

# Routine Molecular Diagnosis of Cancer in Clinical Oncology



“I’m afraid you’ve got cows, Mr. Farnsworth.”

# Why We Need Gene Expression Profiling for All Cancer Patients

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2. To clarify diagnostic distinctions that are problematic using current methods.
3. To deliver newly defined molecular diagnoses that influence treatment choice and/or prognosis.

# Why We Need Gene Expression Profiling for All Cancer Patients

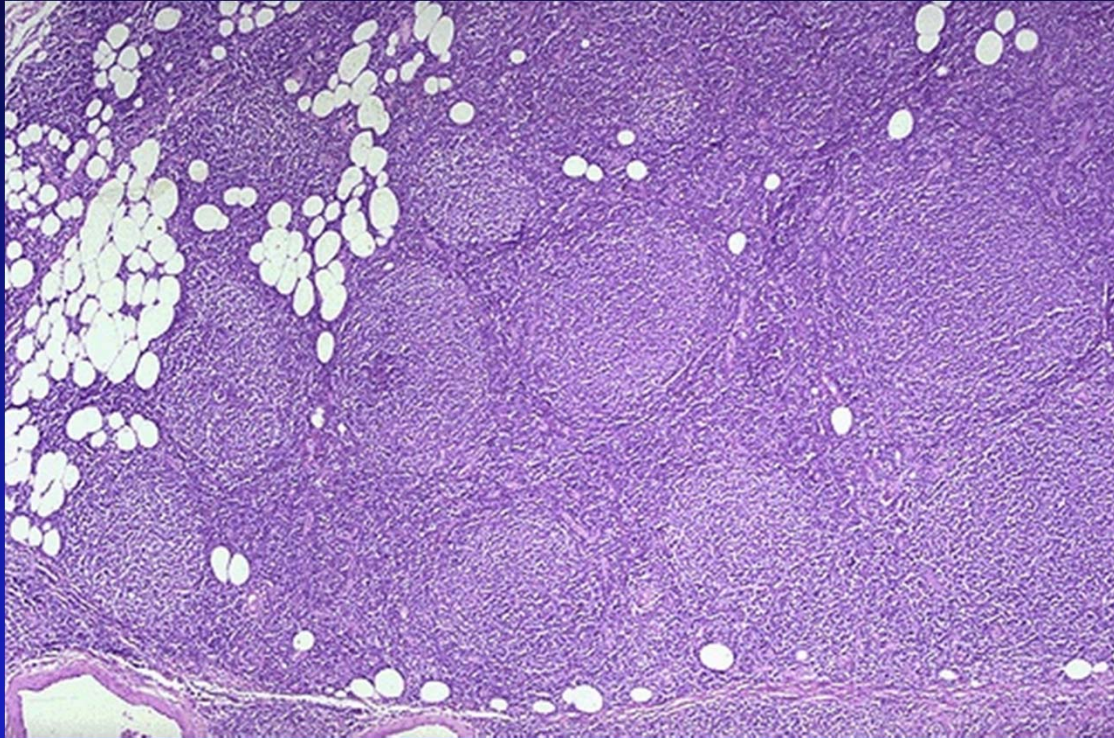
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2. To clarify diagnostic distinctions that are problematic using current methods.
3. To deliver newly defined molecular diagnoses that influence treatment choice and/or prognosis.
4. To translate insights from therapeutic trials that incorporate molecular profiling.

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3. To deliver newly defined molecular diagnoses that influence treatment choice and/or prognosis.
4. To translate insights from therapeutic trials that incorporate molecular profiling.
5. To promote excellence in clinical science.

