# Microarray expression technology in clinical research of non-Hodgkin lymphoma

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## SUMMARY

*Nowadays, in genomocentric era accelerated research of the human genome coupled with advances is enabling the comprehensive molecular profiling of human tissue. Particularly, DNA microarrays are powerful tools for obtaining global view of human non-Hodgkin lymphomas gene expression. Complex information from lymphomas "expression profiling" studies can, in turn, be used to create molecular markers that have diagnostic or prognostic implications. The gene „expression profiling" is not of routine clinical oncology practice, but is used in genomic classification of clinically relevant subgroups of non-Hodgkin lymphoma. The genomics biomarkers have been incorporated into current prognostic models which are based on IPI, R-IPI, and FLIPI. Molecular or pharmacogenomic profiling can be used as new therapeutic targets for patients who are refractory to current therapy. We discus the utility of DNA microarray-based lymphoma profiling in clinical oncology research, and identify future of research in lymphoma evolving fields.*


**Key words:** *Gene Expression Profiling; Microarray Analysis, Lymphoma, Non-Hodgkin; Prognosis; Survival*

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Abbreviations

Non-Hodgkin lymphoma (NHL), Minimum information about a microarray (MIAME), Complementary DNA (cDNA), Comparative genomic hybridization (CGH), Diffuse large B-cell lymphoma (DLB-CL), Gene expression profiling (GEP), Germinal center B-cell like (GCB-like), Activated B-cell like (ABC-like), Primary mediastinal B-cell lymphoma (PMBL), Follicular lymphoma (FL), „Immune response-1" (IR-1), „Immune response-2" (IR-2), Bürkitt's lymphoma (BL), Molecular Bürkitt's lymphoma (mBL), Mantle cell lymphoma (MCL), Peripheral T cell lymphoma (PTCL), Angioimmunoblastic T cell lymphoma (AITL), Anaplastic lymphoma kinase (ALK), Chronic lymphocytic leukemia (CLL), Zeta-associated protein -70 (ZAP-70), B-cell receptor (BCR), Lipoprotein lipase (LPL), Extracelullar matrix (ECM), International prognostic index (IPI), Revised international prognostic index (R-IPI), Follicular lymphoma international prognostic index (FLIPI), Cancer genome atlas (CGA)

## Introduction

Nowadays, in the genomocentric era, research of the human genome using microarray technology is accelerated. Genomics is comprehensive study of the whole genome, genetics products, and their interactions (l,2). The human genome consists of roughly 3 billion basepairs, and current estimate of the total number of genes varies from 20,000 to 25,000 but around 75% genomes contain so-called intergenic DNA or non-coding sequences (l,3). The international Human Map Project identifies the variations in the sequences that are common among humans. The Cancer Genome Atlas (CGA) is a project to determine all genomic changes involved in all types of human cancer (2,3).

Non-Hodgkin lymphoma (NHL) is a heterogeneous, complex, and progressive clonal expansion of B-, T-lymphocytes and rarely NK-cells or their precursors (4). Our taxonomy of lymphomas, which is based mostly on histopathology and immunophenotyping, includes about 30 distinct entities arising from diverse cells types. The genetic complexity of lymphomas probably explains the clinical diversity with traditional methods and genomic expression analysis. Microarrays technique is effective in deciphering this clinical diversity. A number of published studies identify gene expression signatures for major non-Hodgkin lymphoma types and subtypes, and uncover gene expression patterns that correlate with various characteristics of non-Hodgkin lymphoma (4). Microarrays technology identifies molecular profile of individual tumors at the DNA, RNA, and protein levels. For analysis to gene expression profiling to classify NHLs unsupervised and supervised methods are used (5). The Microarray Gene Expression Data Society (www.megd.org) recommends the use of set of criteria (Minimum Information About a Microarray Experiment) (6). There are six major data bases of publicly available information: Gene Bank, EMBL, GEO, NCBI at NIH, DNA Data Bank of Japan, and HuGENet (7).

The era of molecular diagnostics of lymphoma started with the cloning of the immunoglobulin and TCR genes. Later on, a dramatic progress in the development of microarray technology has led to better understanding of pathogenesis and biology of NHLs. A number of molecular abnormalities or markers have been identified that have significant diagnostic or prognostic implications (8). Patrick O Brown published in 1995 the first paper about microarray, which is commonly called a "DNA-or RNA-chip" and Alizadeh et al., in 1999 and 2000 identified three subgroups of diffuse large B-cell lymphoma by using "lymphochip". Nowadays, cDNA and oligonucleotide microarrays platforms are to identify more subtypes non-Hodgkin lymphoma (8).

