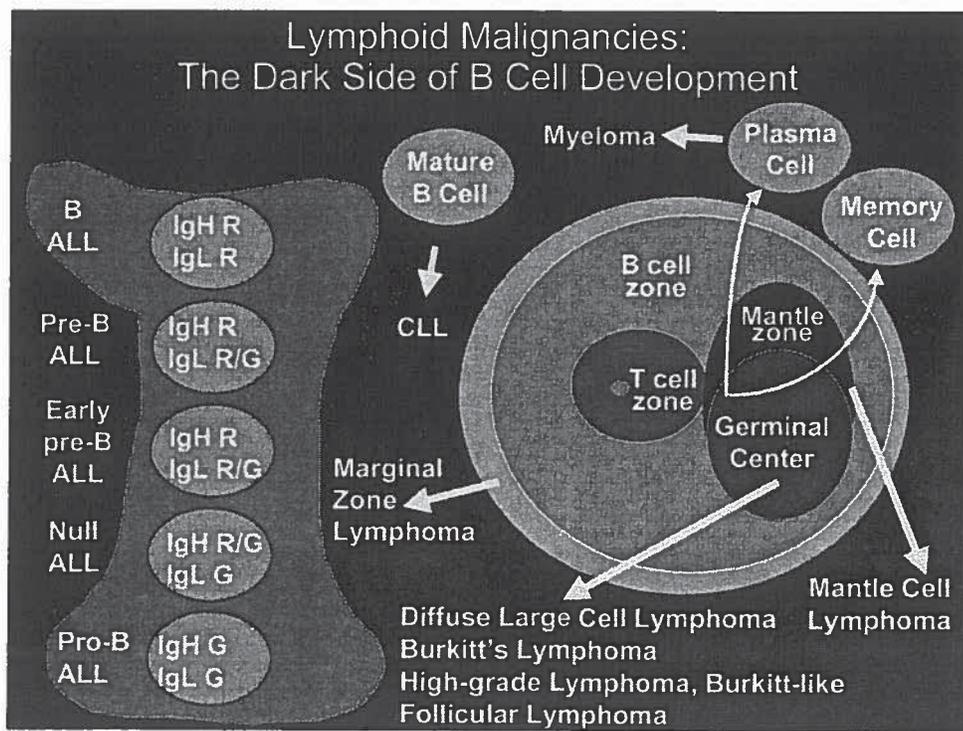


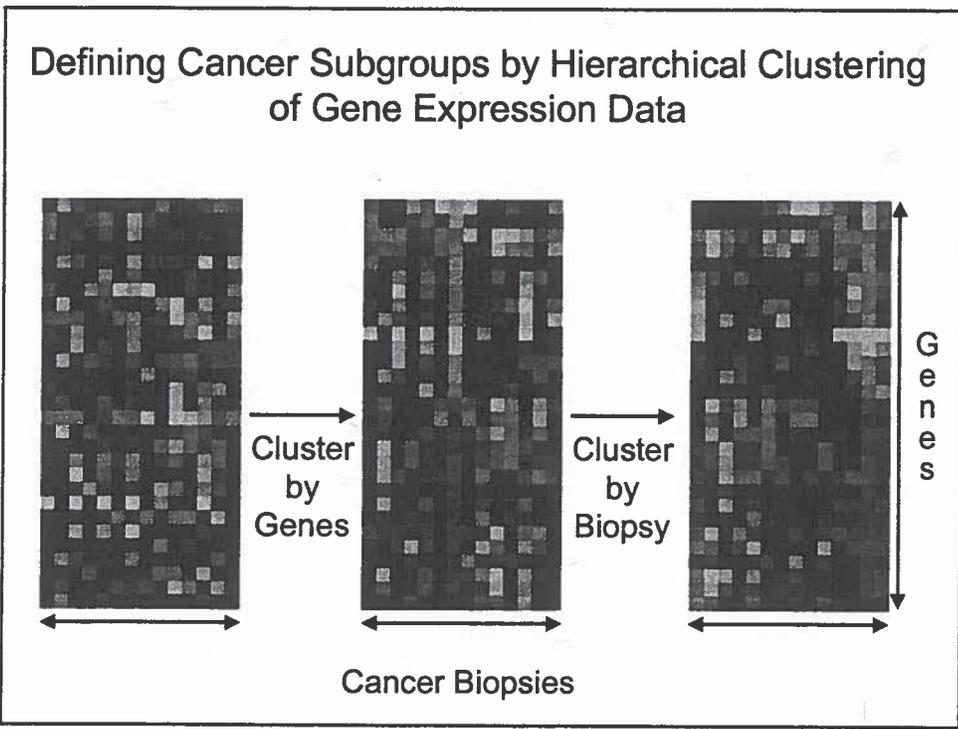
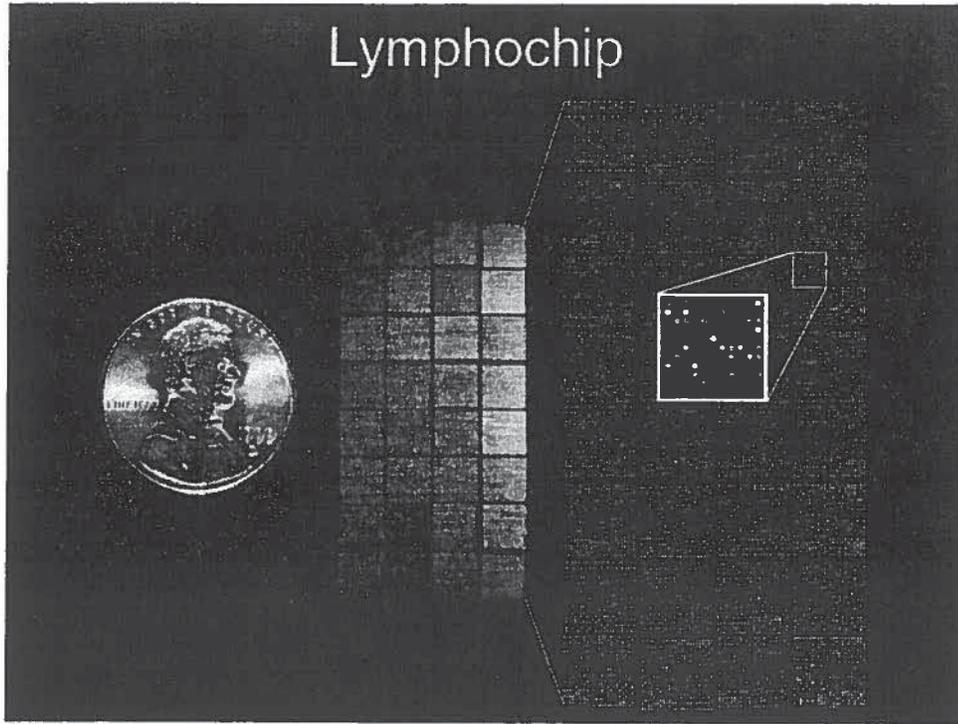
## Towards a Molecular Definition of Cancer

