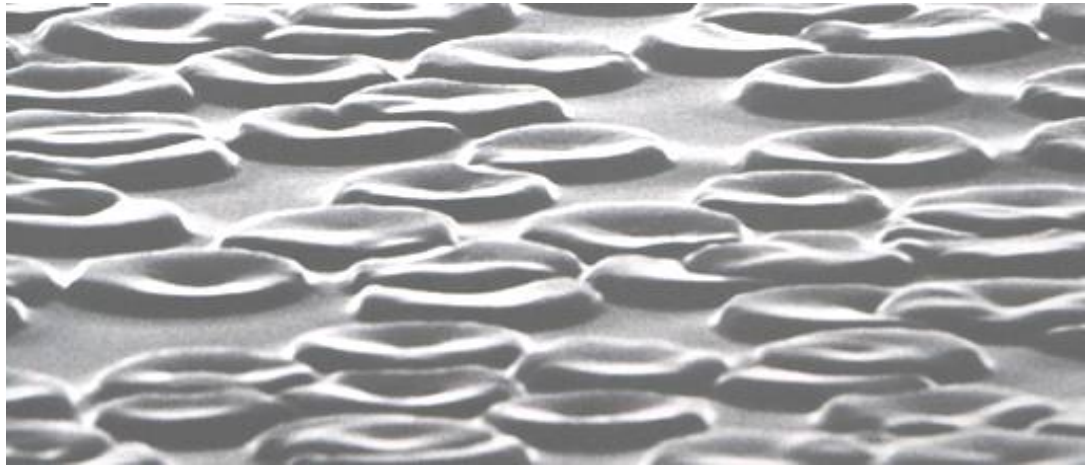
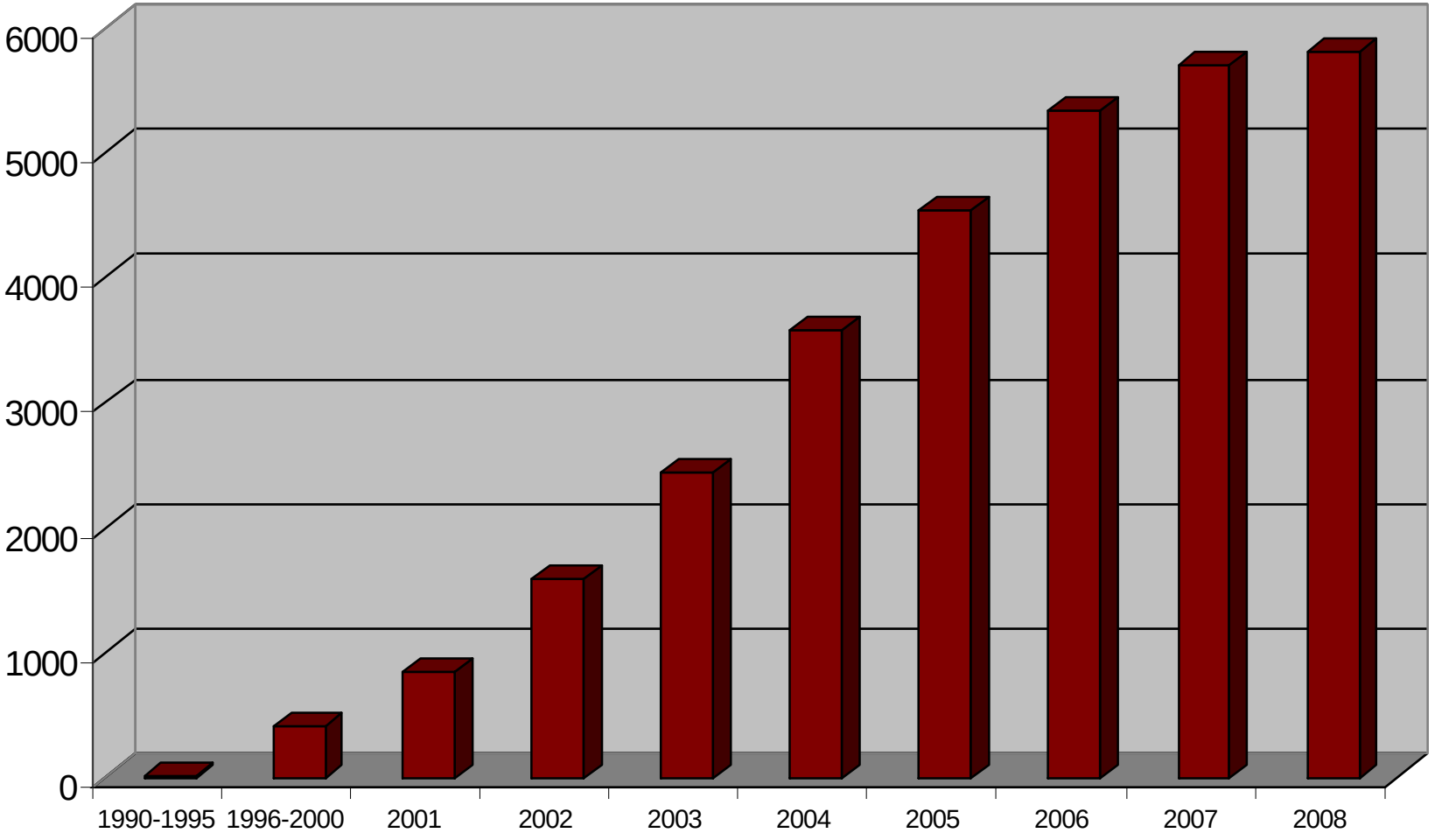


Chip-Technologie: Chance zur individualisierten Therapie von Lymphomen?

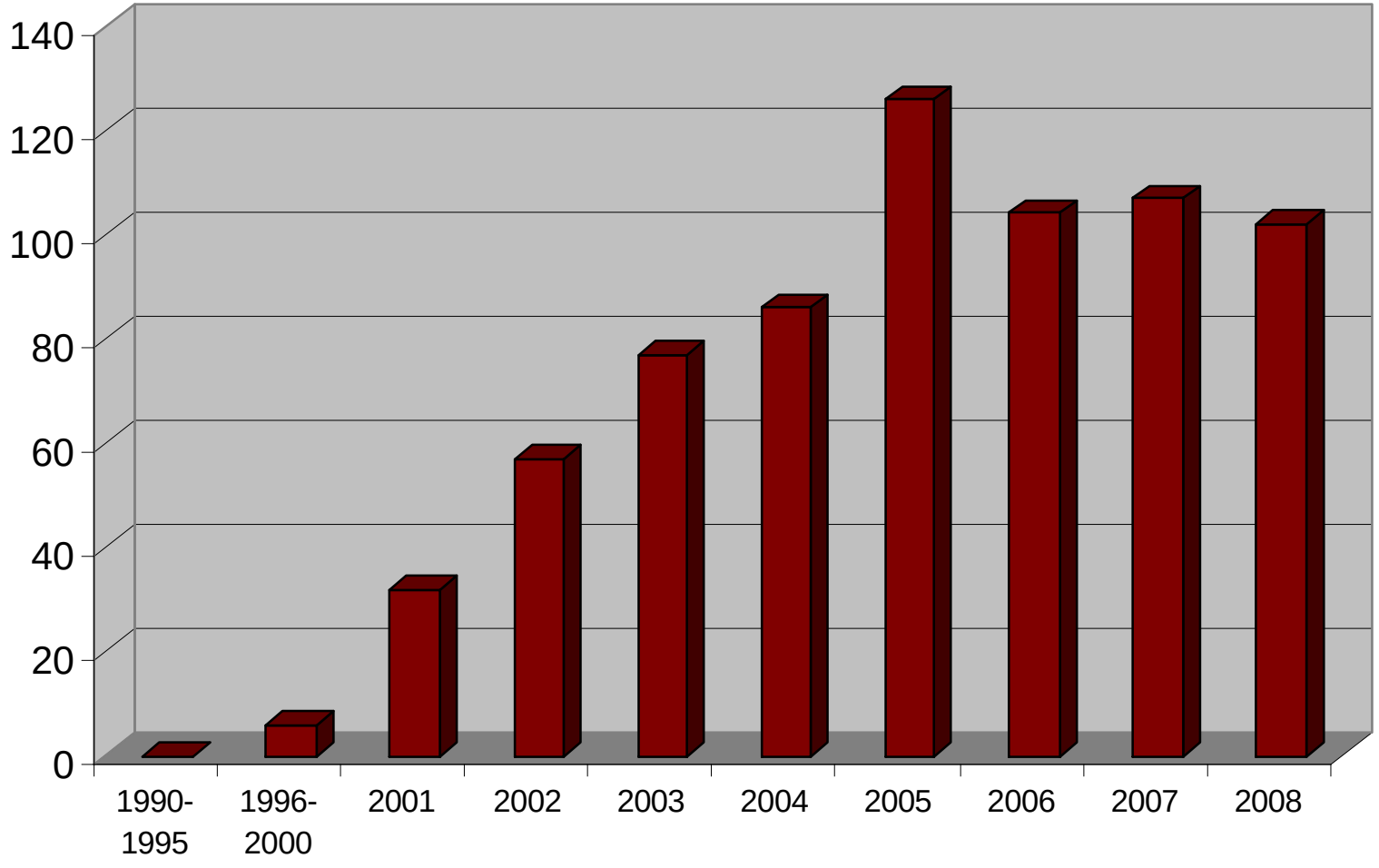
Torsten Haferlach, Münchner Leukämie Labor GmbH