## Expression microarray technology

A DNA microarray is a miniature system containing cDNA fragments that are synthesized directly or spotted on glass or other matrix. Microarrays have been used extensively to simultaneously monitor the expression of thousands of genes from particular tissue or cell type. The technological progress of the cDNA microarrays was extremely rapid and nowadays there are two most commonly used microarray systems complementary DNA (cDNA) and oligonucleotide arrays, which differ in probe materials. The cDNA array probes are usually products of the PCR, generated from cDNA libraries or clone collections and contain from 500 to 5000 bp cDNA (9). These probes are printed on glass slides or nylon membranes as spots at defined locations, typically 100-300 $\mu$m. The benefit of spotted arrays is that they can be made by individual investigators and do not require *a priori* knowledge of cDNA sequences (5,9). The oligonucleotide microarrays can be manufactured by various methods: in situ synthesis method for high-density oligonucleotide arrays used by Affimetrix and Agilent Technologies, and the contact (pins) and non-contact (ink-jet) printing methods of presynthesized oligonucleotide probes. The in situ synthesis is a powerful method: the process can achieve extremely high spot densities (spot size of 5 $\mu$m in 2005) and the probe sequence can be chosen more or less randomly for each synthesis. Oligonucleotides offer greater specificity than cDNAs because they can by tailored to minimize chances of cross-hybridization. Sequences up to 25-70 bp nucleotides have been used effectively. Major advantages of this assays is uniformity of probe length and the ability to discern splice variants (9). The data analysis of microarray experiment is a multi-step and complex process which contains: image analysis, signal adjustment and data normalization. Image analysis software is used to calculate the intensity of each spot or probe on the array and to store these measurements as numerical values in the text file. For statistical analysis and visualization of gene expression data a large number of commercial and non-commercial software tools have been developed (e.g., Gene Spring, Gene Cluster, Cluster, and Treevoew, SAM and d

CHIP) (9). For classification gene expression data used unsupervised clustering or class discovery and supervised clustering or class prediction methods. The expression data can be pictorially summarized, where each row represents single gene, and each column represents expression levels. (7,9). A supervised analysis requires the grouping of patients according to predefined characteristics. In the unsupervised clustering analysis genes or samples are grouped into classes on the basis of the similarity in their expression profiles across cases, tissues or conditions. Microarray analyses are used in clinical oncology: to identify altered genes or biochemical pathways, to identify new class of disease, to predict diagnosis, and classification of unknown samples (10). Microarray technology has a number of limitations (volume tissue, the search for differently expression genes, and statistical analysis) (5,11).

The basic concept of microarray technology is to hybridize preprocessed sequences of mRNA (targets) to the complementary sequences (probes) bound to a solid surface, and to quantify the amount of specifically hybridized target, typically by fluorescence two-color or one-color detection system (Figure 1) (3,11).

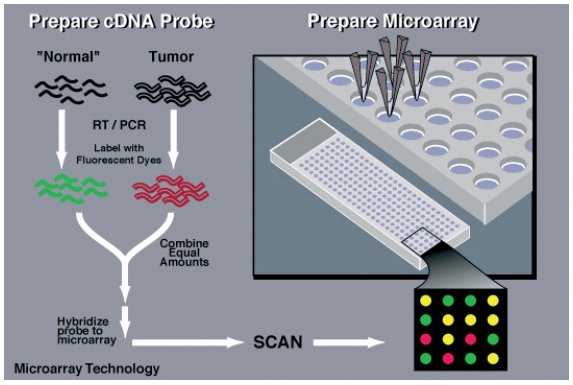


**Figure 1. Schematic illustration of DNA microarray analysis.**
**Source: http://genome.gov/10000533**
**(Courtesy: National Human Genome Research Institute)**

## Gene expression profiling in diffuse large B-cell lymphoma

Primary diffuse large B-cell lymphoma (DLB-CL) is an aggressive malignancy of mature B-lymphocytes and the most common subtype of non-Hodgkin lymphoma in adults. It account for 30% to 40% of all newly diagnosed cases and more than 80% of aggressive lymphomas (12,13). DLB-CL is commonly composed of centrobalst-like and immunoblast-like cells. These cells express the B-cell markers: CD19, CD20, and CD22, and sIg. The approximately 50% of DLB-CL is characterized by chromosomal translocation: t(3;14), t(8;14), and t(14;18). These translocations deregulate expression of Bcl-6(3q21), myc (8q24), and Bcl-2(18q21) genes, as a result of their juxtaposition to the Ig genes (14). DLB-CL represents a heterogeneous entity of a variety of molecular aberration, some of which have been shown to be predicative of outcome. The most frequently aberrations are: p53, myc, rel, Bcl-6, p16, p38MAPK, FLK1, CDK2 chromosomal deletion BLIMP1, and aberrant somatic hypermutation PIM1, PAX5, and Rho. The delBLIMP1 locus on chromosome 6q21q22.1 is frequently identified in ABC-DLB-CL signature, but not in GBC or type 3 DLB-CL signature (15). In DLB-CL the Ig variable region genes commonly undergo somatic mutations. The CD44v6 is expressed predominantly in advanced disease stage and in ABC-DLB-CL in CD44 negative cases. The CD44v6 expression is associated with poor prognosis (16). Iqbal et al., identified in the GCB-DLB-CL translocation t(14;18) group with positive and negative subsets. The translocation t(14;18) was detected in 20% of DLB-CL (17).