Goal: Define homogenous disease entities by gene expression profiling.

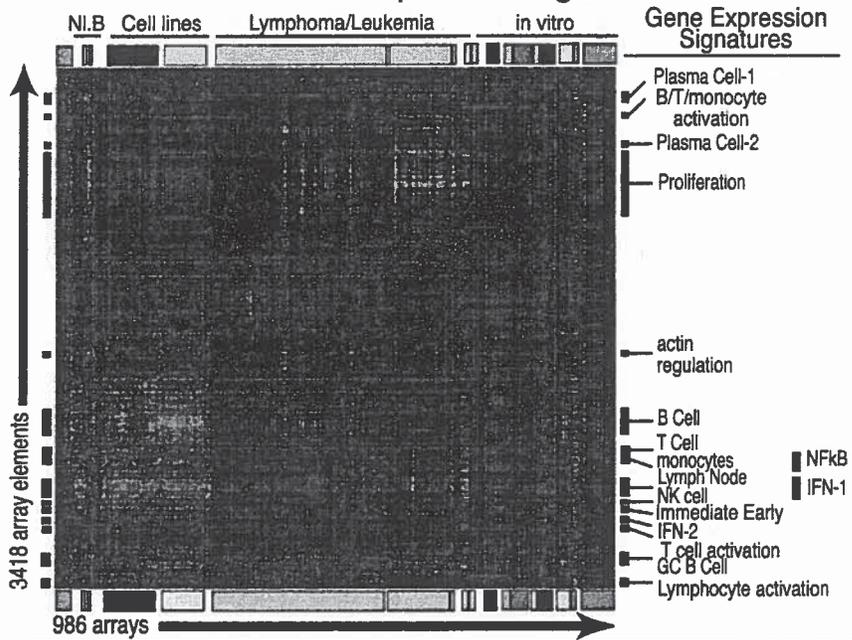
Criteria for a well-defined cancer subtype:

- Common cell of origin
- Common mechanism(s) of transformation
- Uniform clinical behavior
- Utility
  - Defines optimal therapeutic choice for patient
  - Identifies new molecular targets for therapy

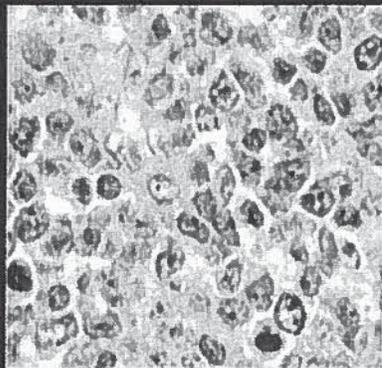




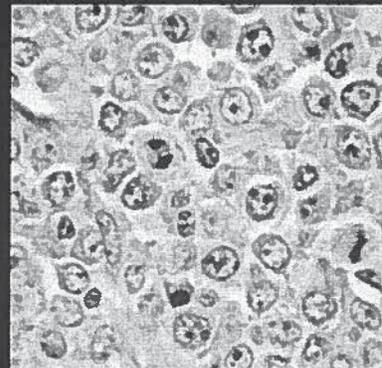
**A Large Compendium of Gene Expression Profiling Experiments  
Defines Gene Expression Signatures**