„Microarrays“ in Medline according to years



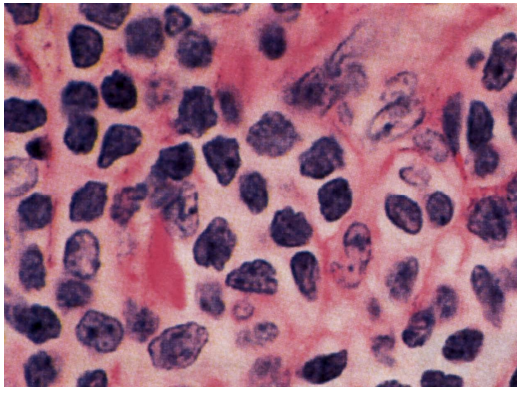
n= 30,243

„Microarrays AND Lymphoma“ in Medline

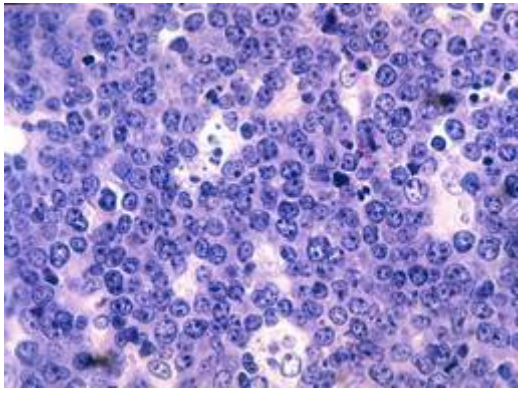


n= 697

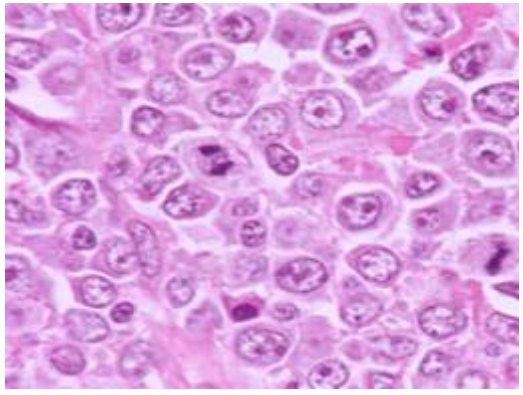
The Diversity of Human Lymphomas



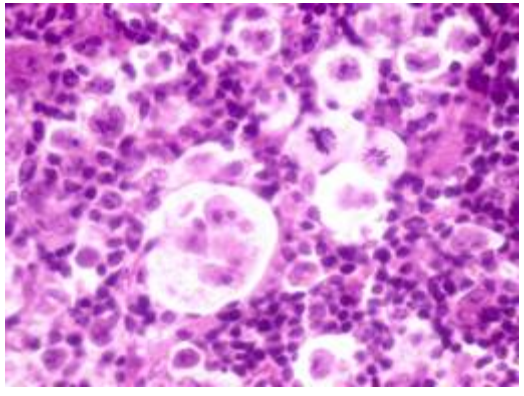
Lymphoma Type A



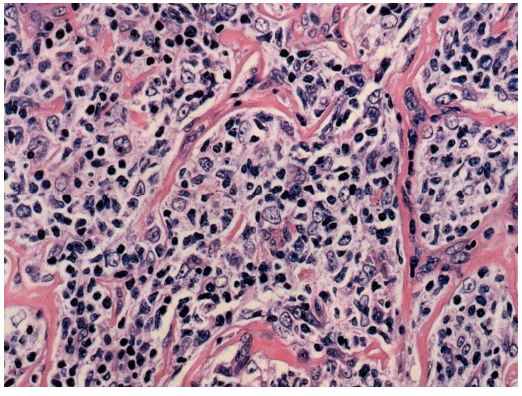
Lymphoma Type B



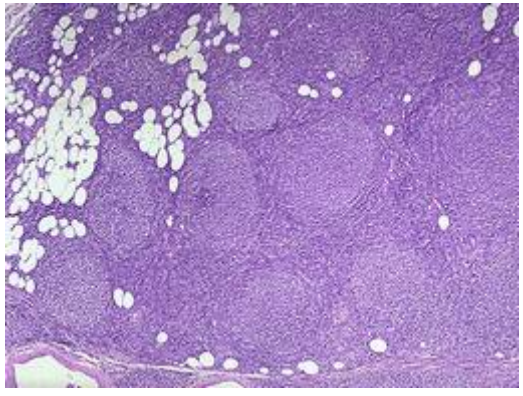
Lymphoma Type C



Lymphoma Type D

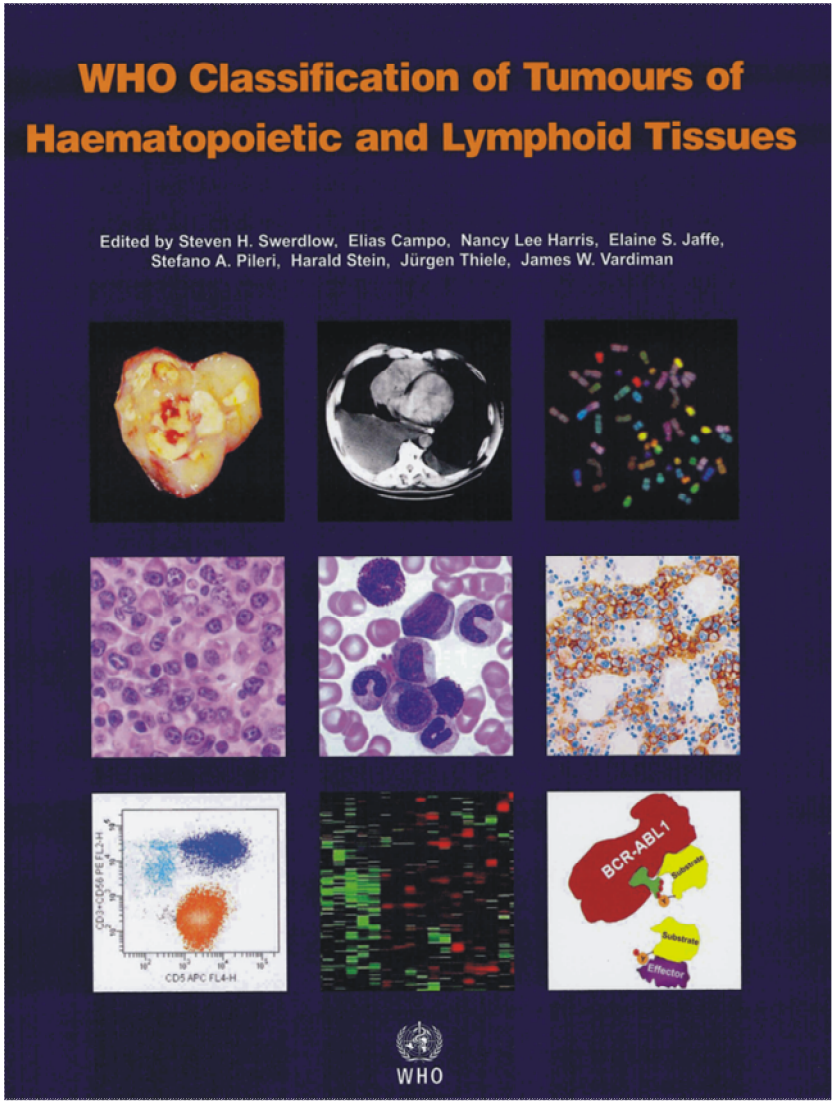


Lymphoma Type E



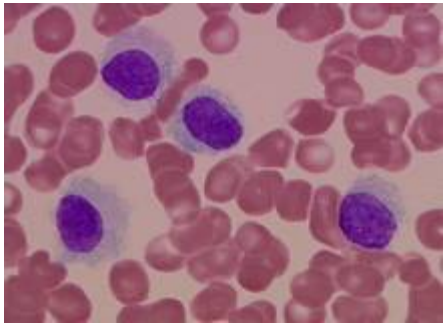
Lymphoma Type F

WHO Klassifikation 2008



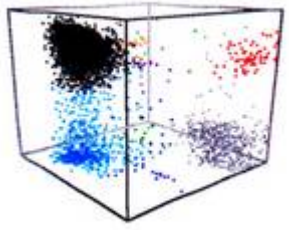
Standards in Diagnosis

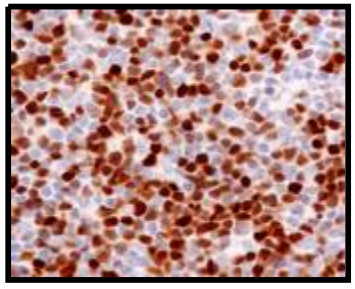
Cytomorphology



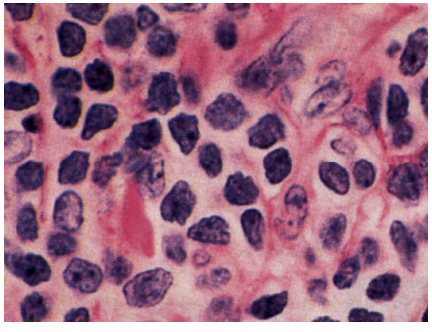
Cytogenetics

Immunophenotype

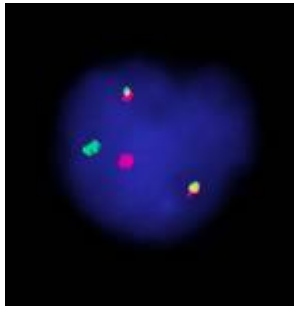




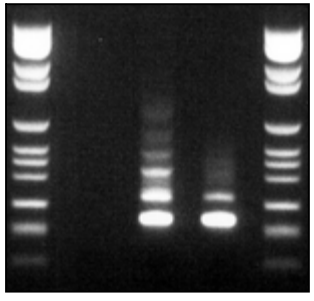
Histology



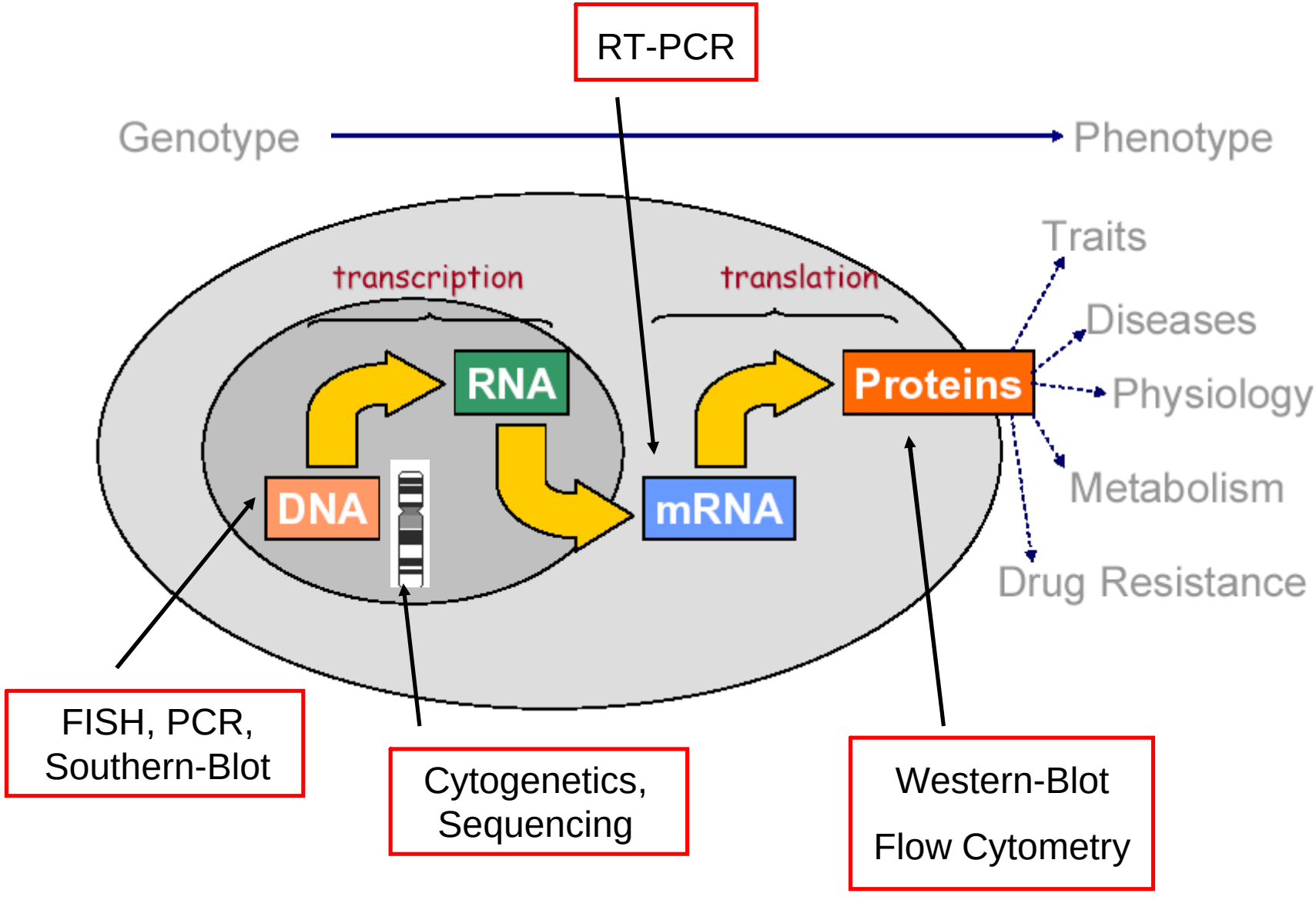
FISH



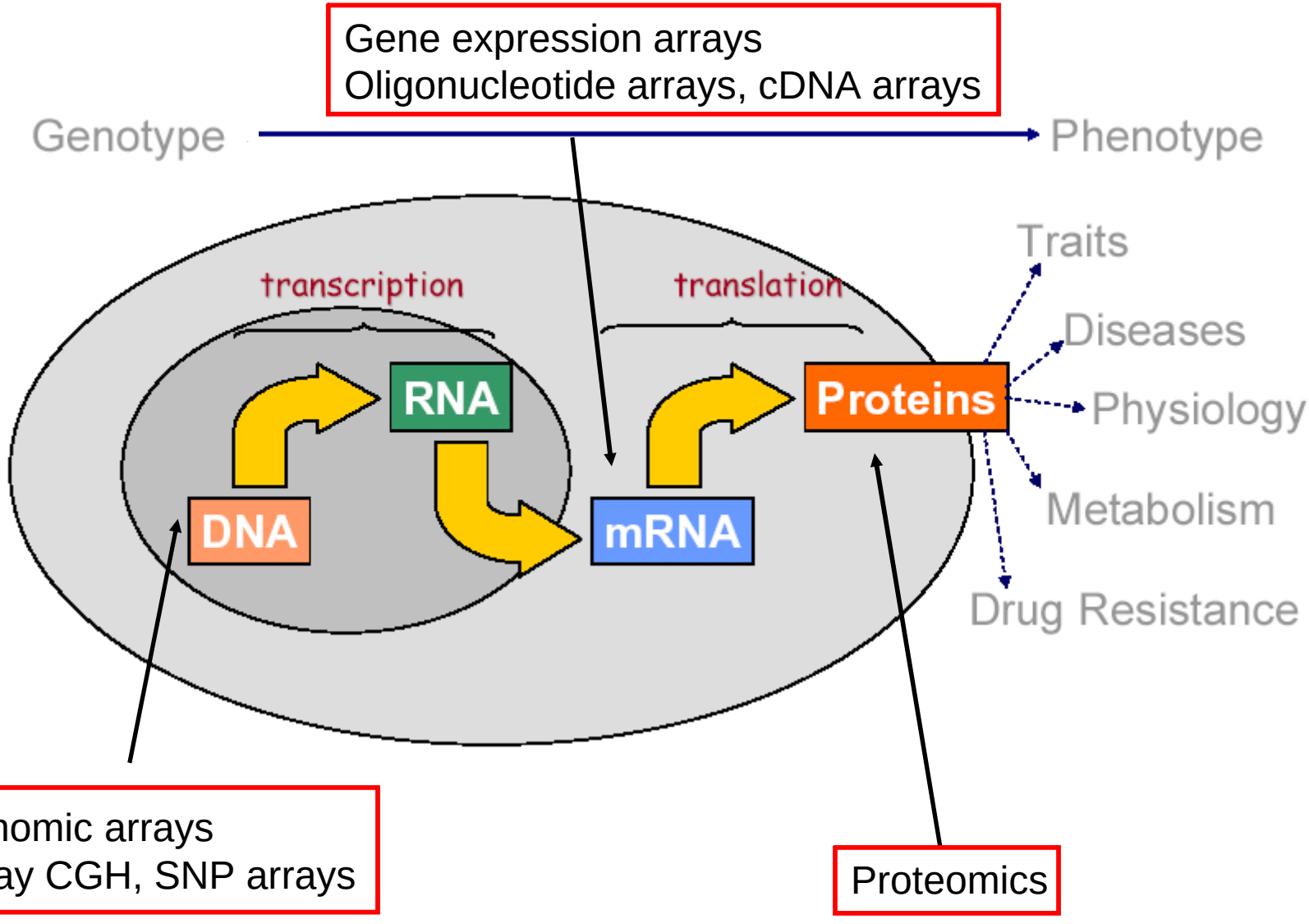
Molecular Biology



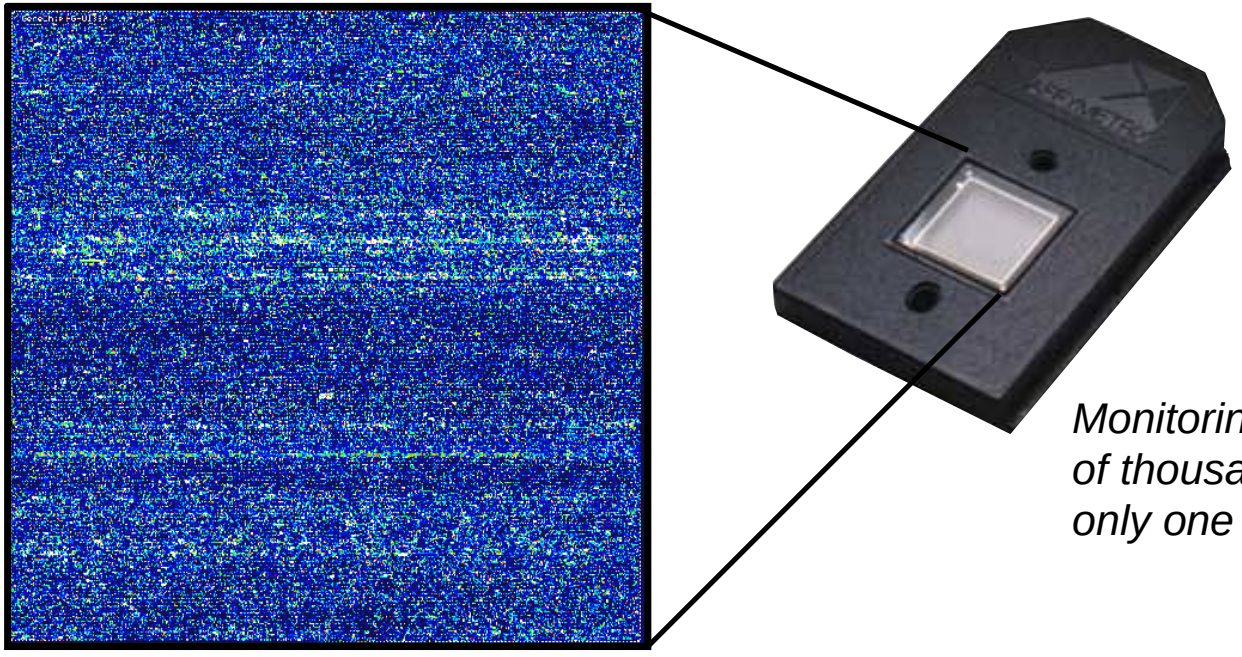
Where do standard methods measure?