**Table1. Molecular subtypes of DLB-CL**

| | GCB | ABC | PMBCL |
|---|---|---|---|
| Median age, year | 58 | 66 | 25 |
| Age older than 60 year, % | 52 | 66 | 9 |
| Female, % | 50 | 40 | 70 |
| Female younger than 35 year, % | 3 | 2 | 35 |
| 5-year survival | 59 | 30 | 64 |

Source: Armitage JO (ref. 18)

At the present time, gene expression profiling is not of routine clinical oncology practice, but it used in molecular classification of clinically relevant subgroups of DLB-CL (Table 1) (18). The genomic markers have been incorporated into current prognostic models which are based on International Prognostic Index (IPI,R-IPI). It contains risk factors significant for the prognosis of overall survival (age, stage, sLDH, performance status, and number of extranodal disease sites) and has been used to stratify patients in risk of treatment failure (19,20). Subclassification of DLB-CL was done by tissue specific microarrays and selected into prognostic subgroups based on cellular origin: germinal center B-cell –like (GCB-like), activated B-cell like (ABC-like), and type 3 or primary mediastinal B-cell lymphoma (PMBL) (21) The DLB-CL subgroups are distinguished from each other by the differential expression of hundreds of different genes, and these genes relate to each subgroup to separate stage of B cell differentiation and activation. These molecular differences suggest that DLB-CL subgroups (GBC, ABC, and PMBL) should be considered as separate diseases (21). Patients with gene expression profiling of GCB have a significantly better survival than the patients with gene expression profiling of ABC. The type 3 has a poor clinical outcome which is similar to the ABC subgroup (Figure 2). Alizadeh et al., created a "fuzzy neural network" for the precise prediction of survival of patients with DLB-CL. In this model four genes are identified (CDIU, AA800/551, AA805661, and IRF4) that could be used to predict prognosis with 93% accuracy. The average 5 years overall survival for all patients was 52%, 76% of GCB-like, and 16% of ABC-like DLB-CL patients (21).

Patients with low expression CD10 have a poor prognosis. However, patients with high CD10 and AA807551 high expression and low expression of AA805611 genes have poor prognosis (21). The gene expression profiling (GEP) of GCB includes many markers of germinal center differentiation (e.g.CD10, CD38, A-myb, OGG1, HGAL, Bcl-6,Bcl-7A, and LMO2) (21,22). The ABC GEP includes genes: IREL (MUM1/LSIRF), CCND2, SYCA3, FLIP, Bcl-Xl, Bcl-2, BLIMP1, and XBP1, and absent expression of Bcl-6 gene. The expression of Bcl-2, CCND2 and SYCA3 correlated with short survival. The Bcl-2 is independent marker of a poor prognosis for patients with DLB-CL. The expression of LMO2, Bcl-6 and lymph node signature correlated with prolonged survival (Figure 2) (23).

Rosenwald et al., showed four gene expression profiling with "17 genes" (e.g. GCB, proliferation, LN, and MHC class II) to constitute a predictor that

correlated with overall survival after chemotherapy. Also, the GCB subgroup has a decreased activity of NF-kB signaling pathway. However, ABC subgroup has a constitutive activation of this pathway. The GCB subgroup had a 5-year survival rate of 60%, while ABC and type 3 of DLB-CL had 35% and 39%, respectively. In GBC subgroup only Bcl-2 translocation and c-rel amplification are detected. The two genes Bcl-6 and HGAL predict overall survival. However, CD10 which is a GCB marker, did not predict overall survival patients in DLB-CL subgroup (Figure 2) (23).

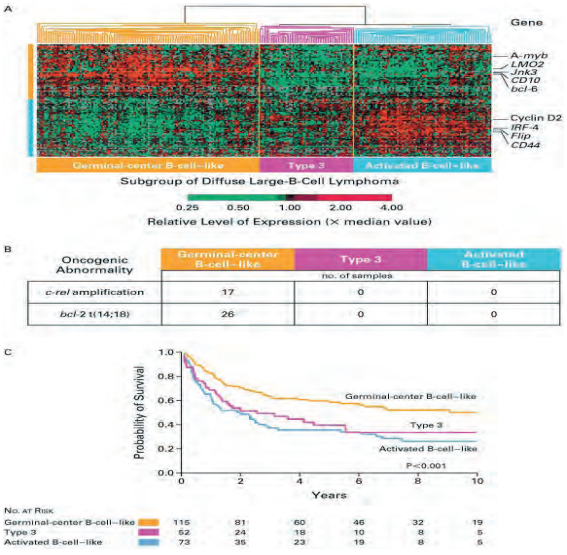


**Figure 2. Gene expressions profiling of diffuse large-B-cell lymphoma (DLB-CL). Panel A shows the hierarchical clustering of diffuse large-B-cell lymphomas. Panel B shows the number of samples with amplification of the *c-rel* locus and *bcl-2* translocations in subgroups of diffuse large-B-cell lymphoma. Panel C shows Kaplan–Meier estimates of overall survival after chemotherapy among the 240 previously untreated patients, according to the gene-expression subgroup (with permission, Rosenwald A, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 2002;346:1937-47. Copyright © 2002 Massachusetts Medical Society. All rights reserved.)**

Also, Wang et al., (24) used first "self organized map" and confirmed better survival for patients expressing GCB profiling than other subgroups. Shipp et al., (25) used the supervised method called "weighted voting algorithm" for distinction of DLB-CL vs. FL and prediction of survival of patients with DLB-CL. Based on this classification they identified "cured vs. fatal/refractory" survival phenotype groups. The "cured" group patients had a 5-year overall survival of 70%, while "fatal/refractory" group had overall survival of only 12%. In fatal/refractory DLB-CL signature overexpression of BCR, PDE4B, NOR1, HMGM, HCK, galectin 3, and BFL1A1 is identified. The overexpression of BFL1A1 is important for reducing the chemosensitivity of DLB-CL patients. This microarray classification is a better predictor for patients' survival than IPI classification (25).

