Epigenetic Immune Cell Profiling in B-Non-Hodgkin lymphoma biopsies

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Introduction

Early detection and differential diagnosis of Non-Hodgkin lymphoma (NHL) is essential for the initiation of suitable therapy options. However, prognosis according to biopsy based sub-classification remains difficult even when accompanied by identification of genetic aberrations or gene expression patterns. Here we present in depth immunophenotyping of NHL biopsies by epigenetic immune cell quantification. This approach can contribute to understanding the tumor microenvironment with the potential to support patient's prognosis.

Method: Epigenetic Real-Time PCR for the Quantification of Different Immune Cell Types

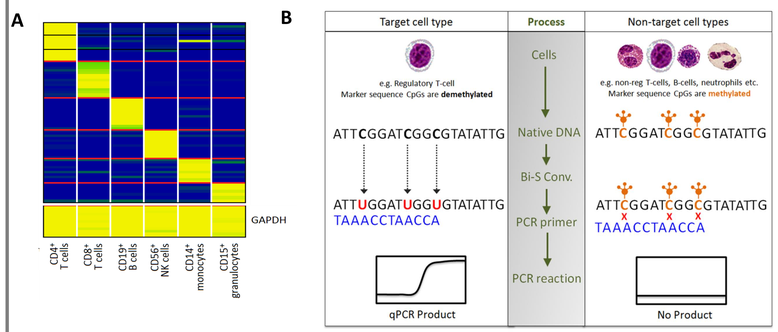

Figure 1: Identification of cell specific demethylated genomic regions and development of qPCR assays for immune cell quantification. **A** Identification of specific demethylated regions specific for various immune cells.[1] **B** DNA-methylation pattern is transferred into the DNA sequence by bisulfite conversion. Primers and probes match only converted, demethylated target sequence. In parallel, the housekeeping gene GAPDH is analyzed. [1,2]

Epigenetic Immune Cell Quantification in Whole Blood of Chronic Lymphocytic Leukemia Patients

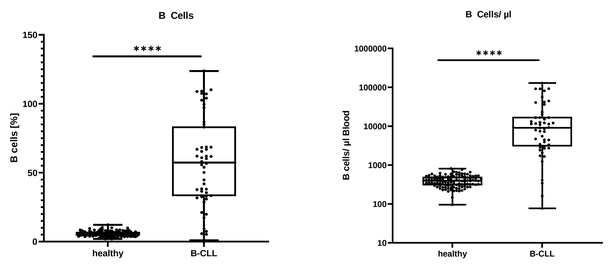

Figure 1: Relative and absolute quantification of B cells in whole blood samples of patients with Chronic Lymphocytic Leukemia (B-CLL). Significant diferences were analyzed using Mann-Whitney test

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- Compared to a healthy cohort, CLL patients show a significantly higher level of B-cells in peripheral blood.
- This led to the consideration if epigenetic immune cell quantification might be useful to characterize and potentially help diagnosing different B cell lymphoma in the affected tissue.

Epigenetic Immune Cell Quantification in FFPE Tissue of different B cell lymphoma entities