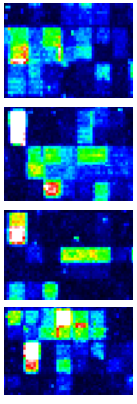
Principles of Genomic Technologies



Applications of Gene Expression Profiling



Monitoring the relative abundance of thousands mRNA transcripts in only one experiment

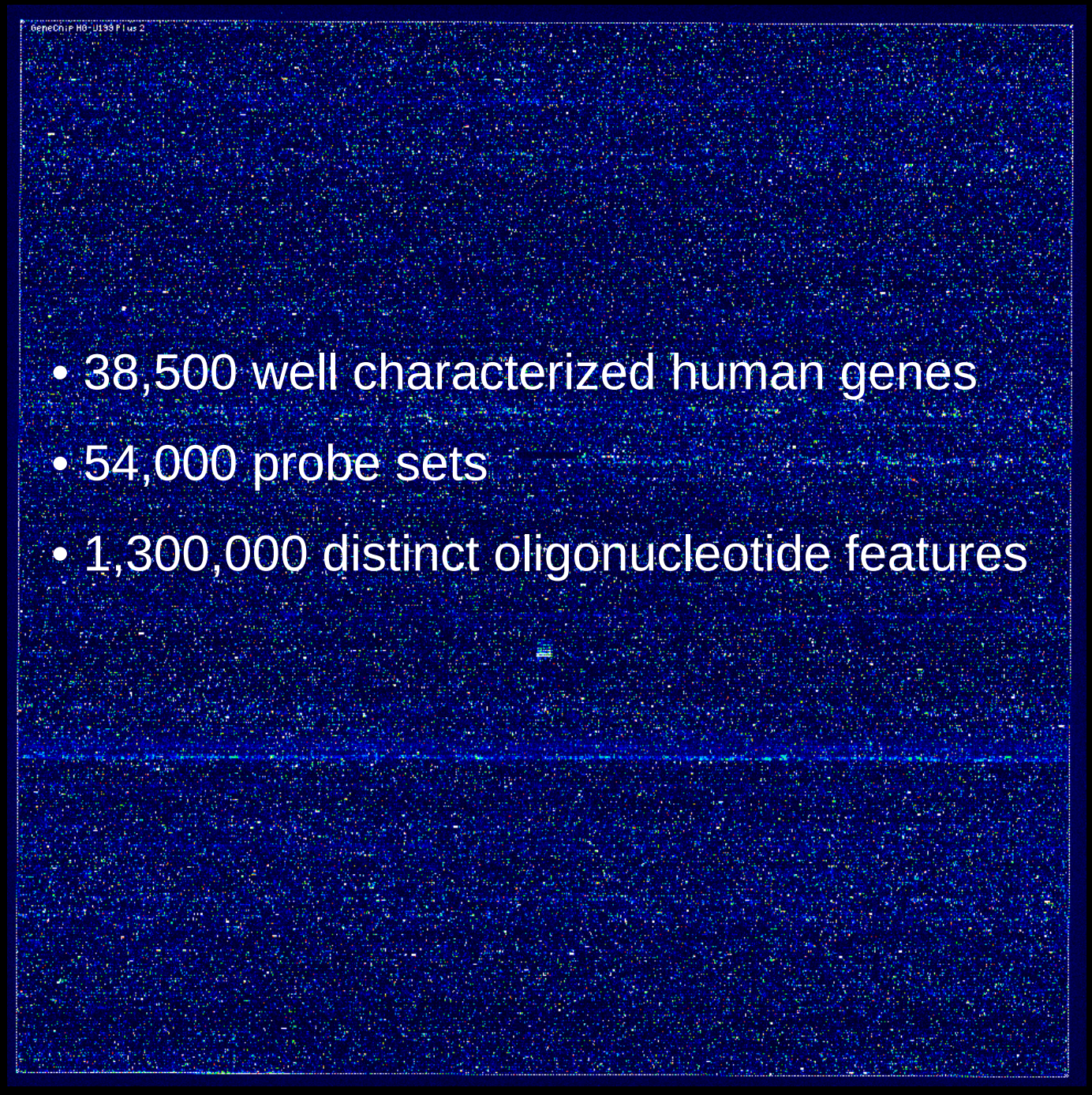


New insights into pathogenic mechanisms

Definition of new entities: class discovery/prognosis

Identification of novel potential therapeutic targets

Diagnostic application


GeneChip HG-U133 Plus 2
• 38,500 well characterized human genes
• 54,000 probe sets
• 1,300,000 distinct oligonucleotide features

Gene Expression Profiling - Advantages

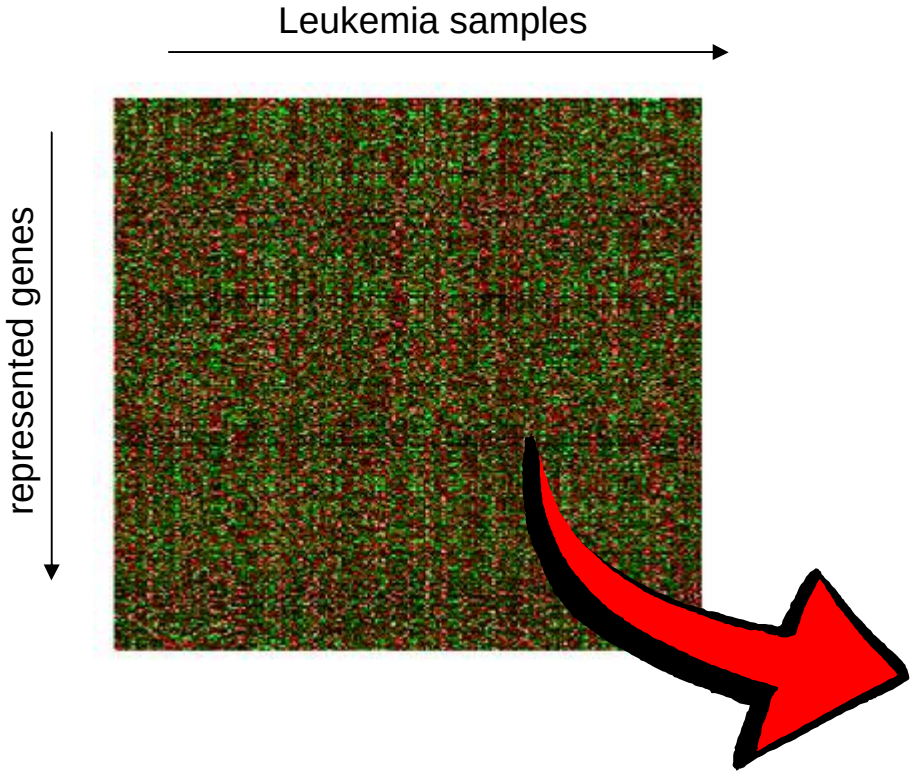
- Genome-wide view of the transcriptome
- Sorting out disease heterogeneity
  (DLBCL, PMBCL, Burkitt lymphoma)
- Building molecular outcome predictors
  (DLBCL, MCL, CLL, FL)
- Defining treatment resistance and response
- Understanding biology (FL)

Gene Expression Profiling - Disadvantages

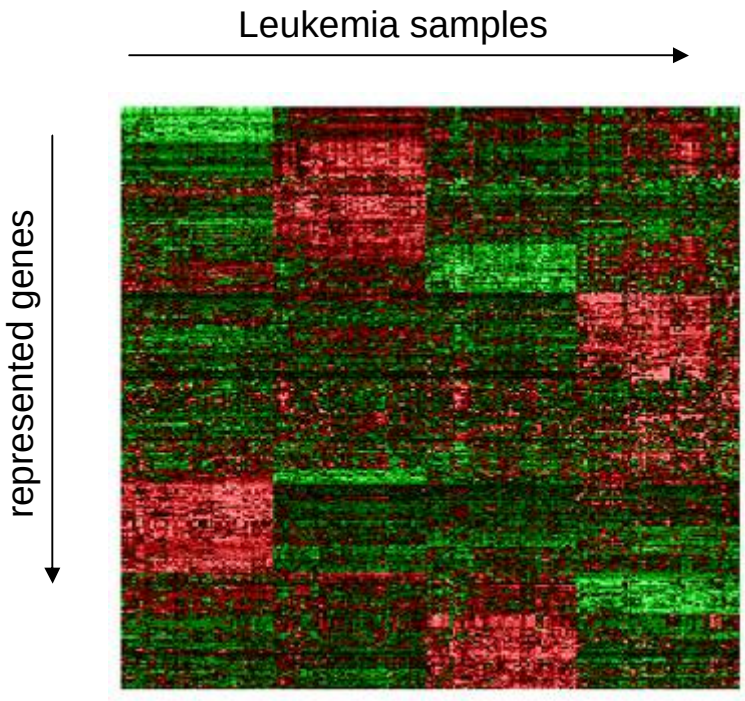
- Nucleic acid hybridization (reproducible?)
- Currently requires frozen tissue
- Tumor cell content must be known
- Measures mRNA and not protein (post-transcriptional control of gene expression?)
- Cost?

Hierarchical Cluster Analysis

Data randomly distributed

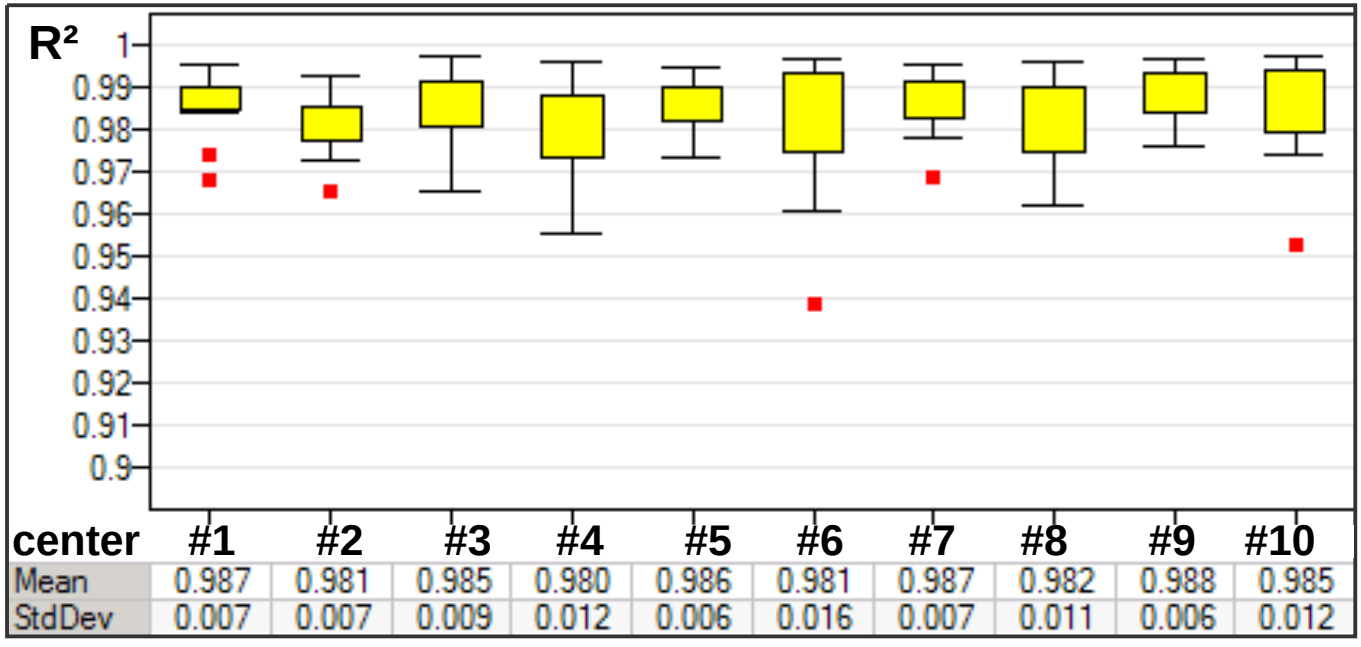


Visualization



High Reproducibility and Concordance

Intra-site correlation (all genes represented on HG-U133 Plus 2.0)



center	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Mean	0.987	0.981	0.985	0.980	0.986	0.981	0.987	0.982	0.988	0.985
StdDev	0.007	0.007	0.009	0.012	0.006	0.016	0.007	0.011	0.006	0.012

Based on six (6) HEPG2 cell line samples for each center:

- 2 samples analyzed during protocol training week (1 µg, 5 µg)
- 4 samples analyzed during proficiency testing (1.5 µg, 3 µg, 5 µg, 8 µg)

➔ 15 different pairwise comparisons for each center

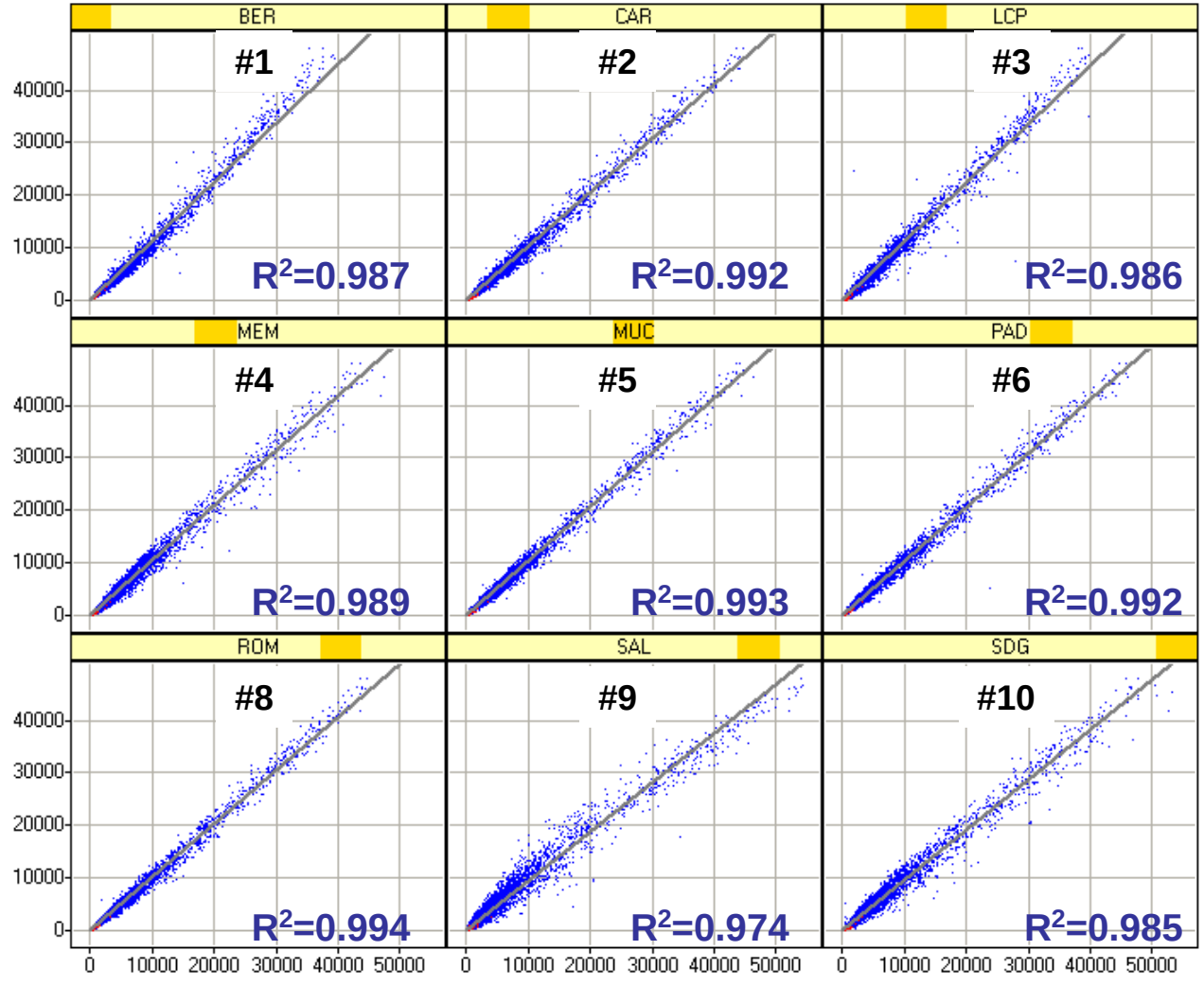
Inter-laboratory Reproducibility

5 µg HEPG2 sample from proficiency testing
(all genes represented on HG-U133 Plus 2.0 microarray)

Example:

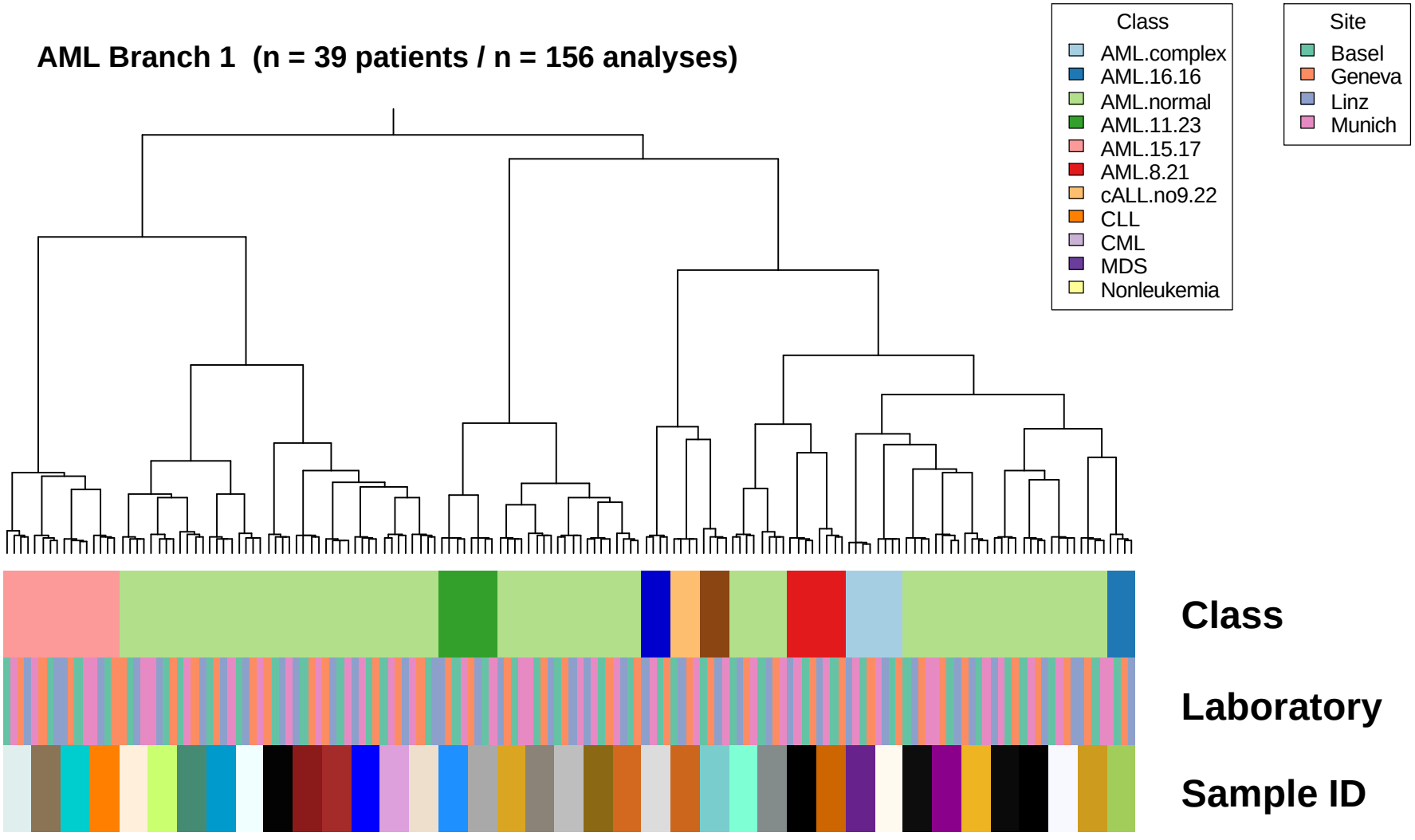
Center 7 vs.

9 other centers



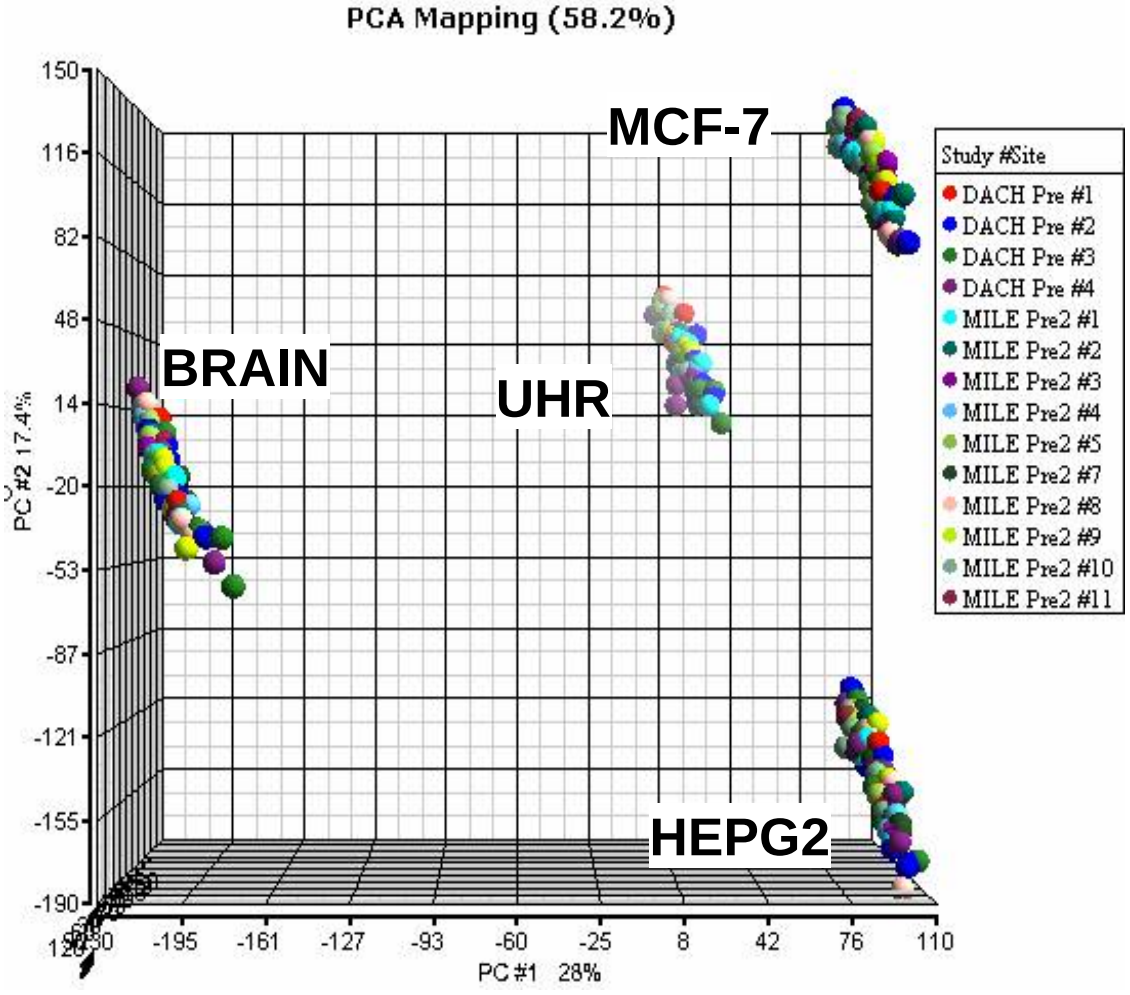
Kohlmann A et al., Br J Haematol. 2008 Sep;142(5):802-7.

Quadruplicates of AML Samples

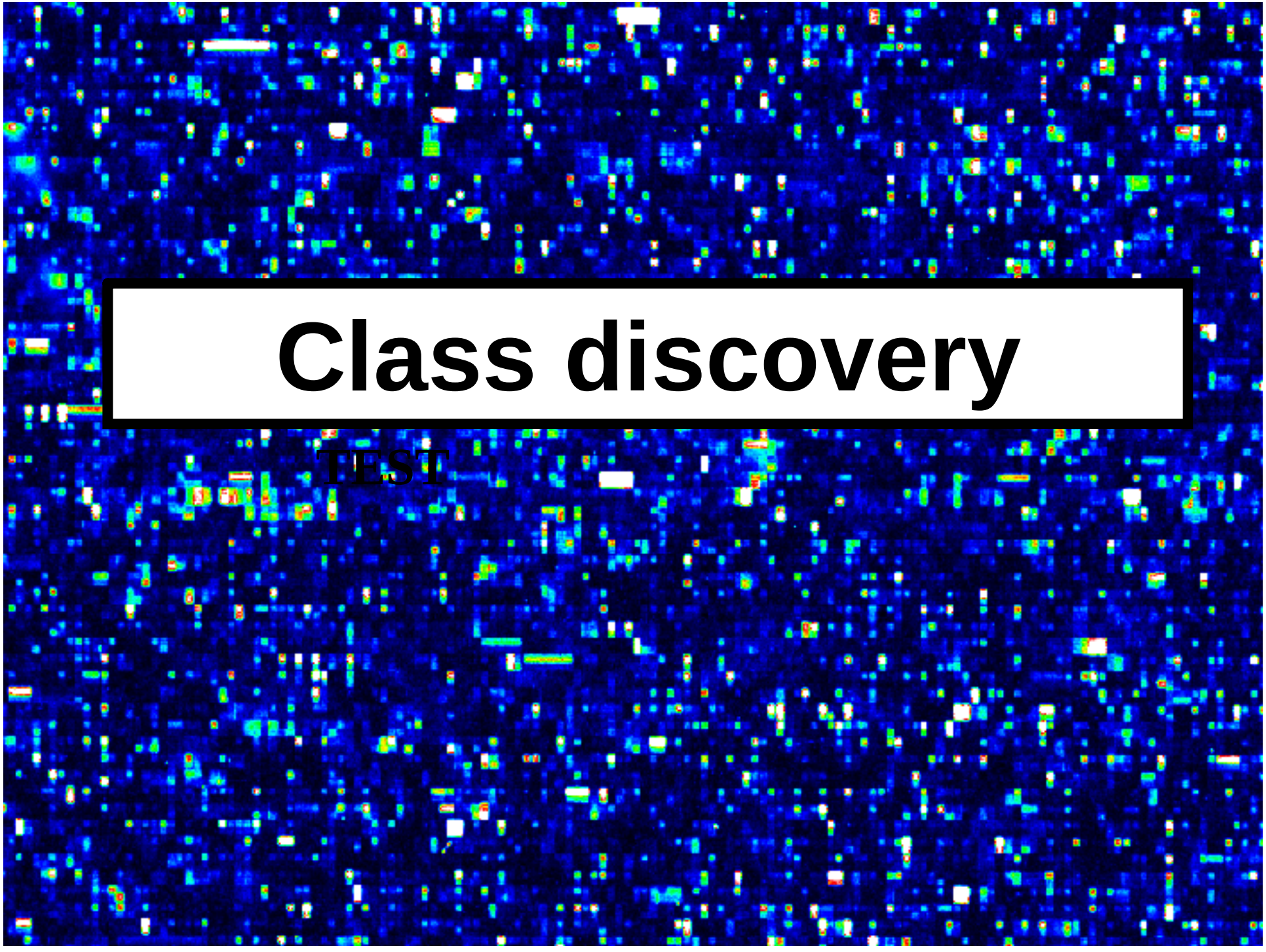


Standardization and Reproducibility

Coloring according to Laboratory



DACH Pre #1	**MUC**
DACH Pre #2	**GEN**
DACH Pre #3	**BAS**
DACH Pre #4	**LIN**
MILES Pre2 #1	**CAR**
MILES Pre2 #2	**MON**
MILES Pre2 #3	**ROM**
MILES Pre2 #4	**SDG**
MILES Pre2 #5	**PAD**
MILES Pre2 #6	**MUC**
MILES Pre2 #7	**SNG**
MILES Pre2 #8	**SAL**
MILES Pre2 #9	**MEM**
MILES Pre2 #10	**LCP**
MILES Pre2 #11	**BER**

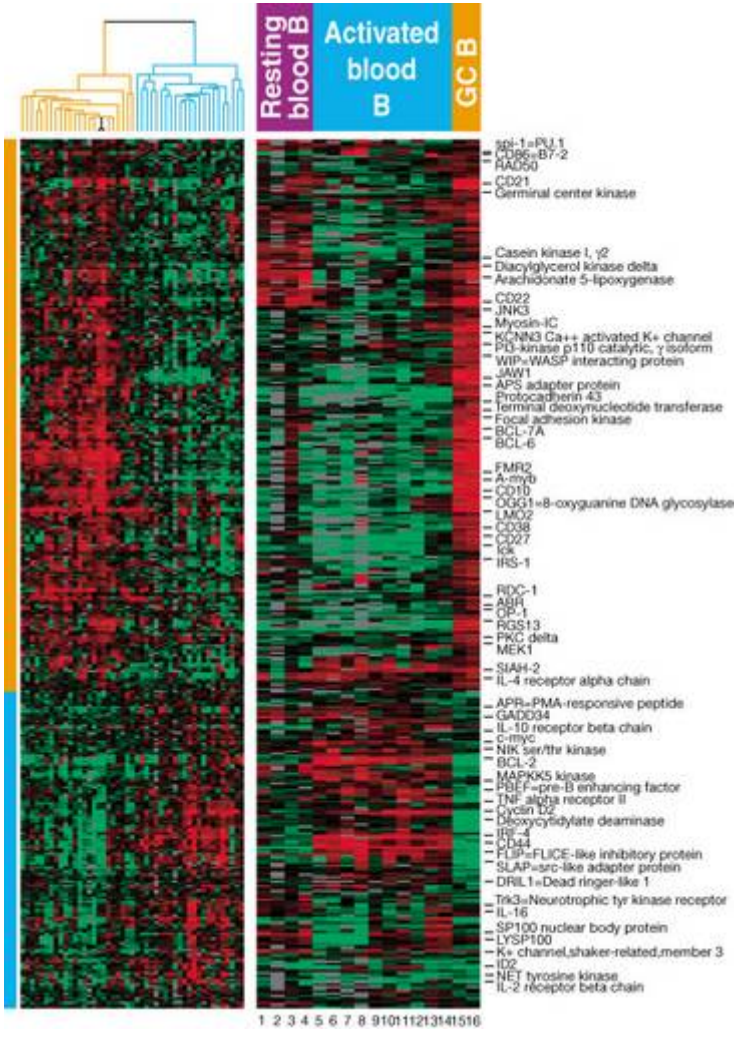

Class discovery
TEST

Distinct Molecular Subtypes of DLBLC

Identification of two molecularly distinct forms of DLBCL which had gene expression patterns indicative of different stages of B-cell differentiation

One type expressed genes characteristic of germinal centre B cells ('germinal centre B-like DLBCL')

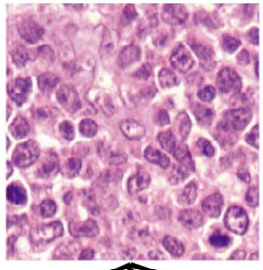
The second type expressed genes normally induced during *in vitro* activation of peripheral blood B cells ('activated B-like DLBCL')