Finally, Lossos et al. (2004) identified with unsupervised analysis clinical prediction model for survival based on "6 strongest predictor genes" (LMO2, Bcl-6, FN1, CCND2, SCY3, and Bcl-2). The expression of LMO2, Bcl-6 (GBC signature) and FN1 correlated with prolonged survival, while the expression of Bcl-2, CCND2 and CCL3 (previously named SYC3-ABC signature) correlated with shorter survival. The identification of the overexpression of these "6 genes" is sufficient to predict survival in DLB-CL patients (26,27).

Wright et al., used "compound covariates" to discriminate between subtypes of DLB-CL (28). Sakhinia et al., identified "indicator genes" (cyclin B1, NPM3, and COL3A1), which were higher in DLB-CL (29).

Colomo et al, and Nyman et al., demonstrated two phenotypes: GCB and non-GCB by immunohistochemical methods with GCB markers (CD10, Bcl-6) and activation markers (MUM1/IRF4 and CD135) (Table 2). Patients with GCB markers had much better survival than patients with activation markers (30-33).

**Table 2. The immunophenotypical profiling of the DLB-CL**

| | | | |
|---|---|---|---|
| GCB | Bcl-2 - | Bcl-6 + | CD 10 + |
| ABC | Bcl-2 + | Bcl-6 - | CD 10 – |
| PMBCL | Coexpression or lack of expression of these three members | | |

Source: Nyman H et al. (ref. 31)

The patients with the non-GCB phenotypes or expression of Bcl-2 or cyclin D2, demand more aggressive therapies. The NF-kB transcription factors are highly expressed in ABC-DLB-CL, but not in GCB-DLB-CL. Because of this, NF-kB pathway is a potential new therapeutic target for patients with ABC-DLB-CL who are refractory to current therapies (34). Millennium Pharmaceuticals tested two beta-carboline derivates as inhibitors of the IKKs and inhibitors of E3 ligase complex and PKC-beta (PS 1145, PDE-4B-enzaturin) (35,36). Deregulated cyclin E is a strong predictor of a poor prognosis and possible target for individualized antitumor therapy (37).

Primary mediastinal large B-cell lymphoma (PMBCL) is a special subtype of DLB-CL, which originates from thymus B lymphocytes. This lymphoma accounts for 2% of all NHLs and histologically it is characterized by fibrosis (38). Clinical manifestation of PMBCL is similar to Hodgkin disease in younger patients (Table 1). Clinical course is aggressive and patients have shorter overall survival than those affected with other types of DLB-CL. The PMBCL have distinctive chromosomal aberration, but gain of 9p is specific for PMBCL, and observed in 75% cases. Mutations in SOCS-1 gene correlate with gains of 9p24 JAK locus. Savage et al., (38) identified a molecular signature unique only for PMBCL; it was termed "46 specific genes". PDL2is the best discriminator of PMBL from other DLB-CL, but it is also highly expressed in Hodgkin lymphoma cells (39). Also, molecular signature of PMBL is characterized by the absence of rearrangement of Bcl-2 and Bcl-6 genes and overexpression of IL-13R alfa. A number of regulators of T-cell activation and downstream effectors JAK2 and STAT1 and increased expression of several genes associated with NF-kB may be the points of attack for future therapeutic agents (38,39). The loss of MHCII expression is correlated with worse outcome in patients with PMBCL (40).

Mircean et al., and Suguro M et al., identified de novo subgroup CD5+ DLBCL that expresses CD5 on the cell surface (41,42). CD5+ is expressed on T cells and subset of B cells. In B-cell neoplasms, CD5+ is expressed on CLL, MCL, DLB-CL and marginal zone of B-cell lymphoma (42). This subgroup comprises 10% of all DLB-CL (negative for CD10, CD21, CD23, cyclin D1 and with predominance of surface IgMkapa) and has a more aggressive clinical course and worse prognosis then CD5- DLB-CL (42). The CD5+ signature shows high levels of integrin beta 1 in lymphoma cells and CD36 in the vascular cells. To differentiate CD5+ subgroup from other types of DLB-CL and mantle cell lymphoma four gene groups are used: metabo-

lism, signal transduction, transcription factors, cell adhesion, and migration. In CD5+ expression profile there is a deregulation of ECM genes (POSTN, COL1A1, CTSK, MMPS, and LAMB3), and upregulation of TRPM genes. The expression of Bcl-6 and CD10 is associated with longer overall survival, but expression MUM1 and cyclin D2 is associated with shorter overall survival. The expression Bcl-2 or FOXP1 did not predict overall survival (41,42). In small number of cases CD5+ signature can also be used in prognosis for mantle cell lymphoma but for differentiation between two gene entities cyclin D 1 expression should be used.