**Diffuse Large Cell Lymphoma:  
One Diagnosis, Many Diseases?**

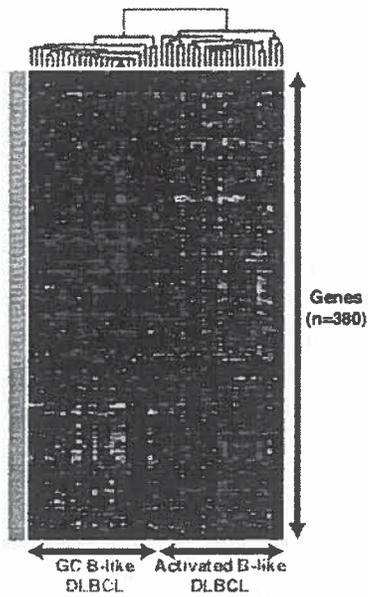


Patient 1

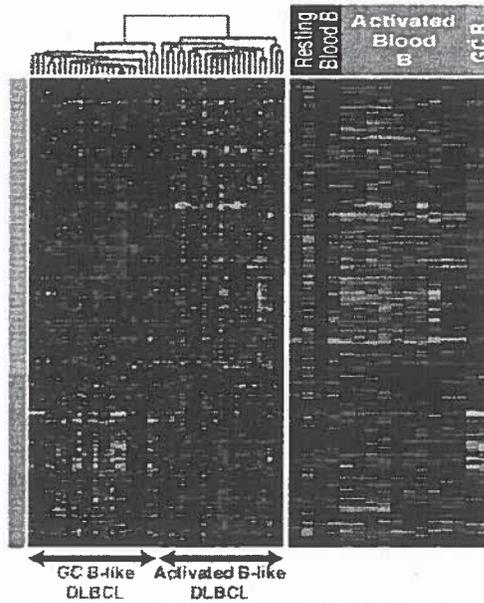


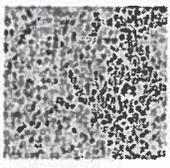
Patient 2

## Two Types of Diffuse Large B-cell Lymphoma Defined by Gene Expression Profiling

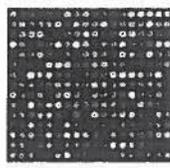


## Diffuse Large B-cell Lymphoma Subtypes Resemble Distinct Stages of B Cell Differentiation and Activation





### Lymphoma/Leukemia Molecular Profiling Project (LLMPP)

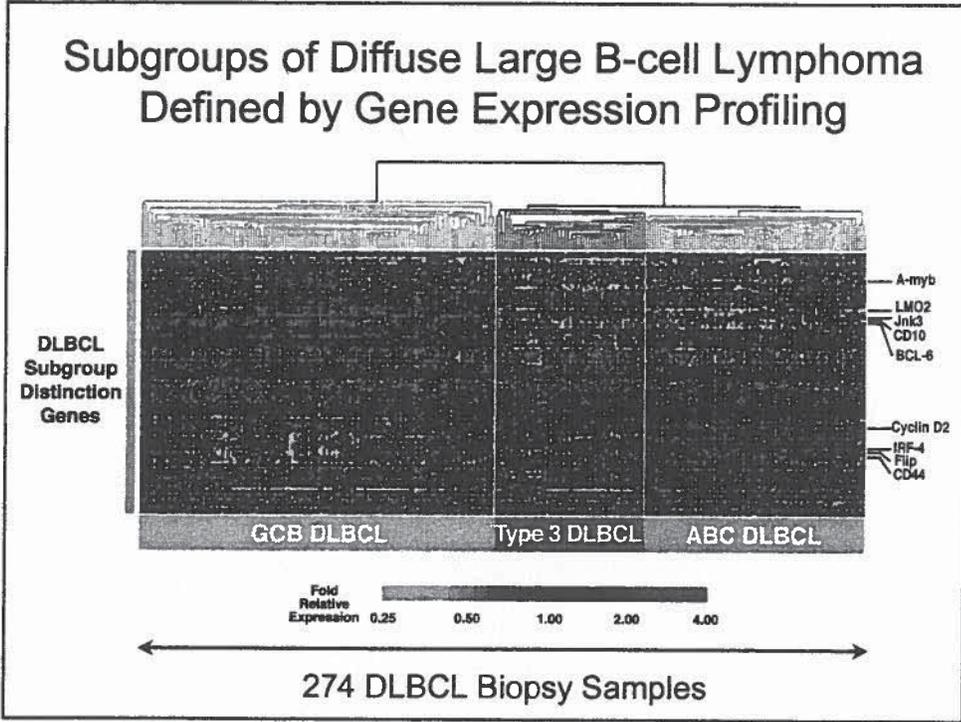


**Goals:**

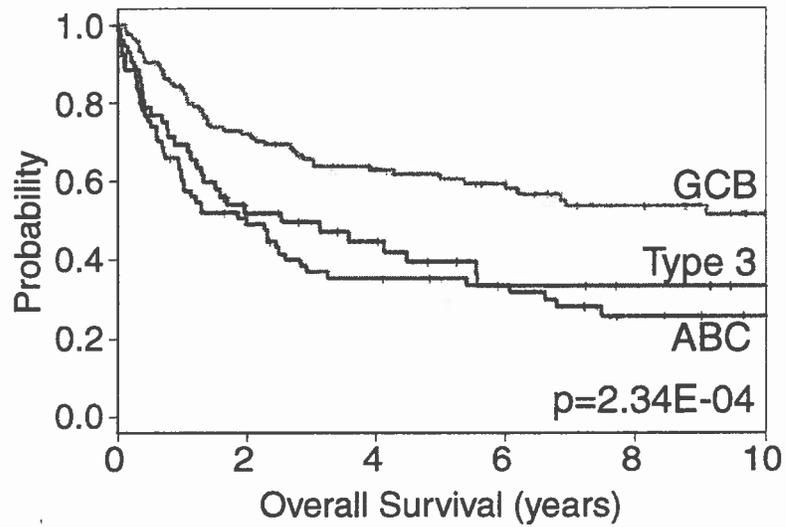
- Establish a molecular classification of human lymphoid malignancies.
- Define molecular correlates of clinical parameters that are useful in prognosis and in the choice of optimal therapy.