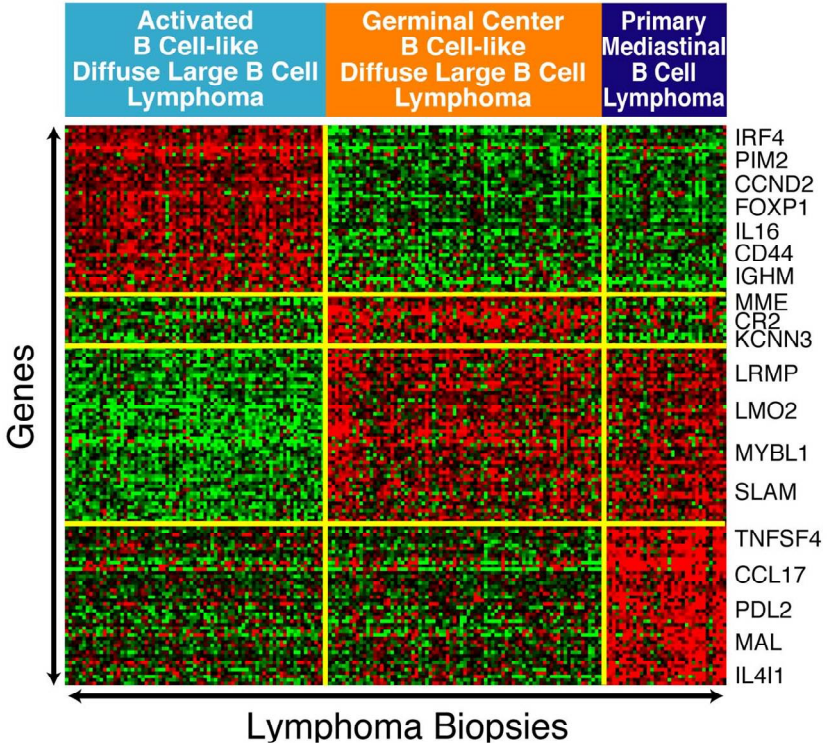


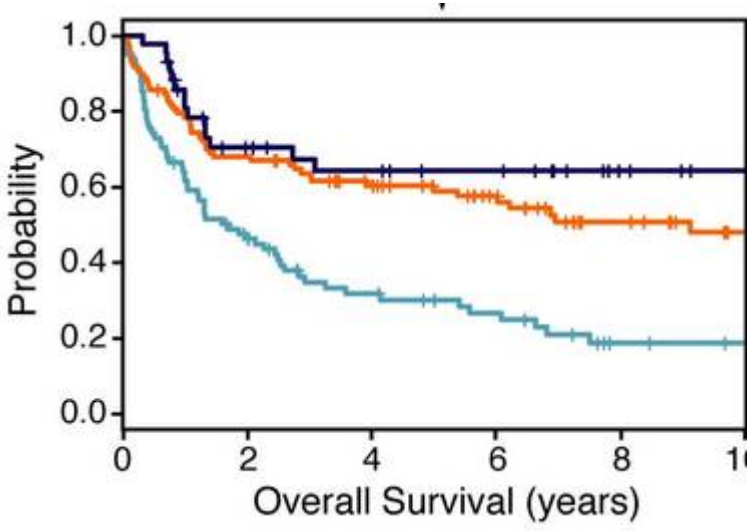
Alizadeh A et al., Nature. 2000 Feb 3;403(6769):503-11.

Molecular Distinct DLBCL Subgroups

DLBCL

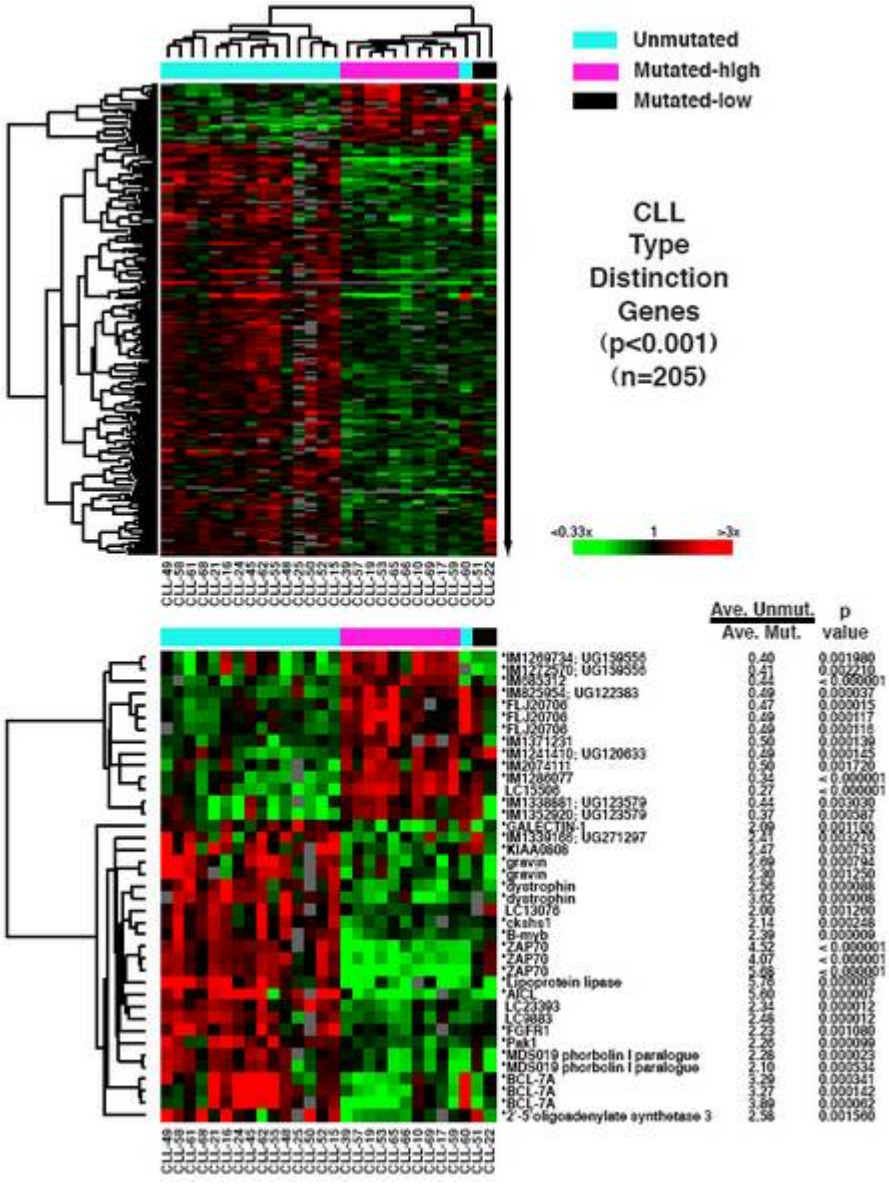






Alizadeh et al., Nature, 2000
Rosenwald et al., N Engl J Med., 2002
Bea et al., Blood, 2005
WHO Guidelines Hematological Tissues 2001

Discovery of ZAP70 in B-CLL



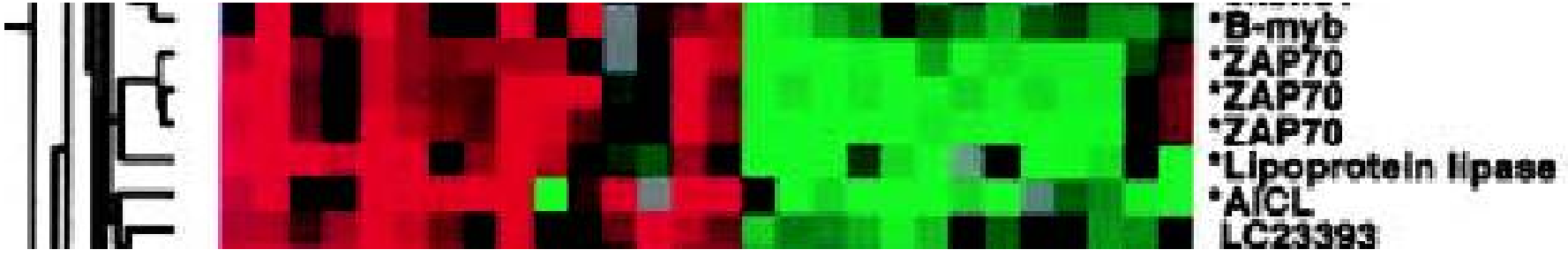
Relation of Gene Expression Phenotype to Immunoglobulin Mutation Genotype in B Cell CLL

The most differentially expressed gene between the CLL subtypes was ZAP-70, a critical kinase that transduces signals from the T cell antigen receptor, and is preferentially expressed in normal T lymphocytes.

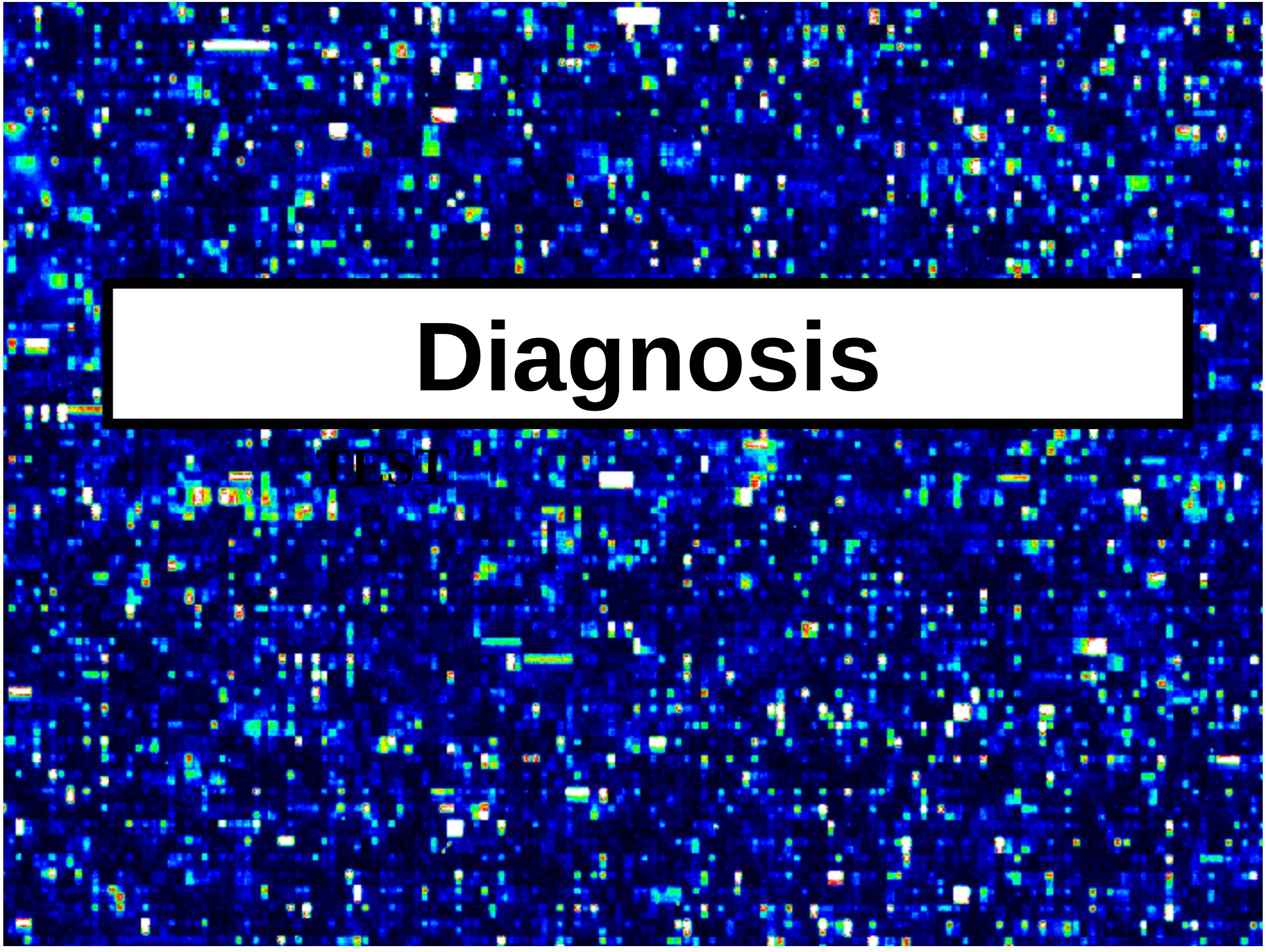
Rosenwald A et al., J Exp Med. 2001 Dec 3;194(11):1639-47.