## Gene expression profiling of follicular lymphomas

Follicular lymphoma (FL) is heterogeneous disease which comprises 20% of all NHLs. About 70% FLs have indolent clinical course. However, 10% of FLs may transform into DLB-CL with more aggressive clinical course. The FL is composed of follicular centre cells (small cells and large cells). The FLs are characterized with t(14;18)(q32;q21) translocation. This translocation leads to the deregulation of the Bcl-2 gene, with overexpression of the antiapoptotic Bcl-2 protein. The Bcl-2/IGH translocation is necessary, but it is not sufficient to cause FLs (45). The additional alteration in genes expression (IL-1, IL-8, and IL-12B) may contribute to malignant phenotype. In transformed FLs are involved genes: CXCL2, NEK2, MAPK1, CD69, DNA polymerase, WEE1, HMGA1, ras, surviving, BIRC5, LDH, and c-myc. FLs are positive for CD10. Clinical prognostic indicators IPI and FLIP (age, sLDH, stage, Hb and number of nodal areas) are limited in to low and low-intermediate risk groups. Also, classical morphological grading system (1,2,3a, and 3b) is not optimal for the choice of therapy (46). Therefore, Björck added to FLIPI index high expression of cyclin B1 as independent prognostic factor which correlates with longer survival (47). Follicular lymphoma is immunological functional disease in which an interaction between nonmalignant immune cells of the microenvironment and tumor cells determines the clinical behavior (48). So far, a number of gene signatures have been identified: "81 gene predictor signature", "37 genes signature", and "indicator genes" signature (46,48). Investigators from NCI have discovered two subsets of genes "survival-associated signatures named IR-I, and IR-2 , whose expression is linked to survival advantage in patients with follicular lymphomas (Figure 3). The overexpression of the immune response-1(IR-1) signature correlated with good prognosis, while immune response-2(IR-2) signature correlated with poor prognosis (Figure 4) (46,48,49).

The immune response-1 signature included genes encoding T-cells markers (CD7, CD8B1, ITK, LEF, and STAT4, osteopontin, MRCOX2, turgen, GRO1, GRO2, NKCT4, LEU13, IFN2, NCF4, CUB C1s, C1qr, TCRbeta, TCRteta, TNF1beta, TNFalfa1, JUNB, FOSGA-beta, p75NTR) and genes that are highly expressed in macrophages (ACTN1, and TNFRF13B). However, the immune response-2 signature included genes known to be preferentially expressed in macrophages, dendritic cells or both (TLR5, FCCR1A, SEP1o, LOM, and CAR1) (46). The FLs signature contains genes upregulated in aggressive phase disease that are involved in cell cycle (CCNE2, CCNA2, CDK2, CHEK1, MCM7) and DNA synthesis (TOP2A, POL03A, HMGA1, POLE2, GMPS, CTPS); genes which reflected increased metabolism (FRSB, RARS, HK2, LDH2); genes involved in signal transduction (FR2B, HCFCR1, PIK4CA, MAPK1) and genes derived from the reactive infiltrate of T cells and macrophages (CD3D, CXCLI2, TM4SF2) (46). Also, CD68 is an independent predictor overall survival. Patients with 15 or more CD68 macrophages per high power filed had a better overall survival than patients with less from 15 CD68 positive macrophages (46). For distinction DLB-CL from FLs used "indicator genes" signatures. In this signatures high levels YY1 gene were associated with shorter survival in FLs and DLB-CL (19,46,49). Besides these complex genomic profiles individual gene markers are also used in the survival prognosis of patients with FLs (49). The FLs genomic expression profile is essential to guide the choice of therapy.

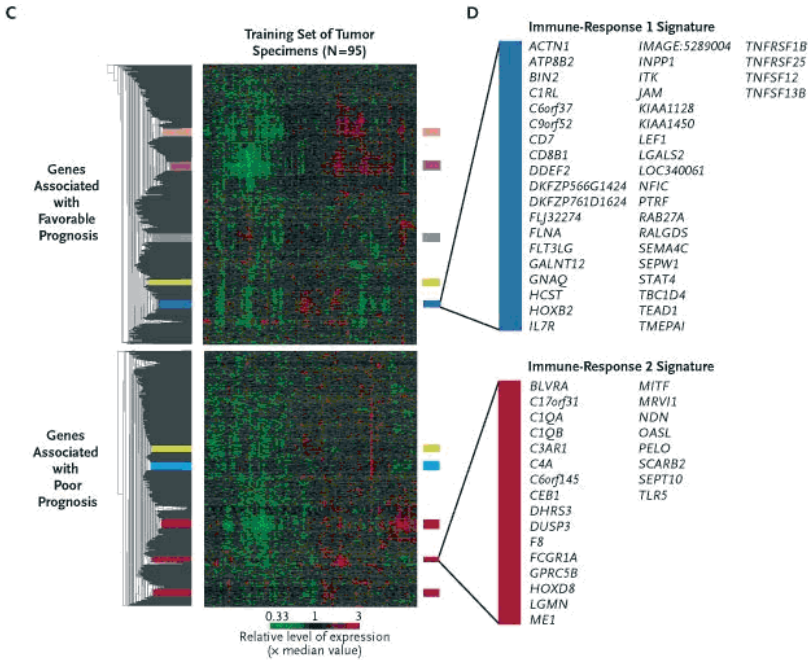


**Figure 3. Genomic expression profiling of follicular lymphoma (with permission, Dave SS, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med 2004;351:2159-69. Copyright © 2004 Massachusetts Medical Society. All rights reserved.)**