Collaborating Institutions

Univ. of Nebraska Med. Center	Univ. of Würzburg
British Columbia Cancer Center	Univ. of Barcelona
Southwest Oncology Group	Norwegian Radium Hospital
National Cancer Institute	Center for Cancer Research



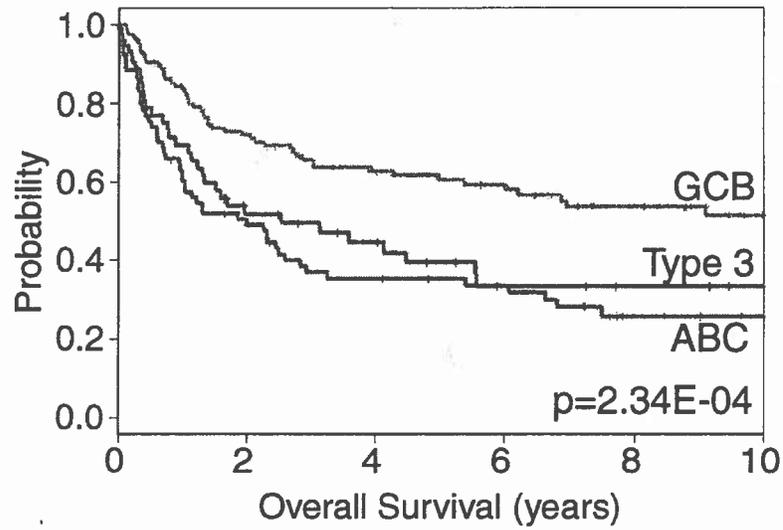
## DLBCL Subgroups Have Distinct Clinical Outcomes



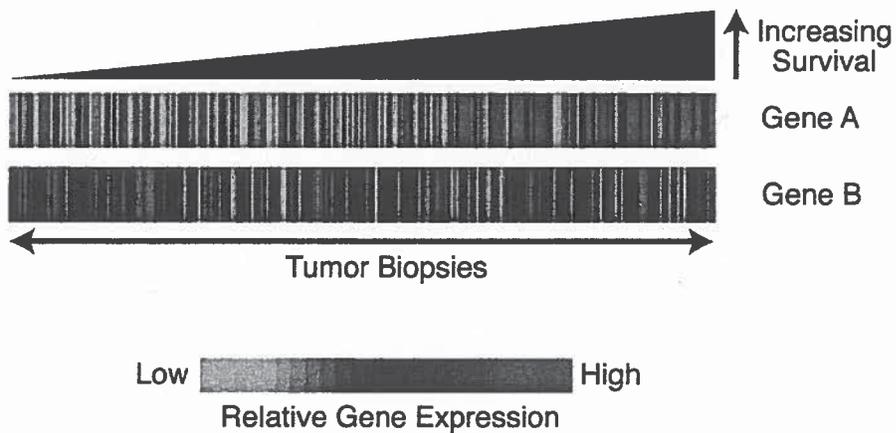
## Diffuse Large B-cell Cell Lymphoma: At least two diseases

	Germinal center B cell-like (GCB)	Activated B cell-like (ABC)
Cell of Origin	Germinal center B cell	Post-Germinal Center B cell
Oncogenic Mechanisms	<ul style="list-style-type: none"> <li>•t(14;18) translocation of BCL-2</li> <li>•Chr. 2p amplification of c-rel locus</li> </ul>	Constitutive activation of NF-kB
Clinical Outcome	Favorable 60% 5-yr survival	Poor 35% 5-yr survival

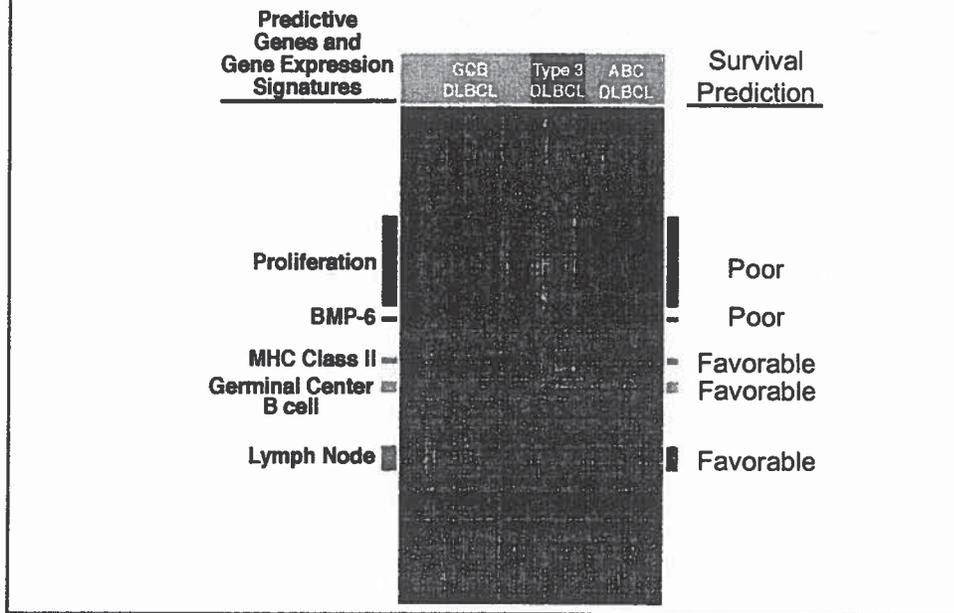
### Remaining Clinical Heterogeneity Within Subgroups of Diffuse Large B-cell Lymphoma