Discovery of ZAP70 in B-CLL

Differential ZAP70 expression was detected in two subgroups of CLL

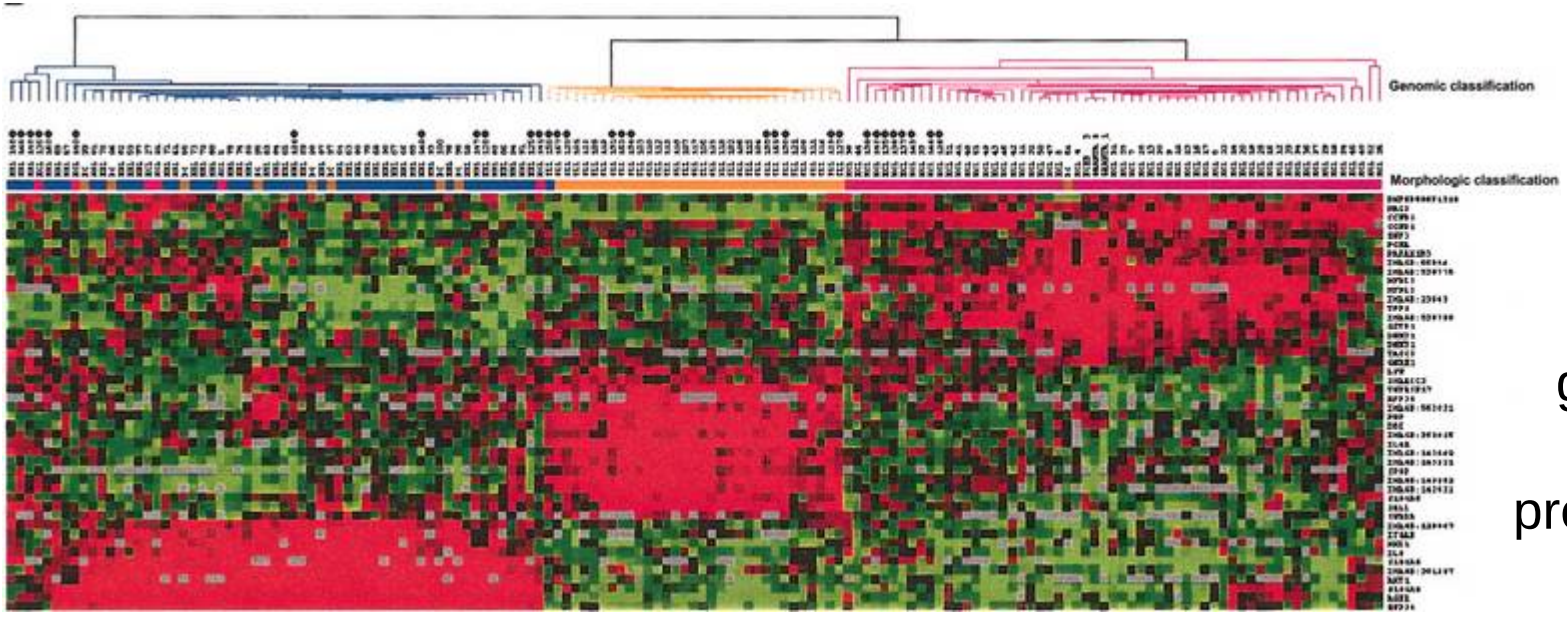


IgVH unmutated CLL IgVH mutated CLL


Diagnosis
TEST

Signatures in SLL, MZL, and MCL

Distinct profiles allowing a molecular diagnosis



n=44 genes for prediction

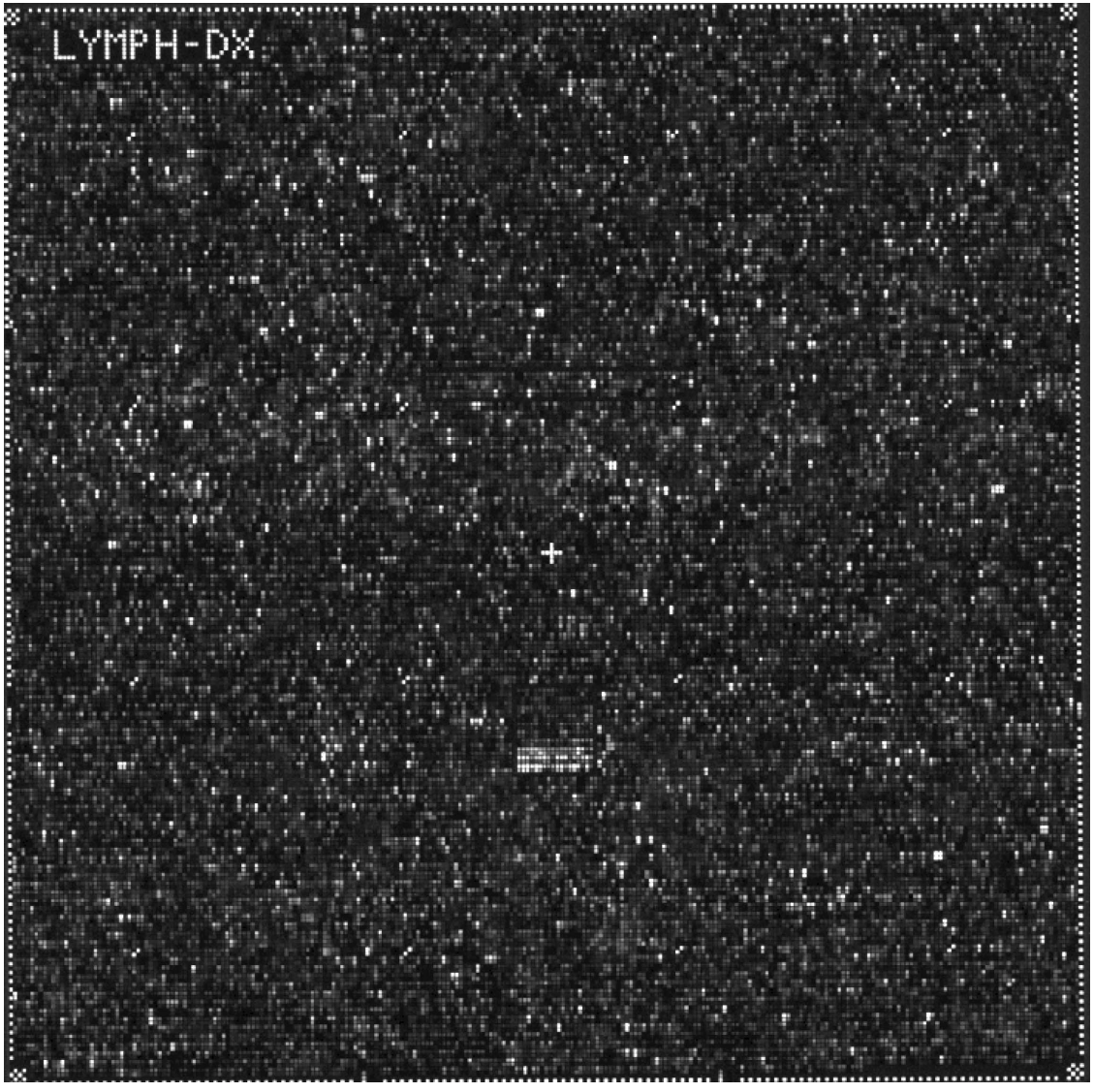
MZL SLL MCL

n=128 non-germinal center small B-cell lymphomas

➡ Specific genomic signatures for non-Hodgkin lymphomas: small lymphocytic lymphoma (SLL), splenic marginal zone B-cell lymphoma (MZL), and mantle cell lymphoma (MCL)

Thieblemont C et al. Blood. 2004 Apr 1;103(7):2727-37.

The LymphDx Custom LLMPP Microarray



~2643 human genes:

Lymphoma diagnostic genes

Lymphoma prognostic genes

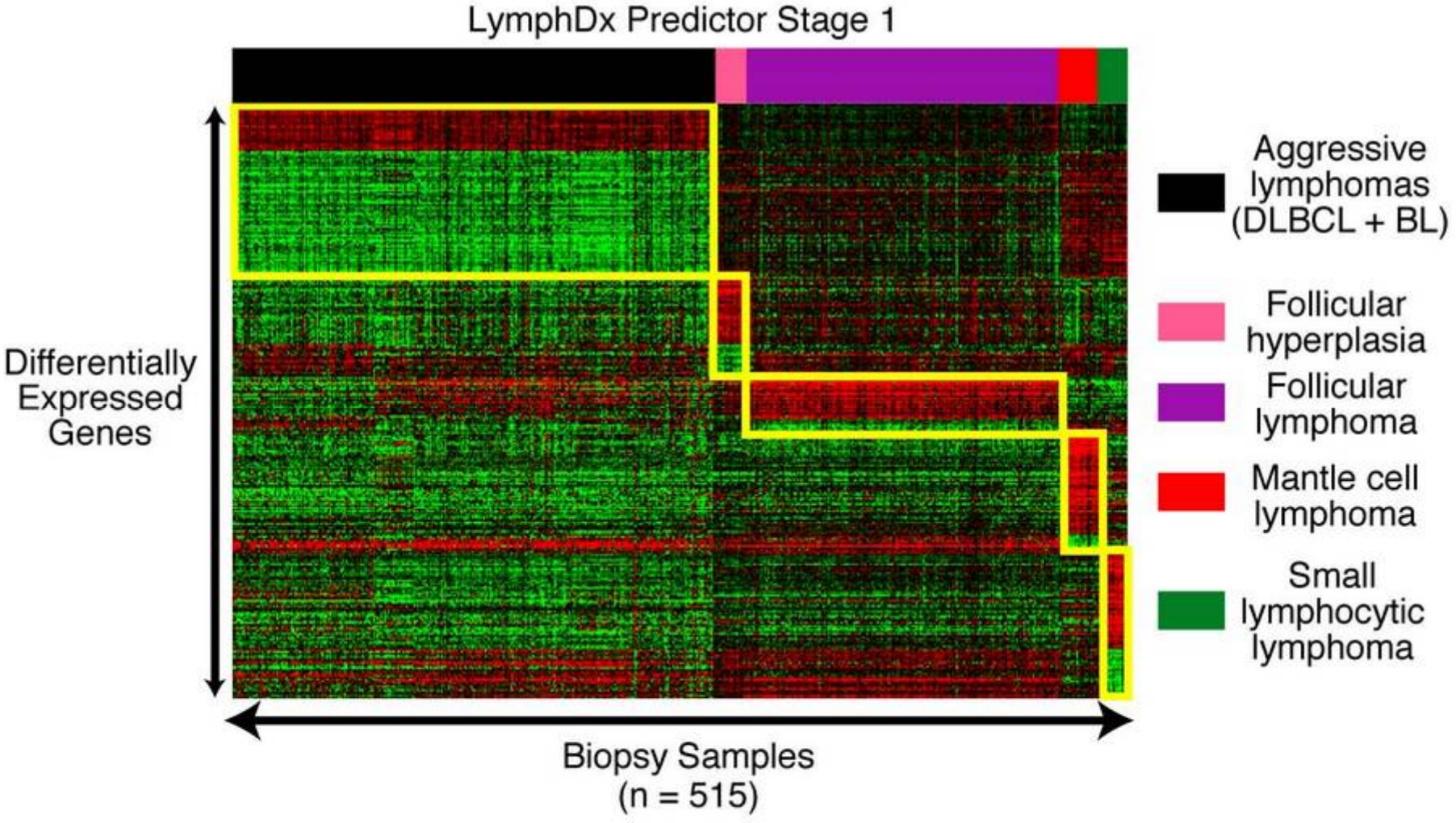
EBV, HHV8, HTLV1
viral genes

Genes encoding all human
kinases
cytokines
chemokines
cytokine receptors
chemokine receptors