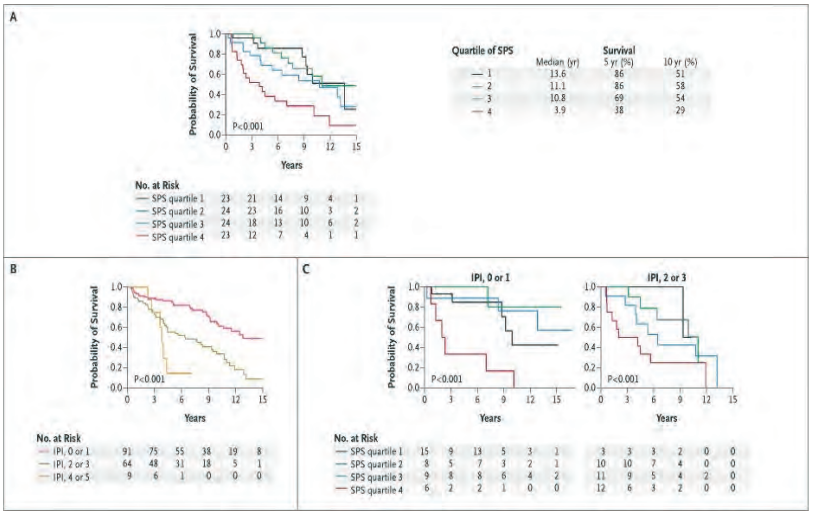


**Figure 4. Survival patients of follicular lymphoma based on the immune-response gene expression signature. Panel A shows overall survival according to the survival-predictor score (SPS). Panel B shows overall survival according to the IPI risk group. Panel C shows overall survival among the patients (IPI) stratified according the quartile of the SPS. (with permission, Dave SS, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med 2004;351:2159-69. Copyright © 2004 Massachusetts Medical Society. All rights reserved.)**

## Gene expression of Burkitt's lymphoma

Burkitt's lymphoma (BL) is a rare and aggressive B-cell lymphoma characterized by a high degree of proliferation of the malignant cells and deregulation of the c-myc gene (1900800) and their kappa and lambda chain IG genes (147200,147200). In 5% to 10% of DLB-CL cases are overlapping in morphological and immunophenotypic features with Burkitt's lymphoma (50). Dave et al., and Hummel et al., identified a characteristic genetic signature that clearly distinguishes this tumor from of DLB-CL (50,51). The "core-group extension strategy" is based on the NSC method by which 58 genes that constitute the molecular Burkitt's lymphoma (mBL) signature have been identified. Some of them belong to NF-kB (e.g. BL2A1, FLIP, CD44, NF-kB1A, Bcl-3 and STAT 3) and serve to differentiate between two subgroups GBC and ABC DLB-CL (51). Based on microarray analysis three major cytogenetic groups are identified: "myc simple, myc complex, and myc negative" (Table 3).

**Table 3. Molecular classification Burkitt's lymphoma**

| Type | Molecular characterization | Clinical outcome | Survival 5-year,% |
|---|---|---|---|
| "Myc simple" | IG-myc fusion and low Chromosomal complexity, Score<6 | Favorable | 76 |
| "Myc complex" | non-IG-myc fusion or IG-myc Fusion and a high chromosomal Complex, score>6 | Poor | 21 |
| "Myc negative" | Comprising myc-negative lymphoma | | |

Source: Hummel M et al. (ref. 51)

Patients with mBL had a favorable prognosis with 5-year survival rate of 75% (Figure 5).

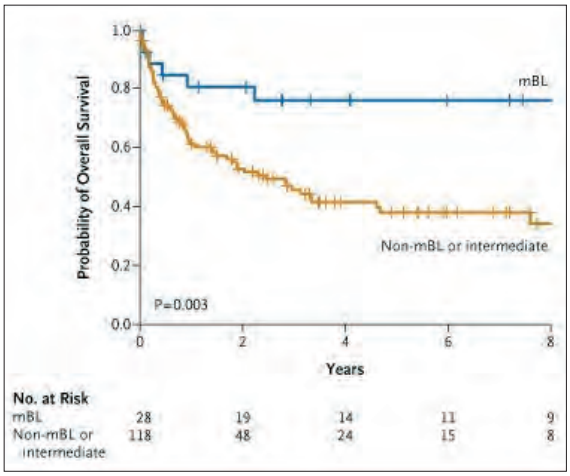


**Figure 5. Overall survival patients of Burkitt's lymphoma (with permission, Hummel B, et al. A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. N Engl J Med 2006;354:2419-30. Copyright © 2006 Massachusetts Medical Society. All rights reserved.)**

The presence of breakpoint of the myc focus is strongly associated with unfavorable prognosis, as compared with the absence of such breakpoint (15% vs. 44%). About 46% cases with IG-myc and non-IG-myc breakpoint had concurrent Bcl-2 and Bcl-6 translocation (52). "The value of molecular profiling to accurately diagnostic Burkitt's lymphoma versus DLBCL will have a major impact on patients because the treatment for these two lymphomas is very different. If Burkitt's patients are treated with intensive therapy, there is roughly an 80% survival rate. However, if they are misdiagnosed with DLBCL, and treated with lower intensity chemotherapy, the survival rate is reversed to 20% or even less" (53).