### Supervised Discovery of Genes that Influence Clinical Outcome in Cancer



## Genes and Gene Expression Signatures Predicting Survival Following Chemotherapy for Diffuse Large B-cell Lymphoma



## Calculating the Outcome Predictor Score

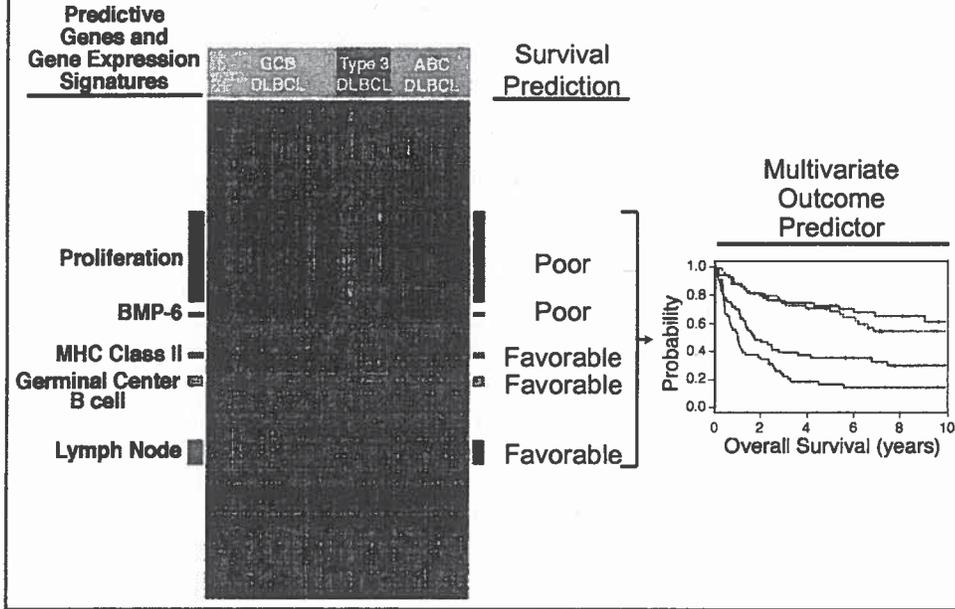
Outcome Predictor Score =

$$\begin{aligned}
 & \text{Proliferation signature average} \\
 & + \text{BMP-6} \\
 & - \text{GC B cell signature average} \\
 & - \text{MHC class II average} \\
 & - \text{lymph node signature average}
 \end{aligned}$$

High score = Poor outcome

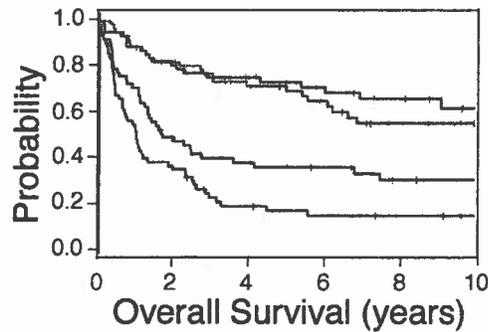
Low score = Favorable outcome

## Building a Gene Expression-Based Outcome Predictor For Diffuse Large B-cell Lymphoma



## A Gene Expression-Based Outcome Predictor for Diffuse Large B-cell Lymphoma

### 5-year Survival

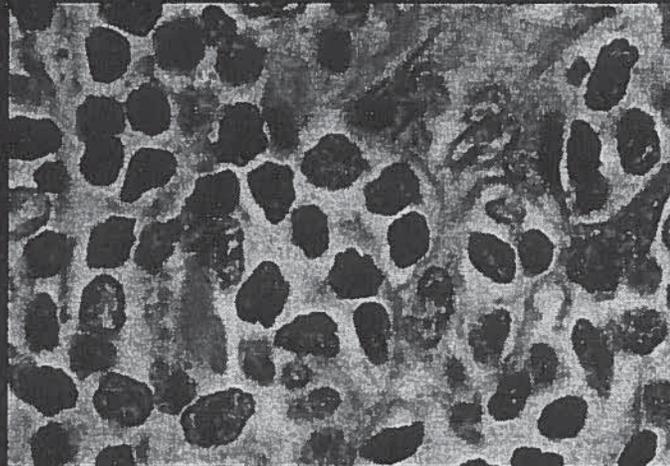


Quartile 1	73%
Quartile 2	71%
Quartile 3	36%
Quartile 4	15%

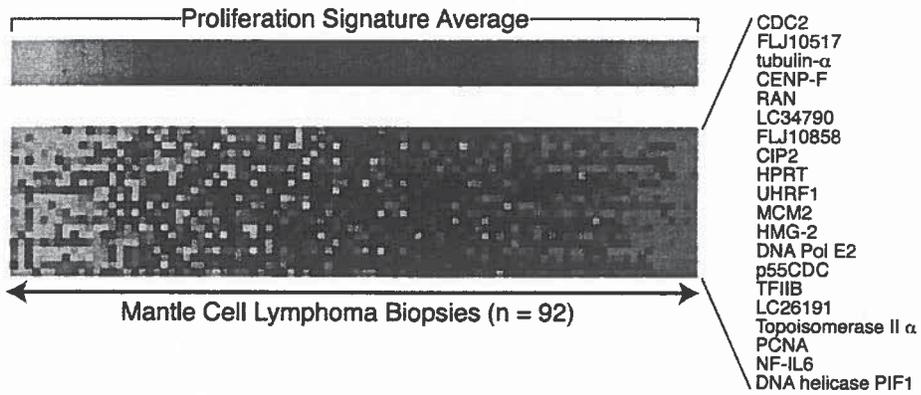
## Gene Expression-Based Prediction of Survival Following Chemotherapy for Diffuse Large B-cell Lymphoma

1. The diagnosis of diffuse large B-cell lymphoma encompasses at least 2 diseases that differ in cell of origin, molecular pathogenesis and survival.
2. Survival following chemotherapy is influenced by a discrete set of biological features of the tumors that are reflected in gene expression signatures.
  - Cell of origin (germinal center B cell vs. other)
  - Proliferation rate
  - Immune response to tumor
3. A clinical test using the 17 outcome predictor genes could identify biologically high risk patients who might benefit from alternative therapeutic approaches.

