# 4367 ESTABLISHING LYMPHOMA TYPE-SPECIFIC CYTOKINE SIGNATURES USING TISSUE-BASED RNA OR PERIPHERAL BLOOD CELL-FREE RNA (cfRNA)

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## INTRODUCTION

Cytokines and chemokine resulting from tumor cells interaction with various immune elements in their microenvironment play important role in lymphoma growth and in response to therapy. They are also relevant for the clinical symptoms and various manifestations of the disease. Evaluating RNA levels of large number of cytokines/chemokines and their receptors in tissue is now possible as a routine using next generation sequencing (NGS). However, it is not known if the tumor microenvironment is reflected in peripheral blood cell-free RNA (cfRNA). Using NGS, we evaluated the RNA levels of 36 cytokines/chemokines and their receptors in tissue samples from patients with various types of lymphoid neoplasms

## AIM

We explored if peripheral blood cfRNA reflects tissue RNA by establishing cytokines/chemokines and their receptors signatures for various types of lymphoma, first in tissue then we tested if using the same signatures but with peripheral blood cfRNA levels we can distinguish between various types of lymphoma in the same fashion.

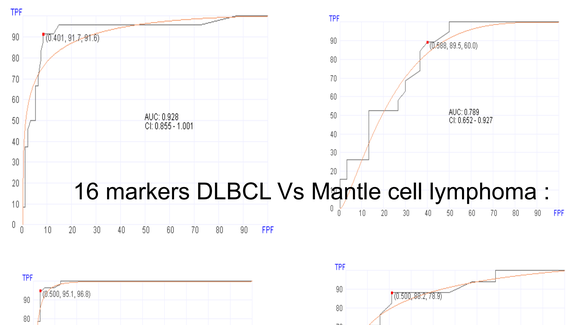
## METHOD

RNA was extracted from tissue samples with confirmed chronic lymphocytic leukemia (CLL) (184), diffuse large B-cell lymphoma (DLBCL)(N=287), mantle cell lymphoma (N=74), and T-cell lymphoma (No=276). In addition, RNA from bone marrow samples with low level B-cell lymphoid neoplasms not otherwise classified (N=750) was used for this study. Peripheral blood cfRNA was extracted from 19 patients with DLBCL, 23 with mantle cell lymphoma, 39 with T-cell lymphoma, 16 patients with CLL and 361 patients with low level B-cell lymphoid neoplasm not otherwise classified. Cellular RNA and cfRNA were sequenced using 1600 gene targeted RNA next generation sequencing (NGS) panel. Only 36 cytokines/chemokines and their receptors are used in generating the models using Bayesian algorithm and random forest. After establishing these models in tissue, we tested them using cfRNA.

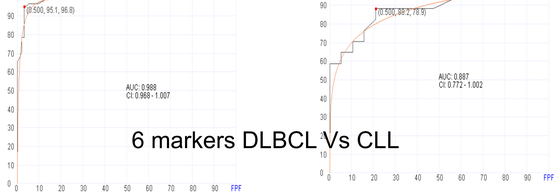
## RESULTS

| Tissue Markers | Mantle (TPM) | DLBCL (TPM) | Log P-Value |
|---|---|---|---|
| TGFBR2 | 6.31 | 5.62 | -11.86 |
| IL21R | 3.59 | 4.65 | -10.12 |
| TGFBI | 4.58 | 5.58 | -9.70 |
| TGFB3 | 2.76 | 3.42 | -6.72 |
| TNFRSF6B | 3.70 | 4.28 | -4.65 |
| TNFRSF10B | 4.46 | 4.82 | -4.03 |
| CTLA4 | 3.23 | 3.75 | -3.23 |
| TNFAIP3 | 6.42 | 6.04 | -2.61 |
| TNFRSF17 | 3.76 | 4.32 | -2.59 |
| CXCR4 | 6.09 | 5.16 | -1.71 |
| IL3RA | 3.50 | 3.83 | -1.47 |
| TNFRSF4 | 4.17 | 4.47 | -1.43 |
| IL2 | 2.17 | 2.42 | -1.07 |
| CXCL8(IL8) | 4.78 | 4.44 | -0.67 |
| CXXC4 | 3.33 | 3.14 | -0.57 |
| IL2RA | 3.04 | 3.12 | -0.41 |

**Tissue RNA** **Peripheral Blood cfRNA**

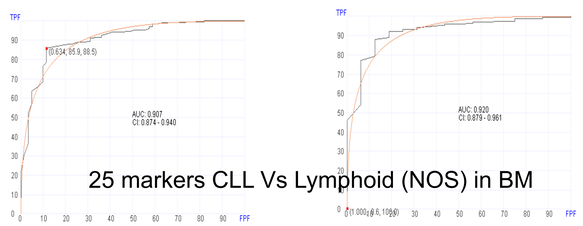



16 markers DLBCL Vs Mantle cell lymphoma :



