1):5.

***Figure 1.*** *Quantification of B Cells in Whole Blood: Healthy Cohort vs Patients With Chronic Lymphocytic Leukemia (CLL)*

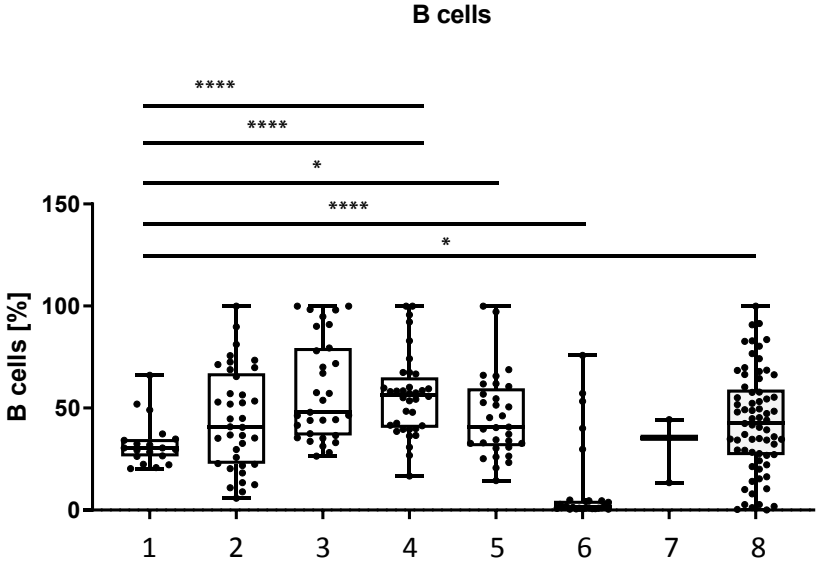



## Results

Relative and absolute quantification of B cells in whole blood samples from patients with CLL showed a significantly higher level of B cells in peripheral blood compared to samples from a healthy cohort (see Figure 1).

When B cells were measured in a wide range of formalin-fixed paraffin-embedded (FFPE) tissue samples from 251 patients with different B-cell NHL entities, B cells were found to be elevated also in tissue, except for B-cell acute lymphoblastic leukemia (see Figure 2). In fact, B-cell acute lymphoblastic leukemia (ALL, group 6 in Figure 2) was characterized by a significant reduction of B cells in the tumor region. ALL is caused by abnormal proliferation of immature forms of white blood cells, and this not fully differentiated type of B cell does not seem to be recognized by the epigenetic assay.

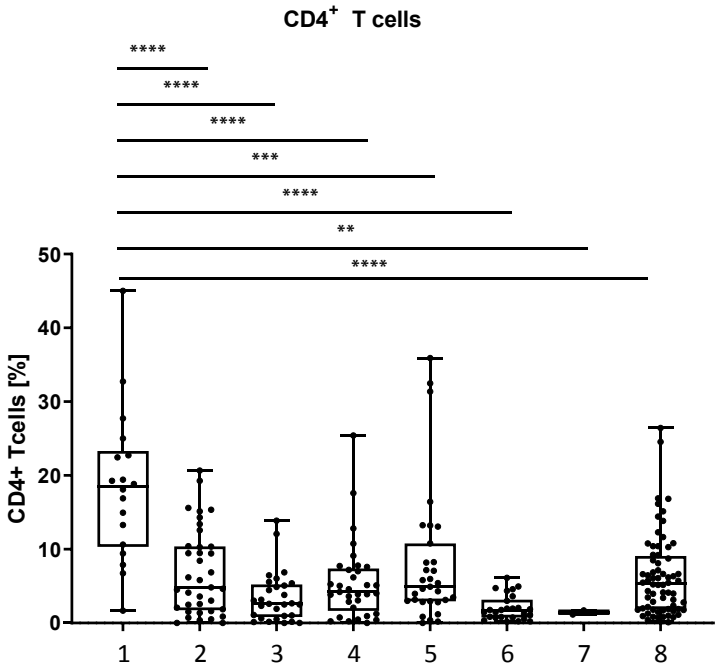
***Figure 2.*** *Relative Quantification of B Cells in Different B-Cell NHL Entities*




1, Healthy lymph nodes (n = 22); 2, marginal zone lymphoma (n = 40); 3, mantle cell lymphoma (n = 32); 4, chronic lymphocytic leukemia (n = 41); 5, follicular lymphoma (n = 33); 6, B-cell acute lymphoblastic leukemia (n = 26); 7, Burkitt lymphoma (n = 3); 8, diffuse large B-cell lymphoma (n = 74).

While an increase in B cells was observed across the majority of B-cell NHL tissue samples analyzed, substantial variation was observed in other immune cell types. All of the B-cell NHL types demonstrated significantly lower levels of CD4+ T cells compared to healthy lymph nodes (see Figure 3), while cytotoxic T cells, regulatory T cells, and natural killer (NK) cells were more heterogeneous.

***Figure 3.*** *Relative Quantification of CD4+ T Cells in Different B-Cell NHL Entities*




1, Healthy lymph nodes (n = 22); 2, marginal zone lymphoma (n = 40); 3, mantle cell lymphoma (n = 32); 4, chronic lymphocytic leukemia (n = 41); 5, follicular lymphoma (n = 33); 6, B-cell acute lymphoblastic leukemia (n = 26); 7, Burkitt lymphoma (n = 3); 8, diffuse large B-cell lymphoma (n = 74).

Further, in mantle cell lymphoma, regulatory T cells and NK cells could be divided into 2 distinct subpopulations, which is of particular interest since this condition can be either indolent or aggressive. However, comparison of immune cell subsets in FFPE tissues from patients with GCB and non-GCB DLBCL showed no statistically significant differences. Together, these findings show that tissue immunophenotyping could potentially be used to further stratify patients with B-cell NHL to support more accurate prognoses.

Learn how Precision for Medicine's global immune monitoring services and solutions, including Epiontis ID, can enhance your projects with Precision's comprehensive immunologic expertise.

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