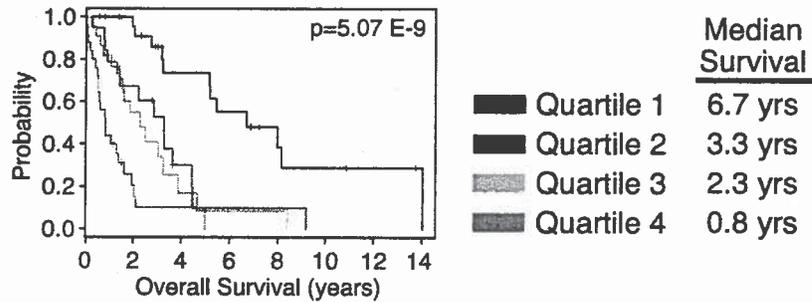
## Mantle Cell Lymphoma



## Variable Expression of Proliferation Signature Genes in Mantle Cell Lymphoma



## Quantitative Measurement of Proliferation Predicts Length of Survival Following Diagnosis of Mantle Cell Lymphoma



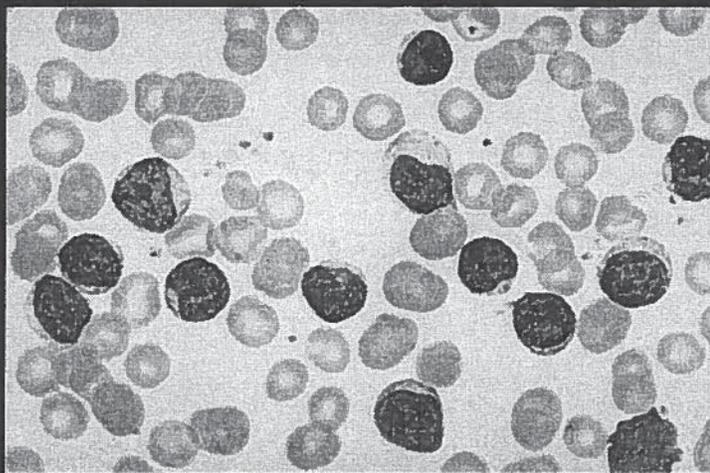
## Survival Prediction in Mantle Cell Lymphoma

1. Mantle cell lymphoma is a cancer caused by dysregulation of the cell cycle.
2. The survival of patients following diagnosis can range from less than 1 year to greater than 10 years.
3. A quantitative measure of tumor cell proliferation can accurately predict the survival of these patients.

High tumor proliferation (median survival 0.8 yr)  
=> Clinical trials of bone marrow transplant or high dose chemotherapy.

Low tumor proliferation (median survival 6.7 yr)  
=> Conservative management.

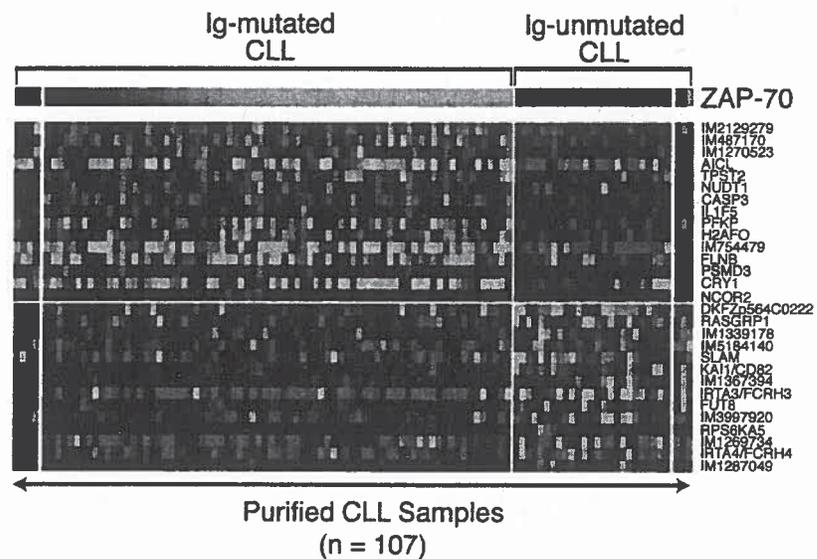
## Chronic Lymphocytic Leukemia



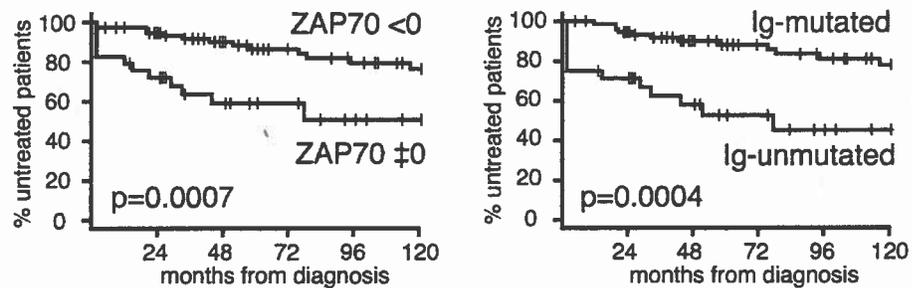
## Two Clinically Distinct Subtypes of Chronic Lymphocytic Leukemia

	<u>Immunoglobulin Mutational Status</u>	<u>Clinical Course</u>
CLL Subtype 1	Unmutated	Progressive Early Rx
CLL Subtype 2	Mutated	Stable Late or no Rx