## Gene expression of Mantle cell lymphoma

Mantle cell lymphoma (MCL) is aggressive type of NHL and comprises about 6% of all NHLs. MCL has worse prognosis with survival about 3 to 4 years (54,55). Mantle cell lymphoma is a specific subtype of NHLs derived from naive CD5+ cells residing in the pregerminal center of primary follicles or mantle zones of secondary follicles (56). MCL is a prototypical neoplastic disease in which a common cytogentic t(11;14) translocation leading to cyclin D1 overexpression is associated with other changes of the clinical, morphological, and molecular variable of this disease. High levels of cyclin D1 are associated with greater proliferation and with poorer survival. The determination of these abnormalities is important for a diagnosis of MCL. Another gene, ATM, which encoding nuclear phosphoproprotein from 370kD plays role in DNA repair and cell cycle control, is frequently mutated on points: ink 2418-19, E2236FS, E423 (55). In addition to these deregulations, alterations have been also identified: deletion CDK, amplification CDK4, transcription repression p16/ink4a, overexpression of BLM-1, deregulation cell cycle and NF-kB pathways, PI3/AKT, and SYK kinases (57,58). With a cDNA microarray specific "MCL signature with 446 genes" has been identified in MCL. These genes include: genes involved in apoptosis, cell cycle, signal transduction, and cell structure. Also, identified are alterations in TNF and NF-kB pathways; overexpression of IL10R, SPARC, osteopontin and BM40 genes; somatic mutation in IGVH and CDC14A, ras, and other genes (59). This characteristic GEP identifies a new subgroup that is cyclin DI negative. GEP signature is also defined for blastoid variant (BV) that is refractory to conventional chemotherapy and associated with a very poor prognosis (60). The GEP MCL-BV increased number of genomic gains and deletions of p16ink4a and p53 genes correlated with poorer clinical outcomes, while 1p21 loss and IGBH mutation were associated with prolonged survival (61). In this signature were identified: gain of TOP1, loss of caspase 7 and RAB27A, and increase of CDK4, IL14alfa (61,62). Also, in MCL-BV were identified overexpression of CD28, B-myb, PIM1, PIM2, DAD1, RSK1, and YY1. The patients with cyclin D1 positive signature and deletion p16/ink4a are associated with the worst clinical outcome (63,64).

## Gene expression of T-cell lymphoma

Peripheral T-cell lymphoma (PTCL) represents about 12% of NHLs. PTCL is heterogeneous group of lymphoma which cannot be classified on basis of morphology or conventional molecular analysis. Based on GEP, specific "tumor profile signature for PTCLs" is identified, which differentiates double positive (CD4+, CD8+) from double negative phenotype form of DLB-CL. In both subtypes genetic alteration in genes for adhesion and matrix remodeling (FN1<LAMB1, COL1A2, COL3A1, COL4A2, and COL12A1) apoptosis (e.g., MOAMP1, ING3, GADD45A, and PDGFR alfa) has been identified. In both genomic profiles NF-kB pathway is activated, which enables the application of target therapy (Gleevec, monoclonal antibody to HGF, STK6, CD52 (Camptah-1H) (65,66).

In angioimmunoblastic T-cell lymphoma (AITL) is detected molecular profiling with overexpression of B cell and follicular dendritic cell related genes, chemokines genes, ECM genes, and overexpression of several genes which characterize normal follicular T helper cells (CXC13, Bcl -6, PDCD1, CD4CL, NF ATC1) (67).

Piva R et al., and Lamant L et al. in anaplastic large cell lymphomas (ALKs) supervised analysis identified two different gene expression profiles: ALK + and ALK – subgroups. The ALKs are characterized by specific chromosomal translocation in which the anaplastic lymphoma kinase gene is fused to the nucleophosmin gene (68). In ALK+ signature there was significant overexpression of Bcl-6, PTP12, CEBPB and SERP1NA1 genes, while in ALK- genes CCR7, CNTFR, IL 22, and IL 21 were overexpressed (69). Systematic screening of these genes can be used for individual target therapy (68,69).

## Gene expression of chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) is malignant B lymphocytes characterized by variable clinical course and overall survival ranging form several months to several decades. Such variability is a consequence of multigenic changes. Wang et al., was the first to discover "CLL signature" from at least ten genes (CD23, FGR, TNFRSF1B, CCR7, IL 4R, PPN12, FMOD, TMEM1, CHS 1, ZNF266, CDS, IL2RA, Bcl-2, WNT3 and ROR1) that can help to differentiate normal B lymphocyte form lymphocyte in CLL (70). In B CLL, the immunoglobulin heavy chain variable region can be mutated or nonmutated. CLL patients with unmutated IGVH genes have a much worse overall survival (range, 79-119 months) then patients with mutated IGVH genes (median survival up to 293) (Figure 6) (71-73).