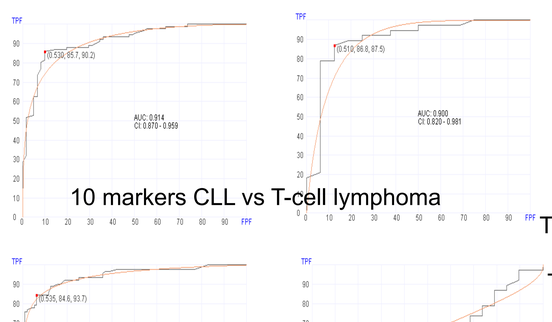

6 markers DLBCL Vs CLL

| Tissue Markers | CLL (TPM) | DLBCL (TPM) | Log P-Value |
|---|---|---|---|
| CCL2 | 2.40 | 6.04 | -Infinity |
| CXCL8(IL8) | 7.31 | 4.44 | -Infinity |
| IL21R | 3.61 | 4.65 | -Infinity |
| TGFBR2 | 6.29 | 5.62 | -Infinity |
| TNFRSF11A | 1.24 | 2.19 | -Infinity |
| TNFRSF6B | 3.22 | 4.28 | -Infinity |

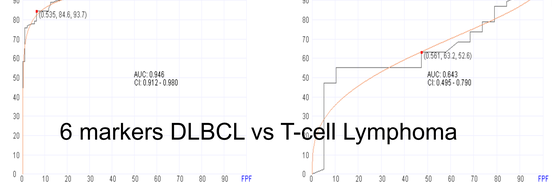



25 markers CLL Vs Lymphoid (NOS) in BM

**Tissue RNA** **Peripheral Blood cfRNA**




10 markers CLL vs T-cell lymphoma




6 markers DLBCL vs T-cell Lymphoma

| Tissue Markers | T-Cell (TPM) | DLBCL (TPM) | Log P-Value |
|---|---|---|---|
| IL21R | 3.63 | 4.65 | -Infinity |
| TGFBR3 | 4.63 | 3.32 | -Infinity |
| TNFAIP3 | 6.93 | 6.04 | -Infinity |
| TNFRSF17 | 1.91 | 4.32 | -Infinity |
| IL1RAP | 3.88 | 2.76 | -15.18 |
| TGFBR2 | 5.88 | 5.62 | -6.06 |

| Bone marrow | CLL (TPM) | Lymphoid, NOS (TPM) | Log P-Value |
|---|---|---|---|
| CCL2 | 2.40 | 4.07 | -Infinity |
| CTLA4 | 4.75 | 3.28 | -Infinity |
| CXXC4 | 3.10 | 0.98 | -Infinity |
| IL3RA | 2.67 | 3.91 | -Infinity |
| TGFBR2 | 6.29 | 5.72 | -Infinity |
| TNFRSF10B | 5.03 | 4.37 | -Infinity |
| TGFBR3 | 4.33 | 3.72 | -15.48 |
| TNFRSF14 | 5.73 | 5.35 | -14.63 |
| IL3 | 1.75 | 0.58 | -13.60 |
| IFNG | 4.31 | 3.34 | -12.81 |
| CXCR4 | 5.27 | 7.71 | -12.32 |
| IL12RB2 | 1.67 | 2.27 | -11.90 |
| IL1RAP | 3.29 | 4.20 | -9.99 |
| IL1B | 3.42 | 4.18 | -9.66 |
| IL2 | 2.48 | 1.86 | -9.51 |
| IL15 | 2.90 | 2.26 | -8.31 |
| TNFRSF10D | 3.29 | 2.98 | -8.13 |
| IL7R | 5.28 | 4.30 | -6.52 |
| CXCL8(IL8) | 7.31 | 6.24 | -5.66 |
| TNF | 5.24 | 4.84 | -4.30 |
| TGFB3 | 2.55 | 2.33 | -3.16 |
| IL6 | 2.63 | 2.70 | -0.50 |
| TNFRSF9 | 2.77 | 2.79 | -0.43 |
| TNFAIP3 | 6.93 | 6.96 | -0.42 |
| TNFRSF6B | 3.22 | 3.21 | -0.33 |

## CONCLUSIONS

- Tissue-based cytokine signatures of various types of lymphoma are unique but, in most cases, the same signatures can be seen in peripheral blood cfRNA.
- Peripheral blood cfRNA reflects tissue microenvironment and can be used as an alternative to tissue.
- Systemic response to DLBCL makes it difficult to distinguish between DLBCL and T-cell lymphoma using the signature established in tissue and a different cfRNA –based signature is needed.

## CONTACT INFORMATION

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