### ZAP-70 is the Most Differentially Expressed Gene Between the Ig-mutated and Ig-unmutated CLL Subtypes



## ZAP70 Expression is Equivalent to Ig Mutational Status in Predicting Time to Disease Progression in CLL



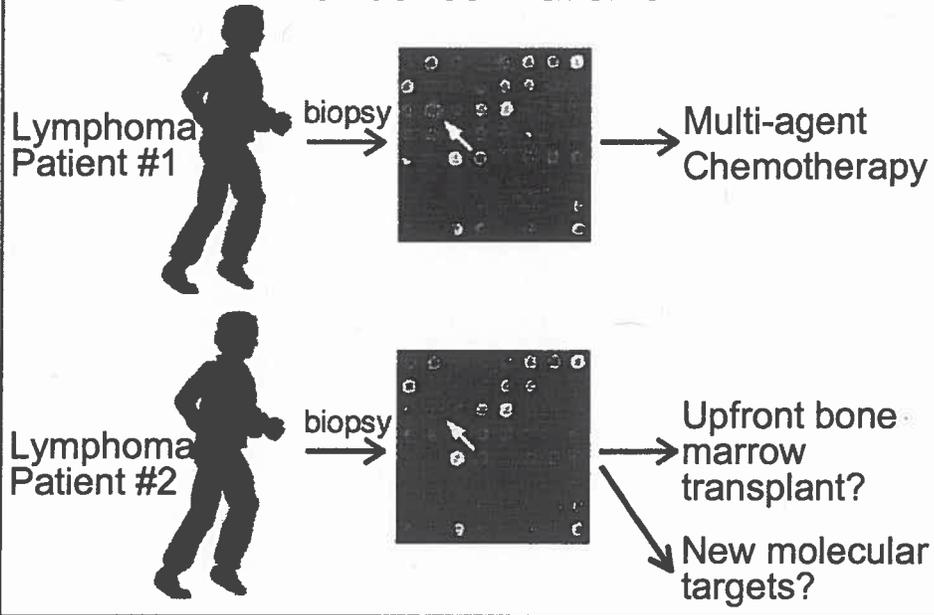
## Molecular Diagnosis of Chronic Lymphocytic Leukemia

1. Two subtypes of CLL can be identified that:
  - a. differentially express over 150 genes
  - b. differ in immunoglobulin mutational status
  - c. are markedly different clinically
2. A clinically useful diagnostic test could be based on ZAP-70 expression.

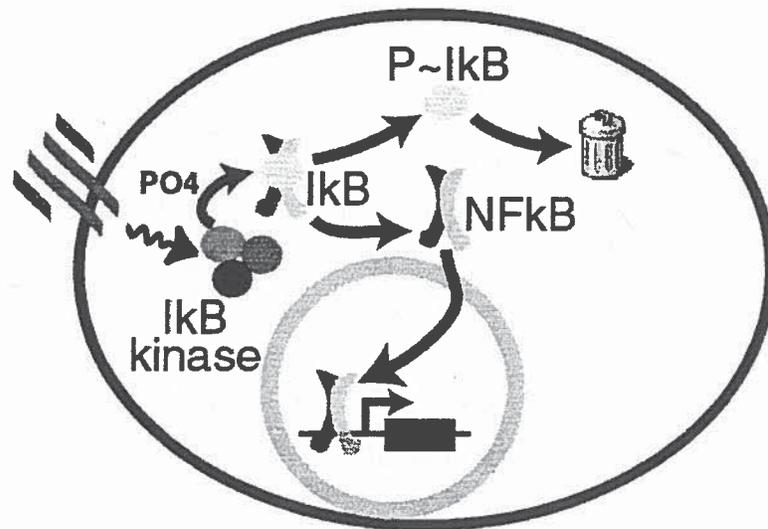
Low ZAP-70  $\Rightarrow$  Watch and wait management.

High ZAP-70  $\Rightarrow$  Early and intensive treatment?

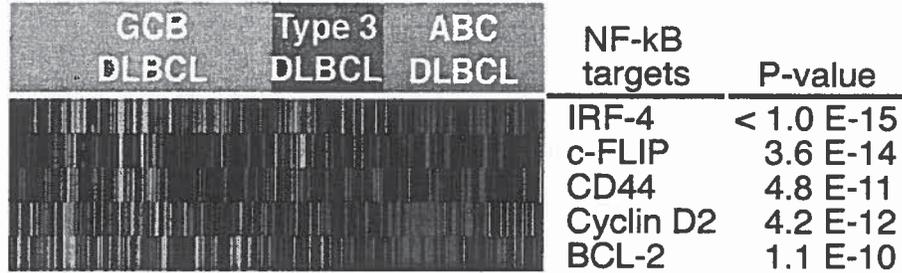
## Microarray-based Treatment Decisions for Cancer Patients



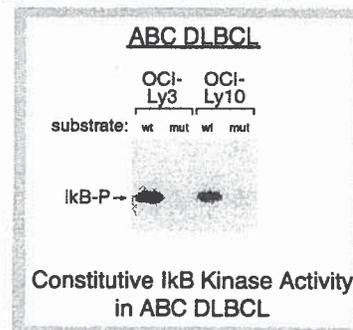
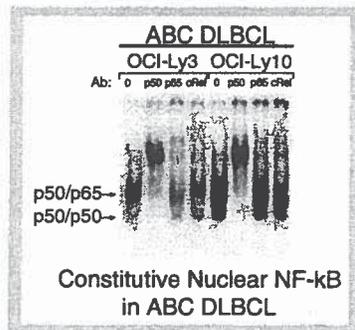
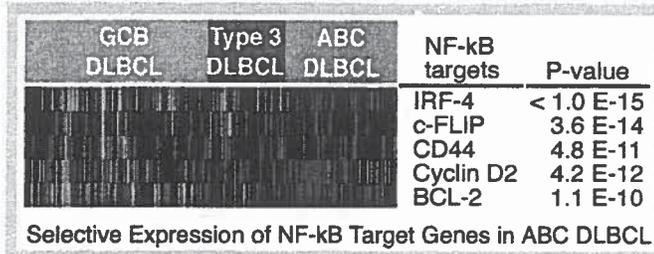
## Activation of the NF- $\kappa$ B Signaling Pathway