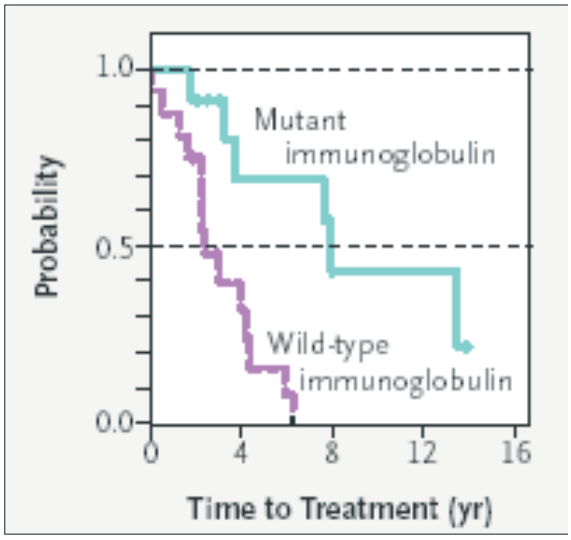


**Figure 6. Overall survival patients with chronic lymphocytic leukemia based on IGVH mutation status. The two curves compare CLL patients, one with somatically mutations IGVH genes (blue), and other with unmutated IGVH genes (purple). (with permission, Staudt LM, et al. Molecular diagnosis of the hematologic cancers. N Engl J Med 2003;348:1777-85. Copyright © 2003 Massachusetts Medical Society. All rights reserved.)**

The BCR stimulation in the IGVH in mutated CLL subtype contributes to the more progressive clinical course. A number of biological parameters, IGVH mutations, CD38 and ZAP-70 expression in leukemic cells are important independent prognostic markers. The high level expression zeta-associated protein-70 (ZAP-70) is the best gene for differentiation in all immunoglobulin unmutated CLL (74). Rosenwald study confirmed that ZAP-70 expression could predict the IGVH mutation status, but RAF1, PAX5 expressed genes may be gold markers for differentiating between these groups and can serve as prognostic markers (75). ZAP-70 and IGVH mutations have similar prognostic information and therefore they can substitute each other. Also, the expression of CD38, IL13, IL18, and L-selection is associated with the poor prognosis patients with CLL (75). Patients with LPL expression in CLL cells have significantly shorter survival time up to 23 months than the patients with LPL- expression with survival period of 88 months (76). Buhl AM et al. has identified a new disease specific gene CLLUR-1 (CLL upregulated gene-1) on chromosome 12q in germinal center B cells in CLL (77). The p53 mutant CLL cells which are fludarabine resistant have aggressive clinical course and deletion of 11q (ATM gene) and correlates with a lower response rate to fludarabine and early response after autologous stem cell transplantation (78). About 10% of patients with CLL have mutations in genes for miRNAs. Thirteen miRNAs genes were identified in CLL. They represent a unique genetic specific profiling and could potentially be useful to distinguish between the two types (mutation and nonmutation) of CLL (79). The overexpression of miRNA-21 and miRNA-155 is identified in CLL, but identified in about 11% of CLL patients decreased miRNA15a/miRNA-16 and miRNA-146 exists. Thirteen out of several hundred miRNAs genes correlate with ZAP-70 expression and IGVH mutations status (80,81).

## Conclusion

In postgenomic era, microarray technology has been of great help in identification of molecular heterogeneity of identical histological types of NHL and initiation of new genomic classification of this disease. In addition, microarray technology has enabled discovery of new and specific genomic expression profiles of DLB-CL, FL, BL, MC, T-cell lymphomas, CLL, etc., and helped the creation of supervised and unsupervised predictive and prognostic models. Genomic expression analysis can precisely identify molecular variants of DLB-CL, FL, MCL or blastoid variant of this histological type. It can also give additional information necessary for establishing of differential diagnosis of DLB-CL from PMBL and of PMBL from mediastinal type of Hodgkin disease. Apart from these genomic signatures, microarray analysis has revealed individual genetic markers such as cyclin E, Bcl-2, Bcl-6, CD44v6, PDL2,CD68, which are in correlation with disease prognosis. Nowadays, the transfer of these basic researching into hematooncological practice is gradual and it relies mainly on traditional REAL classification and prognostic indexes. Robotization of microarray technology and its limitations in clinical practice have initiated the transfer of genomic signatures into the area of immunohistochemical and cytofluorometric detection of prognostic genetic markers. For example, immunohistochemical expression of bcl-2, bcl-6 and CD10 can be used for identification of DLBCL subgroups as well as with the lymphochip. Genomic researching and initial epigenetic analyses have contributed to better understanding of molecular biology and pathogenesis of lymphoma and enabled the development of the concept of individual approach in the treatment of certain lymphomas. Further progress in genomic researching of lymphomas is directed to the improvement of taxonomy and development of individual targeted therapy.

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**Conflict of interest**

We declare no conflicts of interest.

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