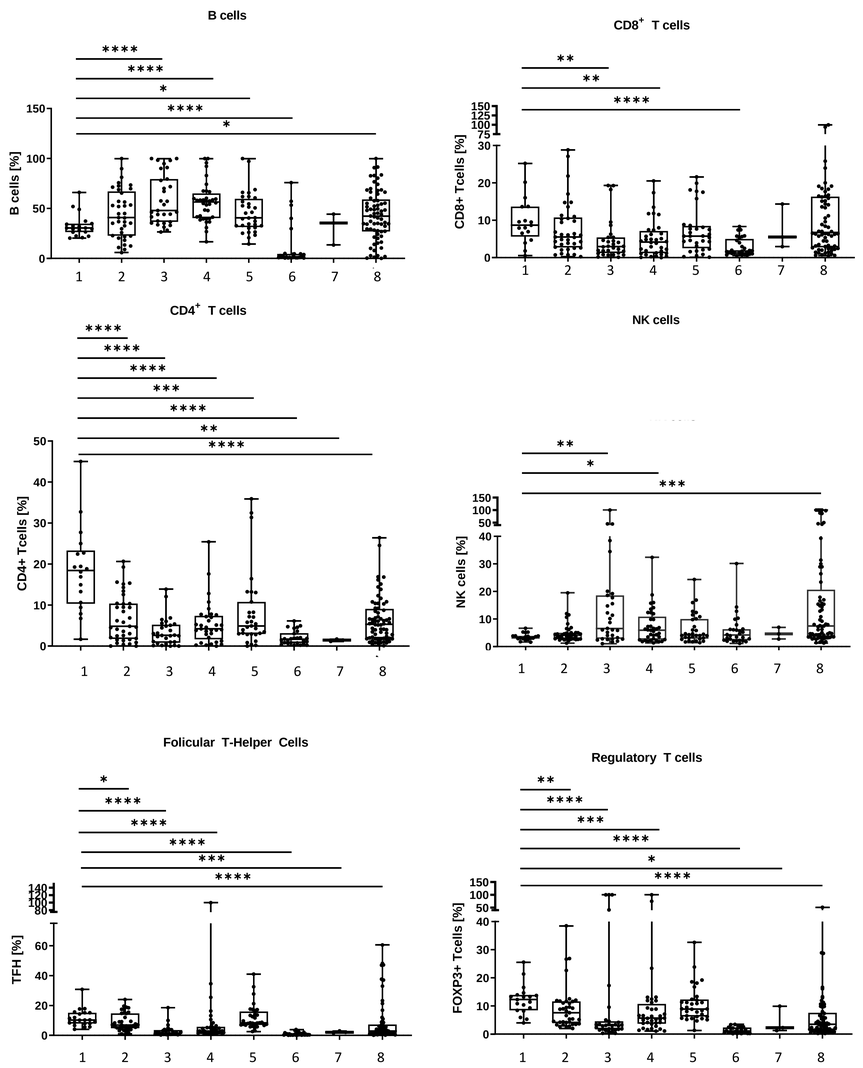

Figure 3: Different immune cell subsets were analyzed in formalin-fixed tissue samples of 251 patients with different B cell Non-Hodgkin lymphomas and lymphnodes from healthy individuals. 1: Healthy Lymphnodes (n=22); 2: Marginal zone lymphoma (n=40); 3: mantle cell lymphoma (n=32); 4: Chronic lymphocytic 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). Significant diferences between the entities were analyzed using Mann-Whitney test

- Similarly to what was observed in whole blood, B cells are elevated in most lymphoma entities except Burkitt Lymphoma and B cell acute lymphoblastic leucemia. In contrast, the latter is characterized by a significant reduction of B cells in the tumor region.
- All analyzed lymphoma types have significantly lower levels of T helper and T follicular helper cells.
- Regulatory T cells, Cytotoxic T cells and Natural Killer cells are more heterogenious in different entities.

Distinction of "germinal center B-cell-like" and "non-germinal center B-cell-like" Diffuse large B cell lymphoma

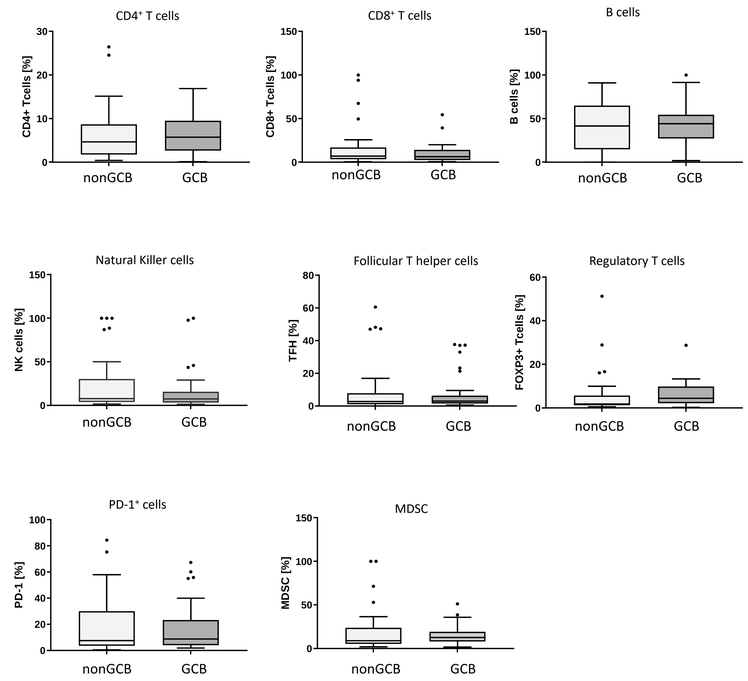

Figure 4: FFPE Tissue from patients with Germinal center B-cell like (GCB) and non-GCB Diffuse Large B cell lymphoma were analyzed and compared. No statistical significant differerence was observed between both patient groups.

Conclusion

Epigenetic immune cell quantification allows immune cell profiling from archived samples such as formalin-fixed tissue. This enables the analysis of tumor microenvironment for example in B cell Non-Hodking Lymphoma.

Here we present the characterization of different NHL entities.

The increase of B cells observed in whole blood of B-CLL patients was confirmed in a majority of tissue samples from patients with different B cell lymphoma.

Beside this universal trend, a substantial heterogeneity within the entities was observed. Especially regulatory T cells and NK cells can be divided in two fractions in patients with mantel cell lymphoma. This is of particular interest as this lymphoma can either be indolent or aggressive. Thus immunophenotyping in tissue could provide help for further stratification of patients. For Diffuse Large B-cell Lymphoma no difference was found between GCB and non-GCB subtype although the latter has an inferior prognosis.

Reference

[1]**Baron et al.**, Epigenetic immune cell counting in human blood samples for immunodiagnostics**.** Sci. Transl. Med. 2018 Aug; 10 –pp1-11

[2]**Wieczorek et al.,** Quantitative DNA methylation analysis of FOXP3 as a new method for counting regulatory T cells in peripheral blood and solid tissue. Cancer Res. 2009 Jan 15;69(2):599-608