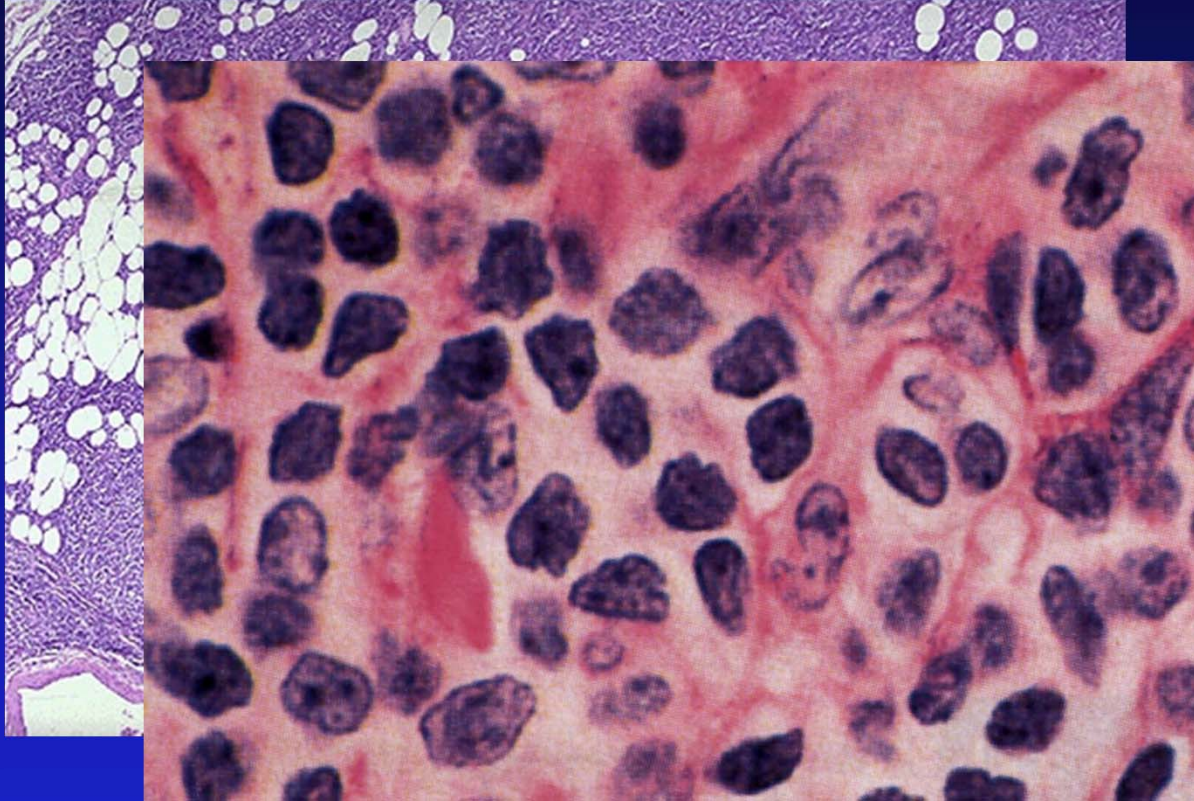
# The Diversity of Human Lymphomas

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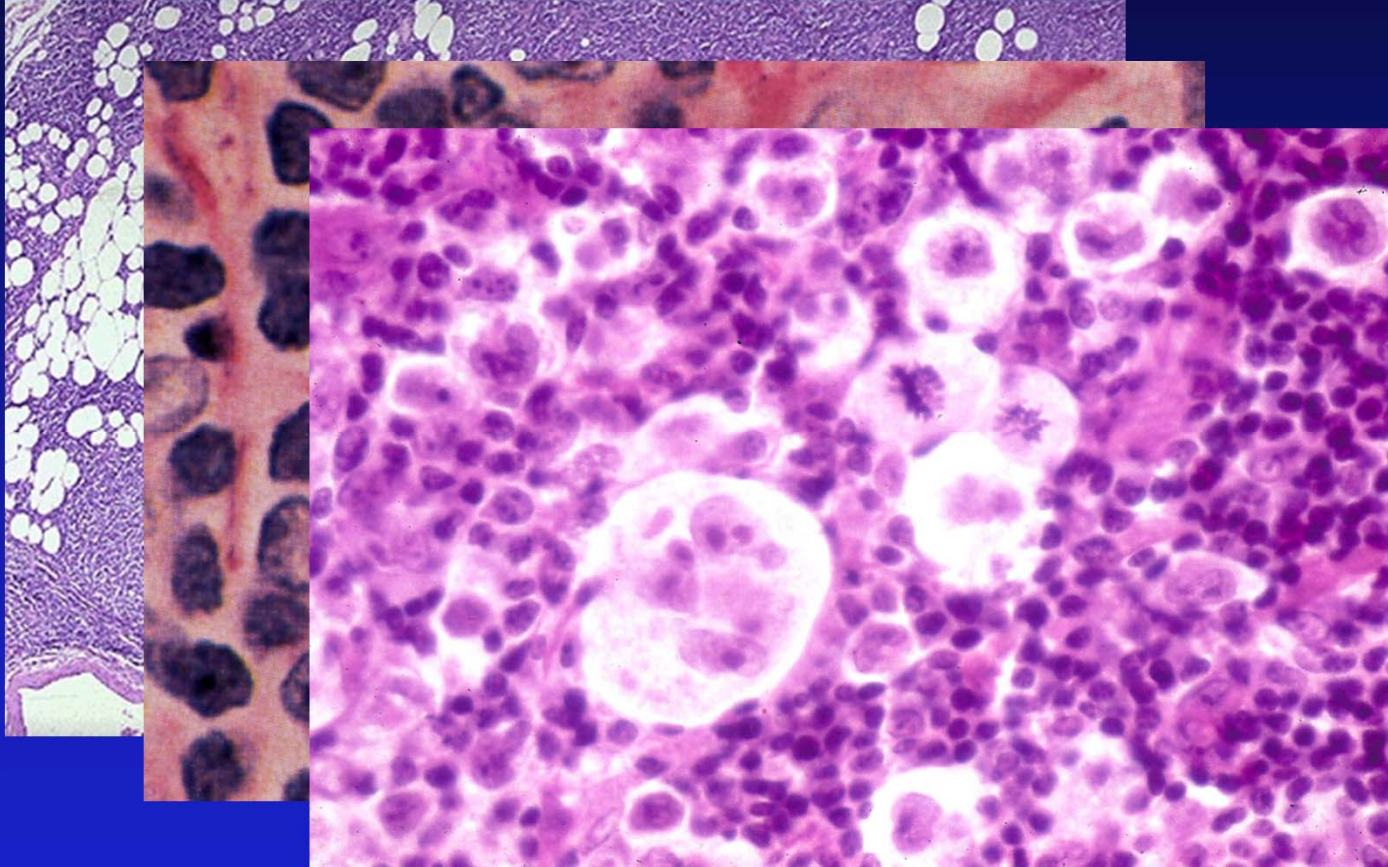