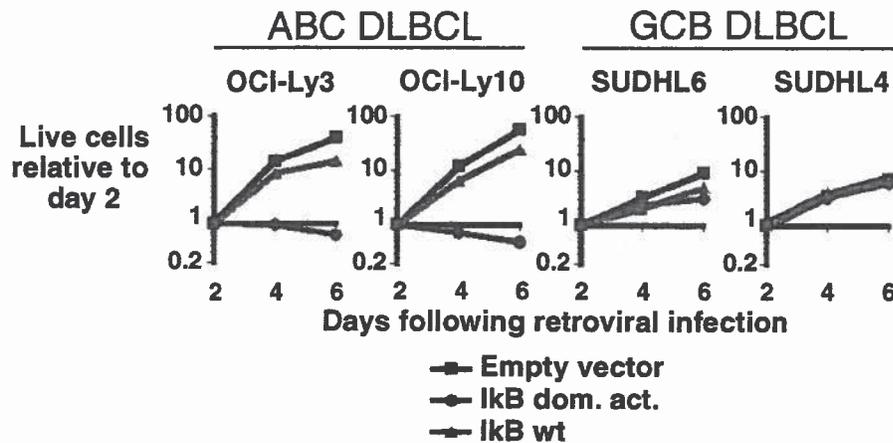
## Preferential Expression of NF- $\kappa$ B Target Genes in Activated B Cell-like DLBCL



## Constitutive Activity of the NF- $\kappa$ B Pathway in Activated B cell-like Diffuse Large B-cell Lymphoma

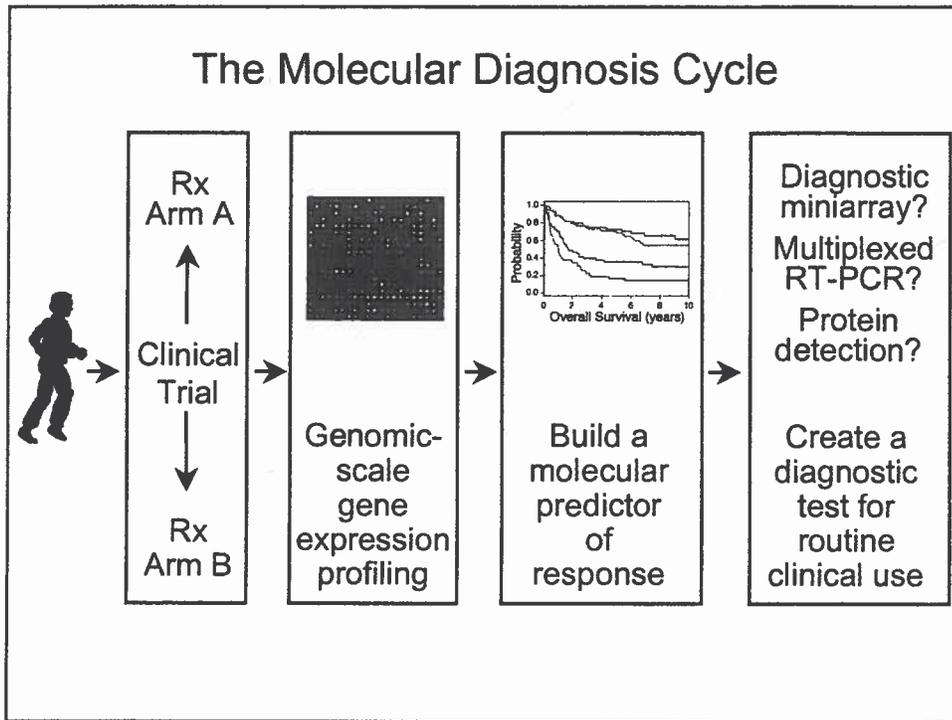


## Inhibition of the NF- $\kappa$ B Pathway Kills Activated B cell-like Diffuse Large B Cell Lymphoma Cells



## Molecular Targets in Cancer from Gene Expression Profiling

1. The NF- $\kappa$ B pathway is constitutively active in activated B-like DLBCL due to I $\kappa$ B kinase activation.
2. Dominant inhibition of the NF- $\kappa$ B pathway kills activated B-like DLBCL cells.
3. The NF- $\kappa$ B pathway is a new therapeutic target for the most clinically intractable subgroup of DLBCL.
4. These results provide a rational basis for a clinical trial of an inhibitor of the NF- $\kappa$ B pathway, Velcade/PS-341, in DLBCL patients.



### Genomic-scale Gene Expression Profiling in Cancer Clinical Trials

1. Clinical trials either test a new treatment regimen or an old regimen in a new setting.
  - => Previously defined molecular predictors of survival may not apply.
  - => A broad molecular screen for correlates of survival will allow a new outcome predictor to be generated for each arm of the trial.
2. Molecular profiling may identify patient subsets that respond better to one treatment arm than the other.
  - => The first step towards patient-specific therapy.
  - => May prevent a promising new drug from being discarded.
3. Molecular profiling will allow clinical trials to be compared with respect to patient enrollment.
  - => May reconcile conflicting results from similar trials.
  - => Gives a scientific basis for clinical trial design and analysis.

## Impediments to Molecular Profiling in Clinical Trials

1. Most cancer patients receive their first consultation and diagnostic biopsy in a community setting.  
=> Molecular profiling in a clinical trial would require a second biopsy which may be infeasible.  
And who pays?
2. Biopsy specimens must retain integrity of biomolecules (RNA, DNA, protein)  
=> Frozen biopsy material ideal but how to store?  
=> New methods may allow the preservation of biomolecules at room temperature.
3. Physicians and patients do not currently appreciate the value of molecular profiling of cancer.  
=> Patient and physician education is paramount.  
NCI-designated cancer centers could lead the way.

## LLMPP DLBCL Study-Consortium Representatives

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