Invariantly expressed
control genes

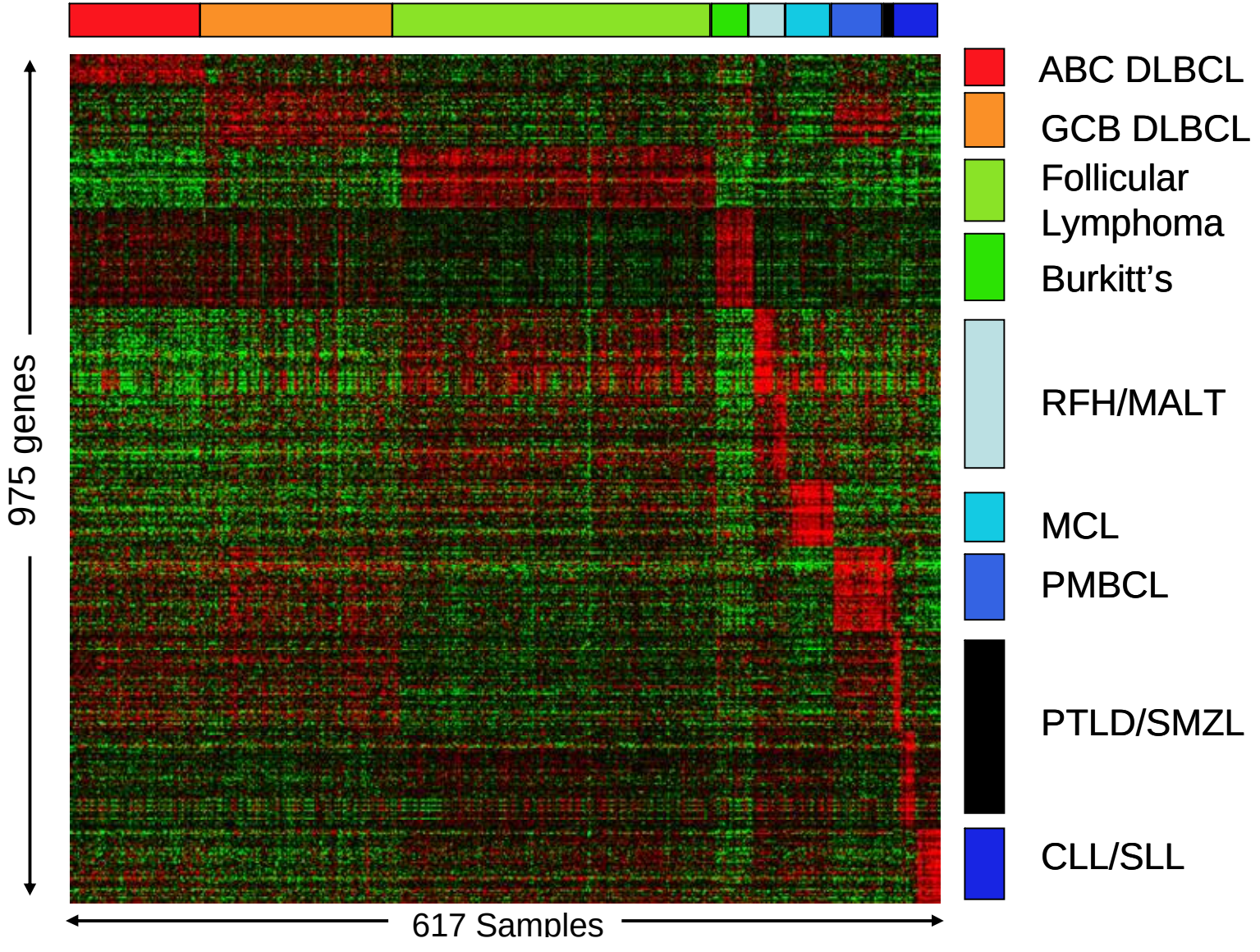
Courtesy of Dr. Lou Staudt, LLMPP group

Microarray-based Diagnosis of Lymphoma

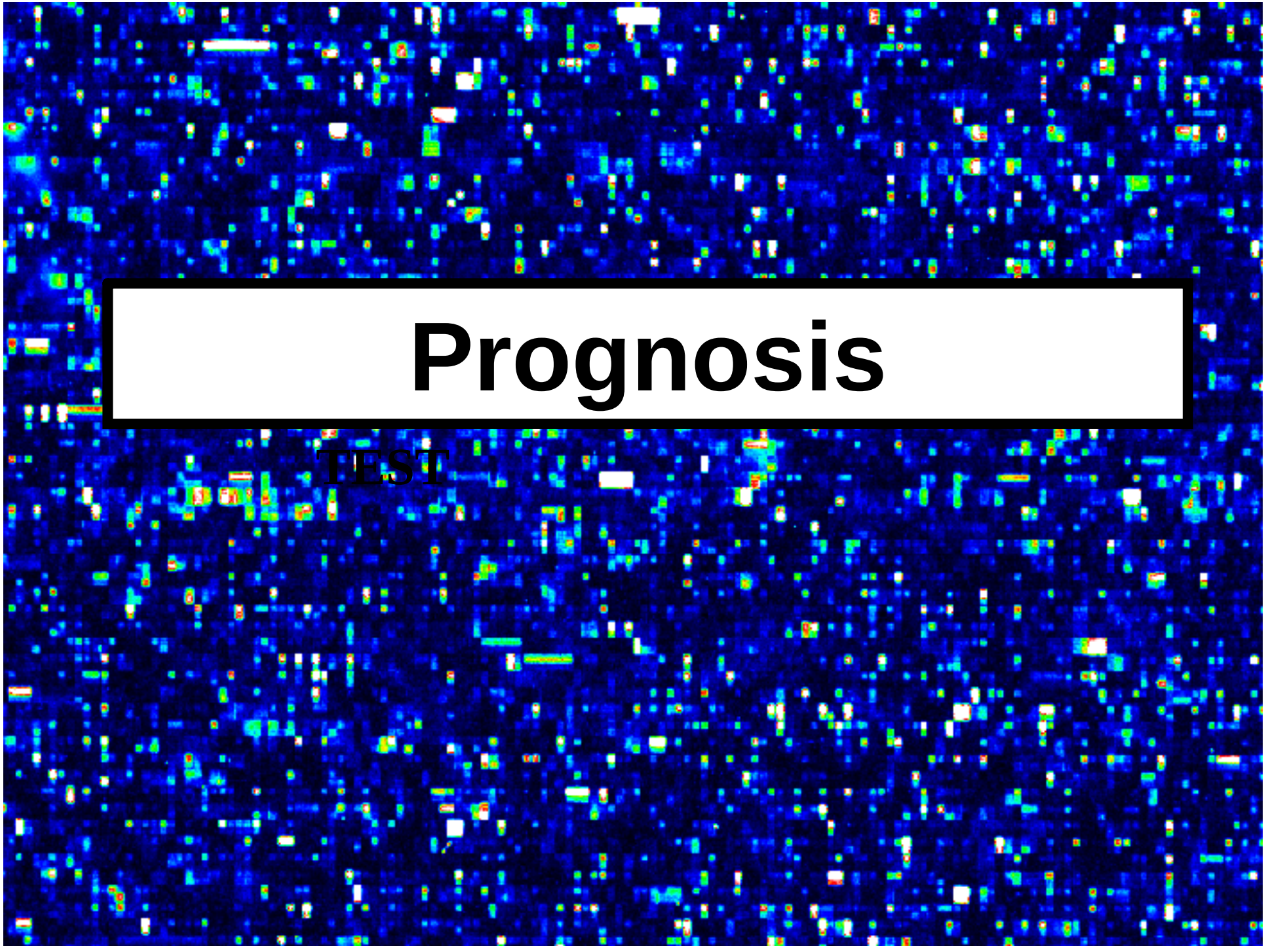


Courtesy of Dr. Lou Staudt, LLMPP group

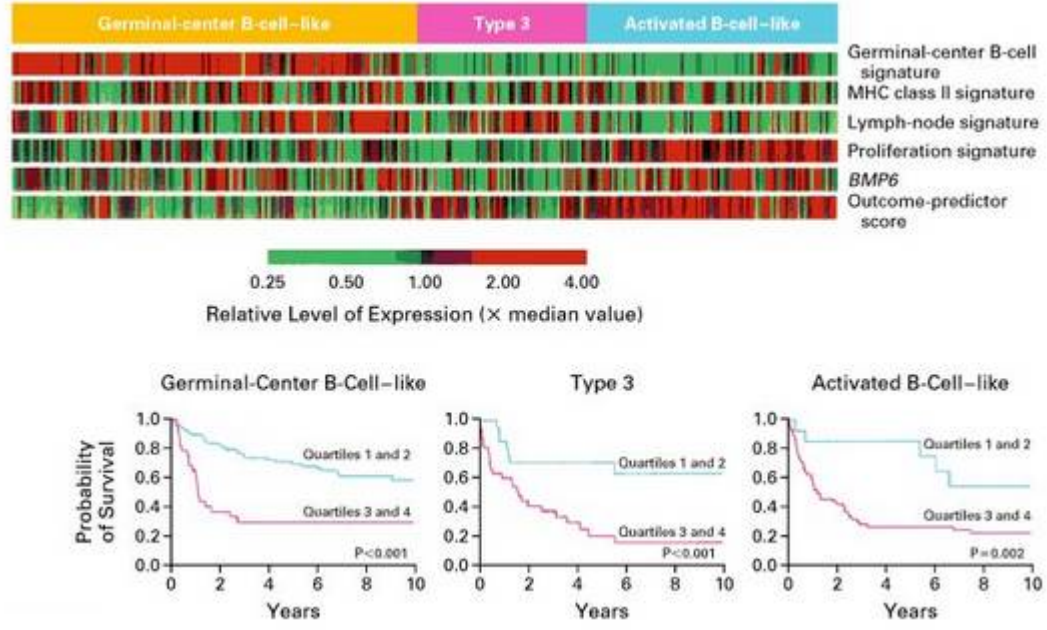
Molecular Diagnosis of Lymphoma Subtypes



Courtesy of Dr. Randy Gascoyne, LLMPP group


Prognosis
TEST

Survival Prediction for DLBCL



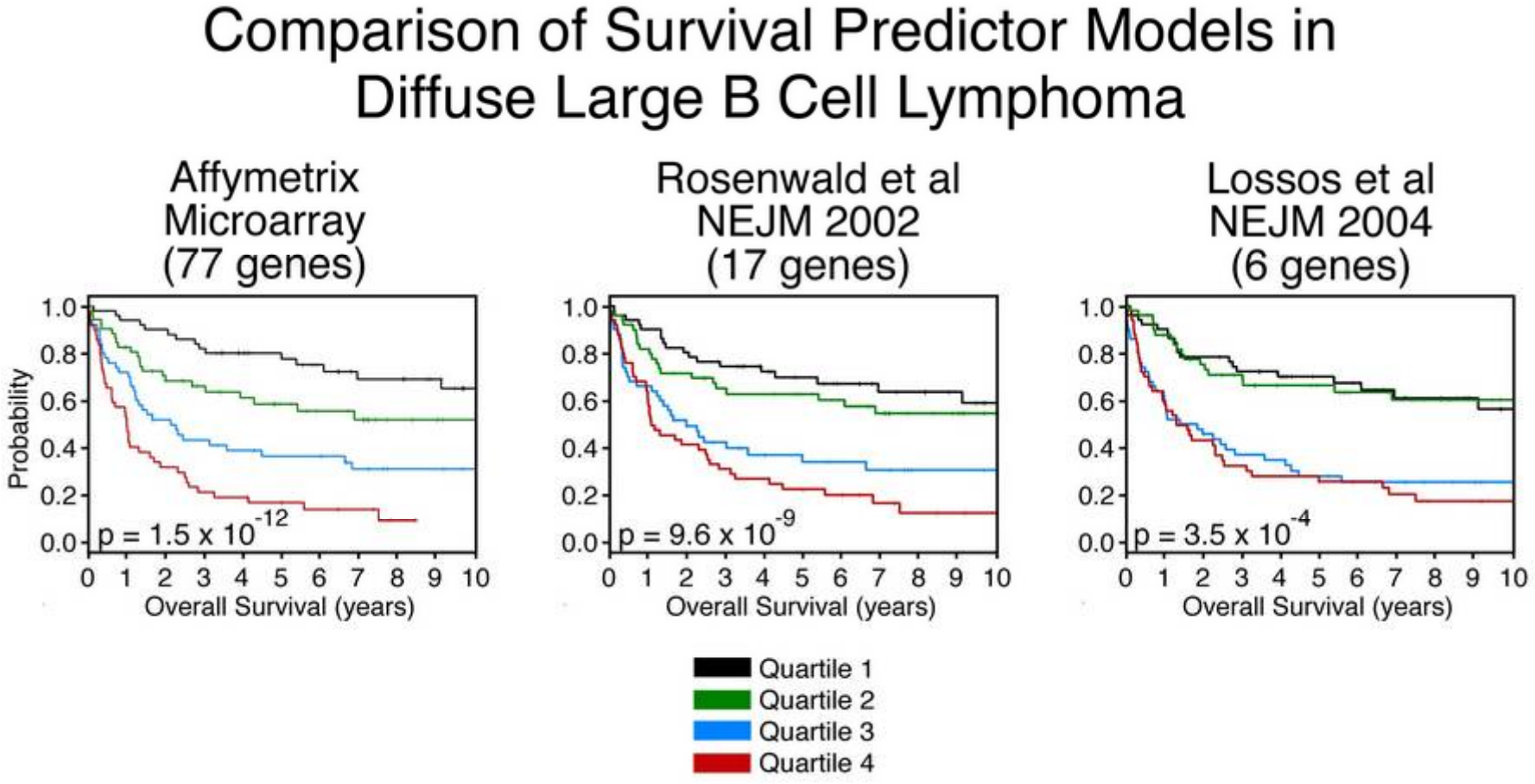
3 Subgroups with distinctive gene expression profiles

Gene expression patterns that were associated with survival in a preliminary group of 160 patients (tested in a validation group, n=80 patients)

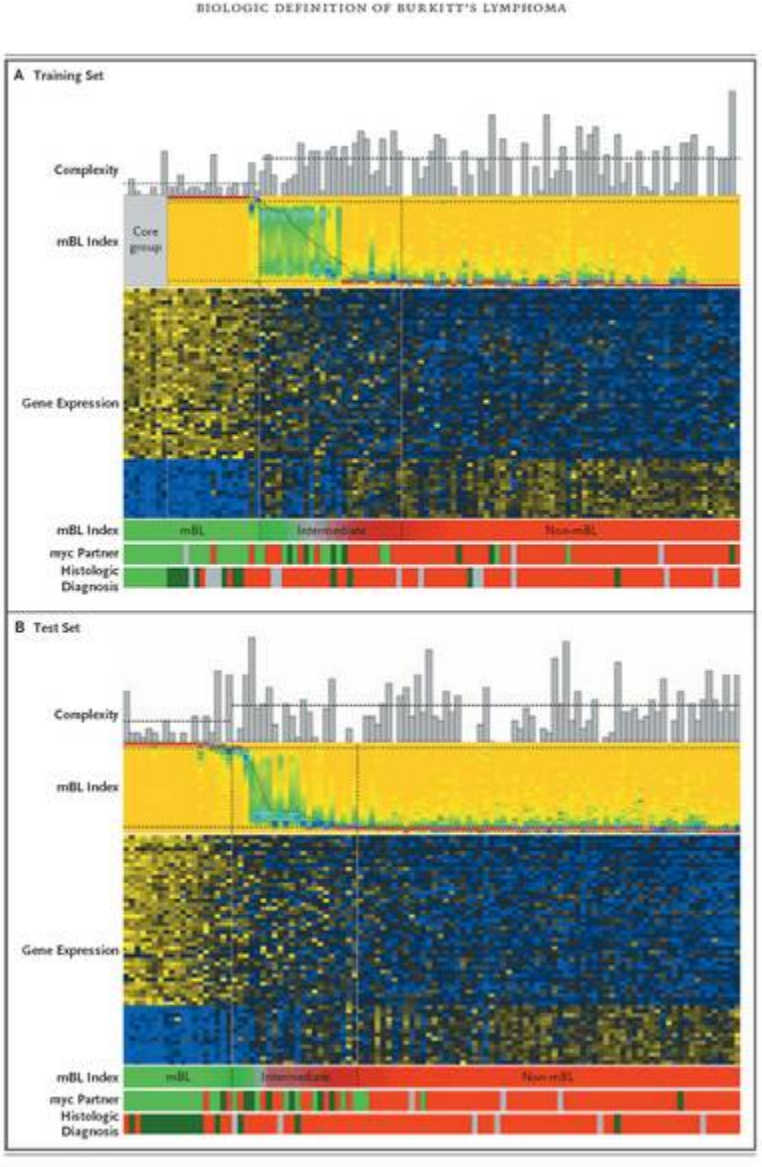
DNA microarrays can be used to formulate a molecular predictor of survival after chemotherapy for diffuse large-B-cell lymphoma

Rosenwald A et al., N Engl J Med. 2002 Jun 20;346(25):1937-47.

Comparison of Classifiers for DLBCL



Biologic definition of Burkitt´s lymphoma



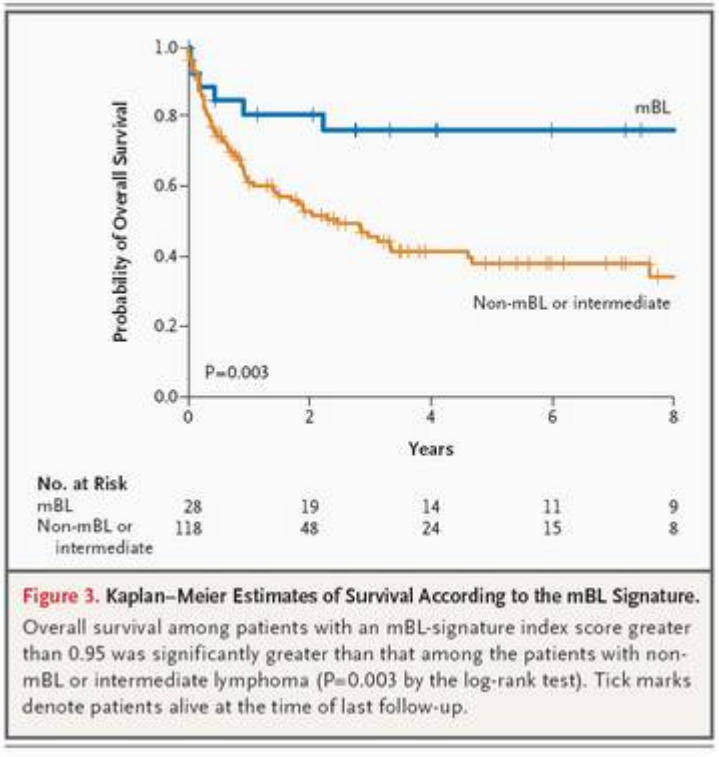
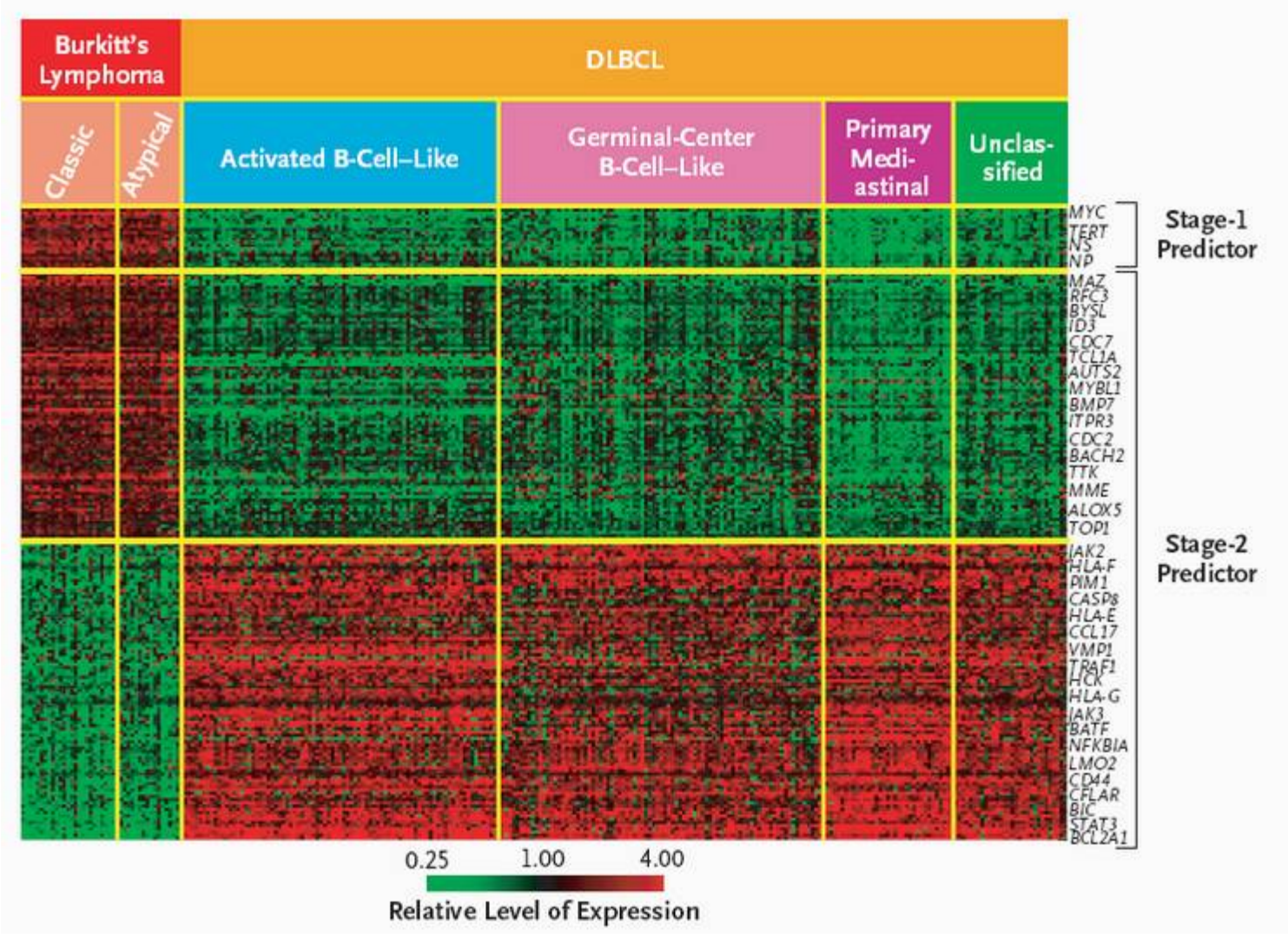


Figure 3. Kaplan–Meier Estimates of Survival According to the mBL Signature.
Overall survival among patients with an mBL-signature index score greater than 0.95 was significantly greater than that among the patients with non-mBL or intermediate lymphoma (P=0.003 by the log-rank test). Tick marks denote patients alive at the time of last follow-up.