# The Diversity of Human Lymphomas

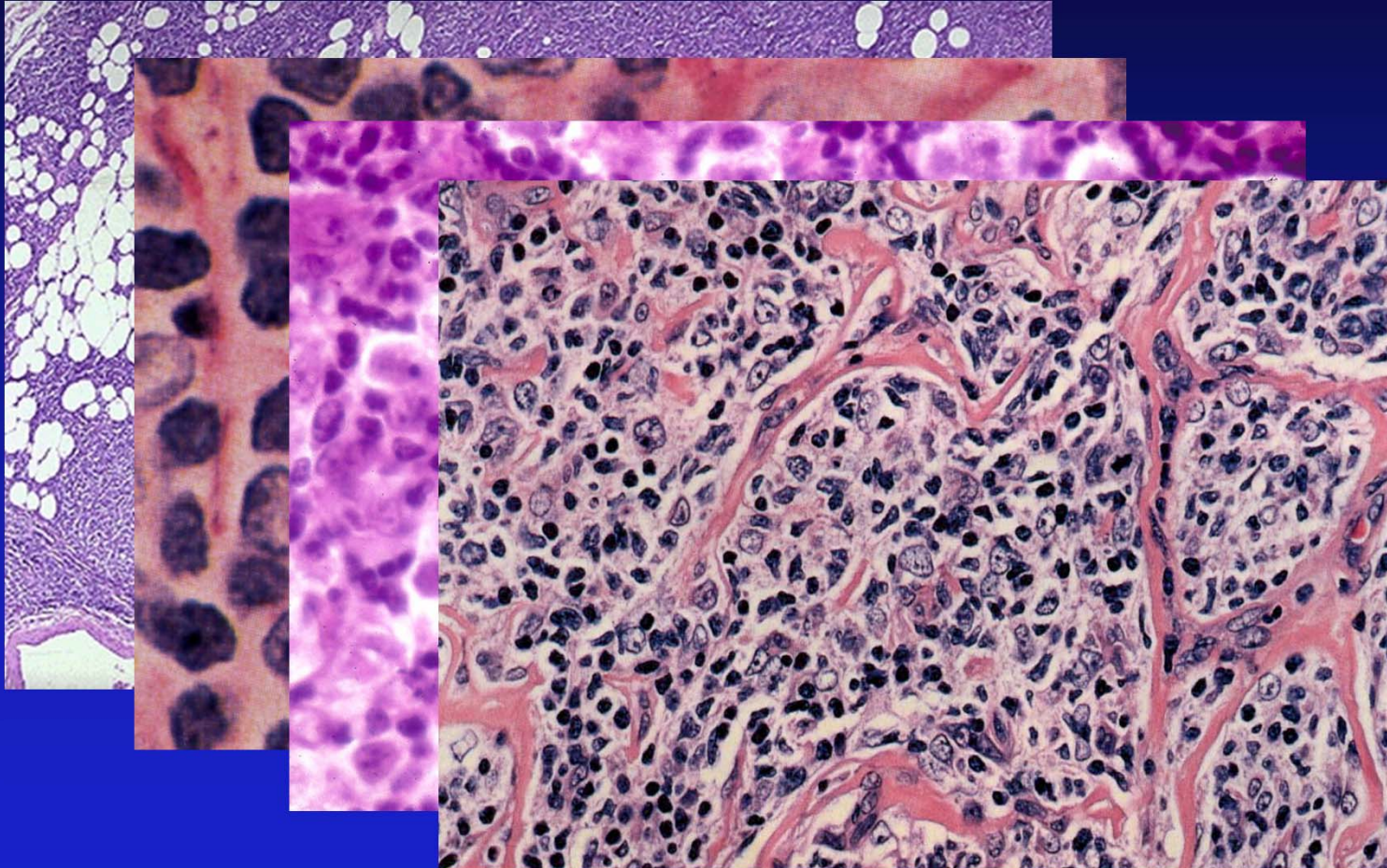


# The Diversity of