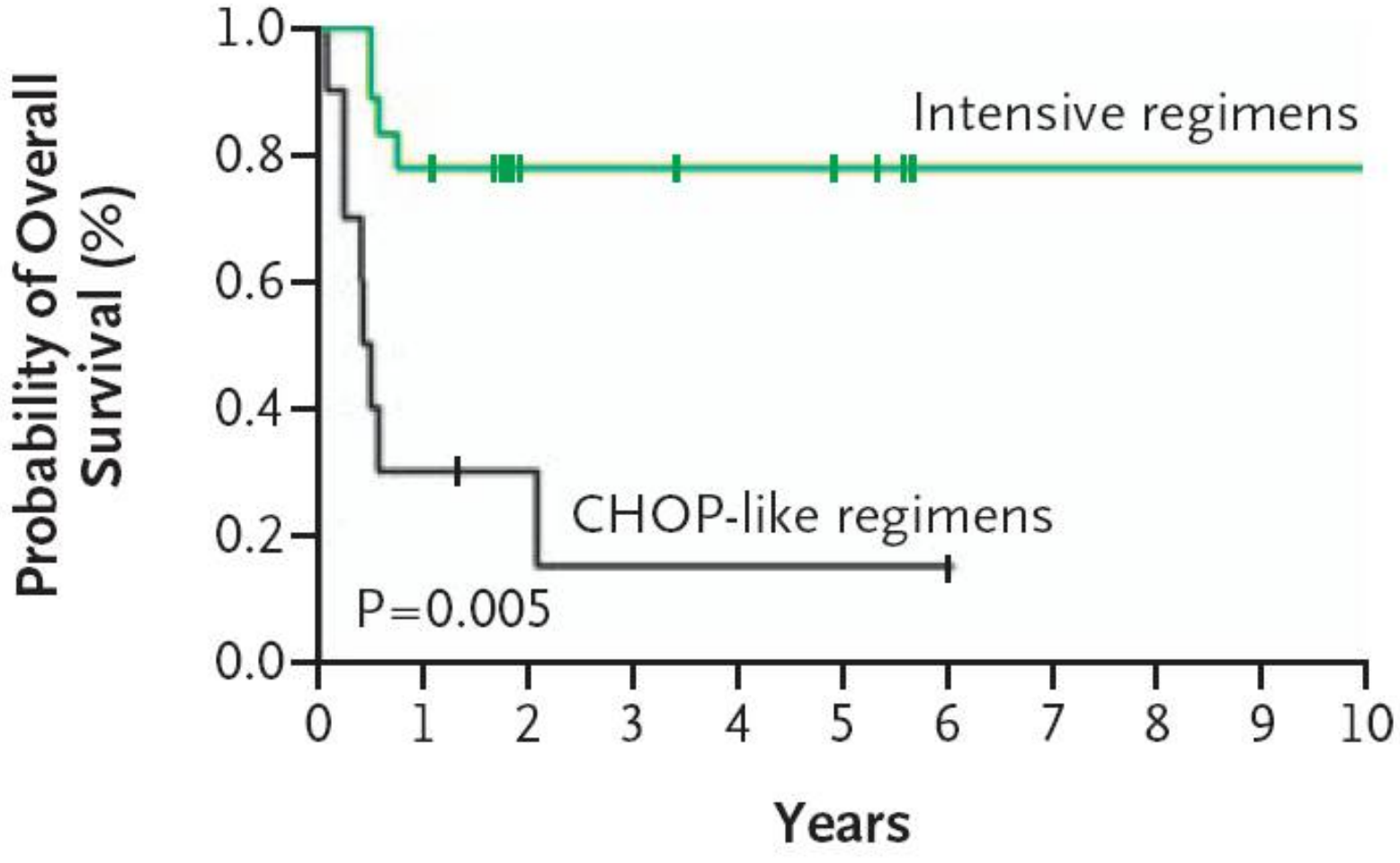
Hummel et al., N Engl J Med. 2006 Jun 8;354(23):2419-30.

Molecular diagnosis of Burkitt´s lymphoma



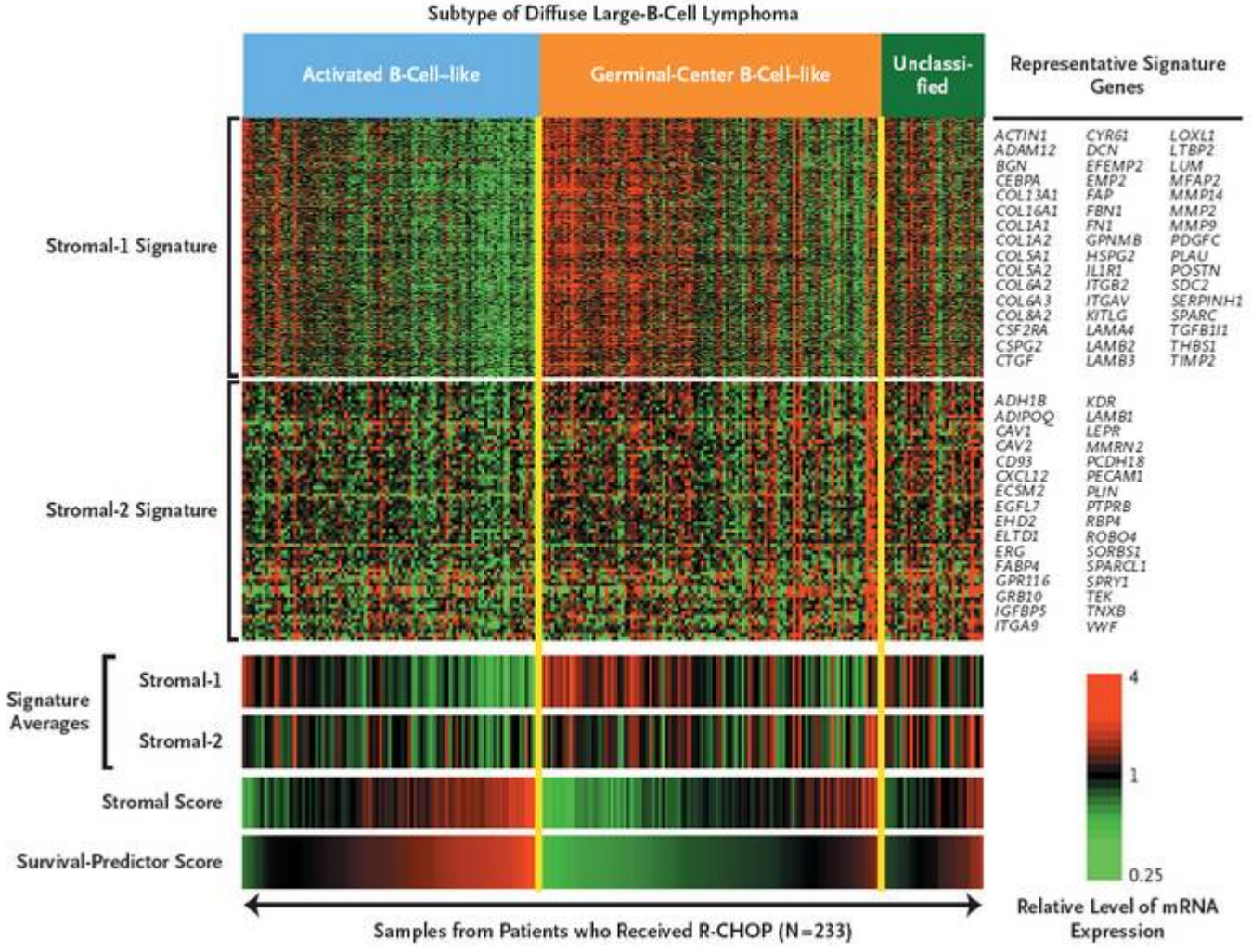
Dave S et al., N Engl J Med. 2006 Jun 8;354(23):2431-42.

Molecular diagnosis of Burkitt´s lymphoma



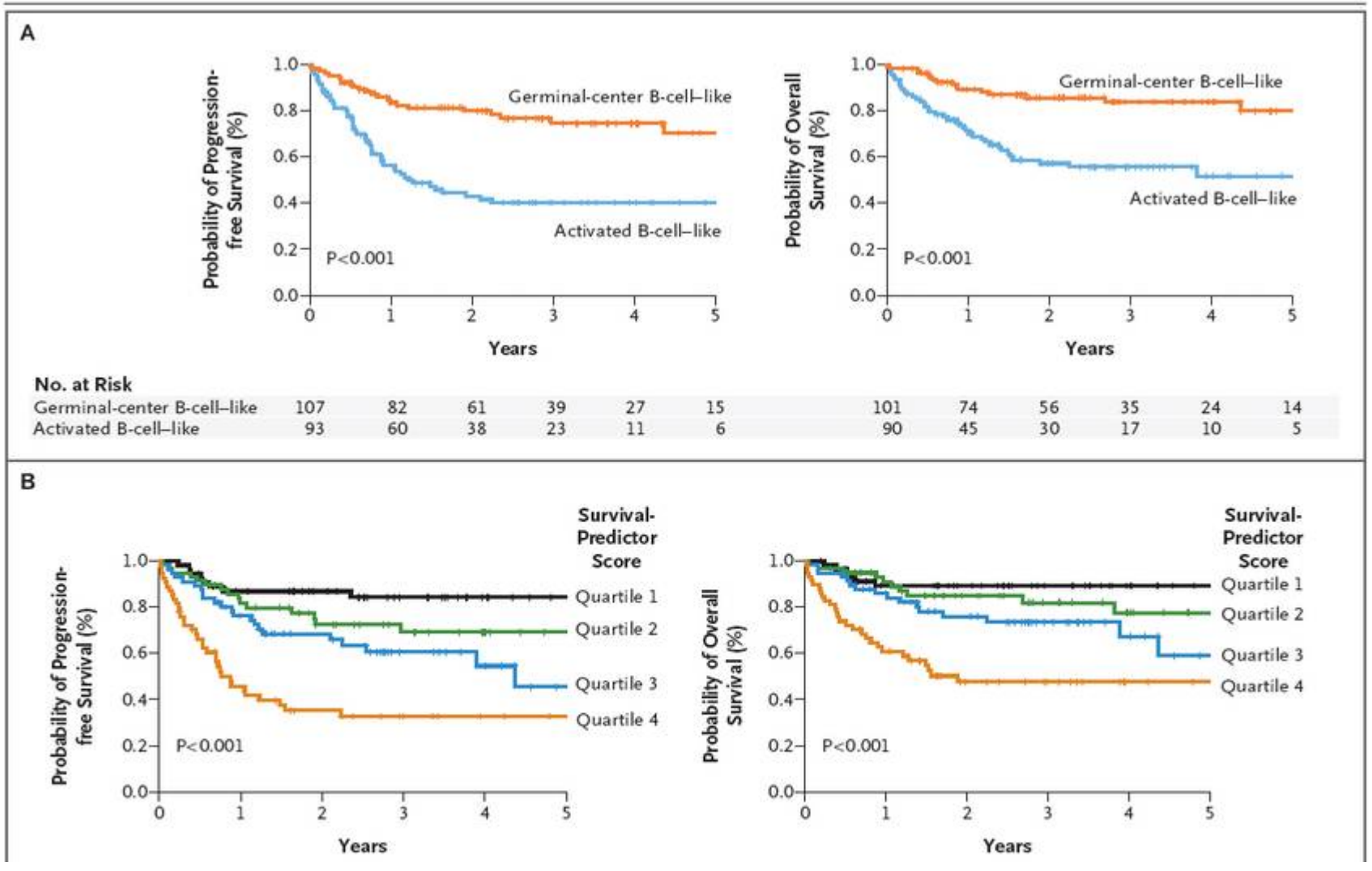
Dave S et al., N Engl J Med. 2006 Jun 8;354(23):2431-42.

Stromal Signatures in large-B-cell Lymphomas



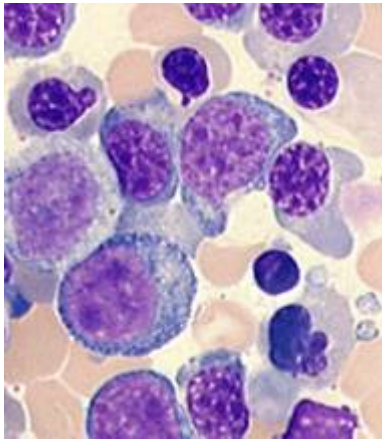
Lenz G et al., N Engl J Med. 2008 Nov 27;359(22):2313-23.

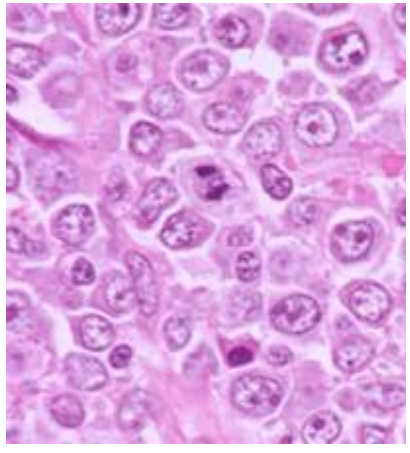
Survival in large-B-cell Lymphomas



Lenz G et al., N Engl J Med. 2008 Nov 27;359(22):2313-23.

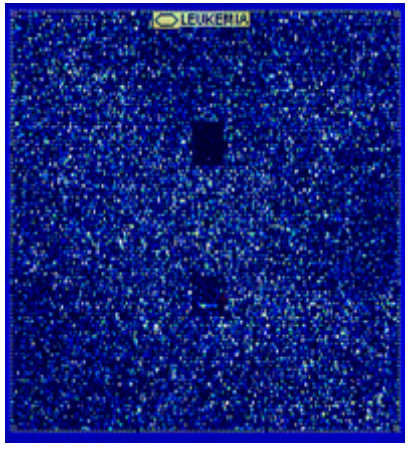
Next-Generation Molecular Tests ?

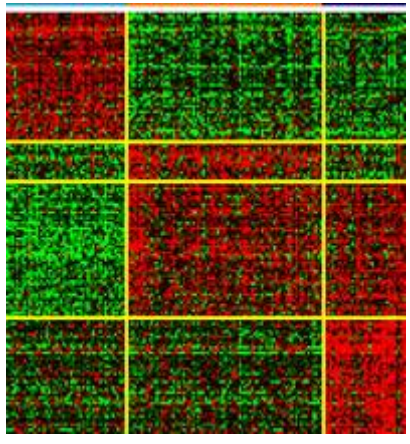




"Diagnosis is a system of more or less accurate guessing in which the end-point achieved is a name. These names applied to disease come to assume the importance of specific entities, whereas they are for the most part no more than insecure and therefore temporary conceptions."

T. Lewis
Reflections upon medical education
Lancet *1944 i:619-621*





Zusammenfassung

Microarrays erfassen die Expression von 38.000 Genen und messen mit sehr hoher Reproduzierbarkeit

Bei den Lymphomen finden sich sehr gute Beispiele für eine diagnostische und prognostische Anwendung

Auch neue Subgruppen lassen sich finden

Ansprechen auf Therapie kann prognostiziert werden