Human Lymphomas



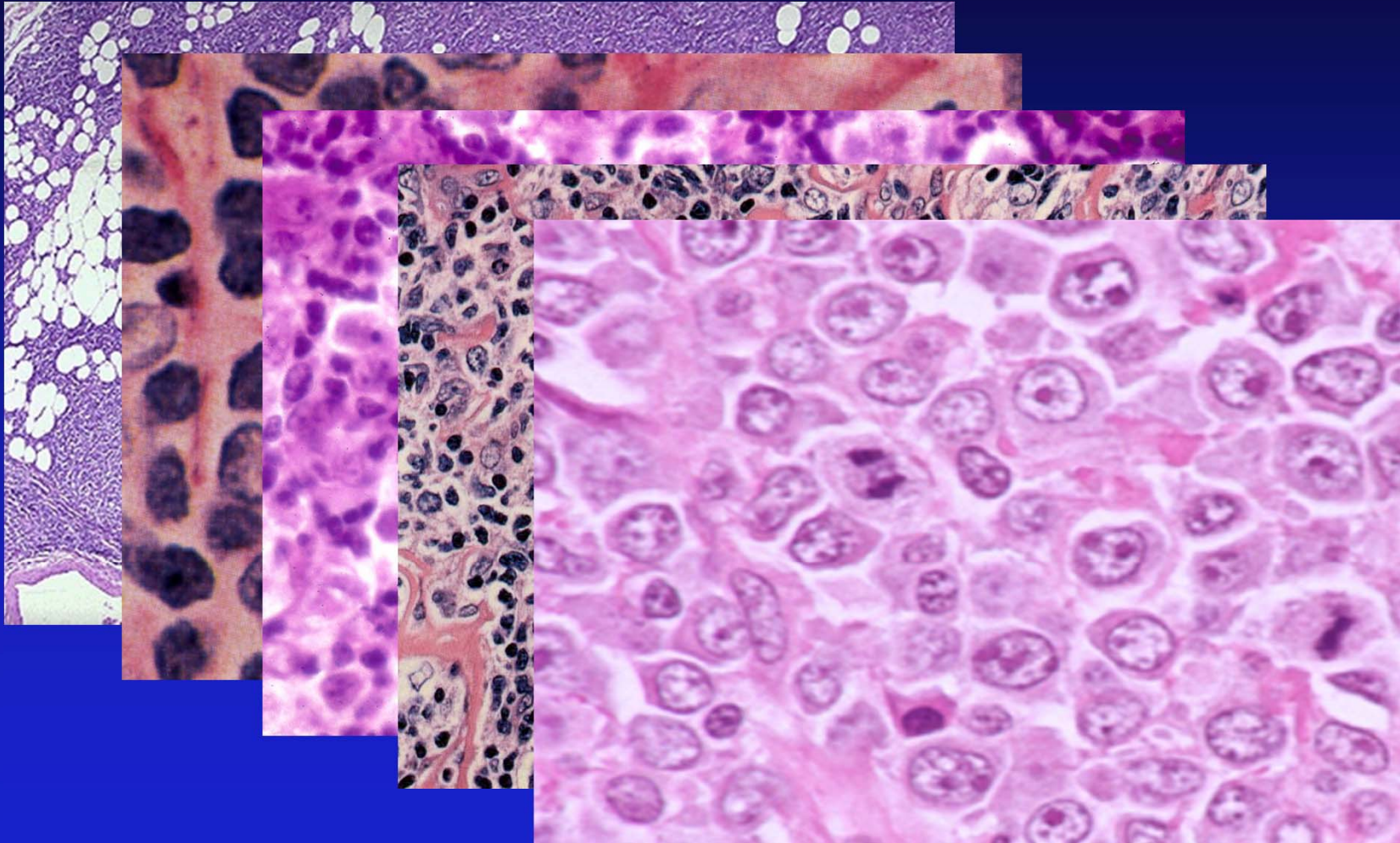


# The Diversity of Human Lymphomas



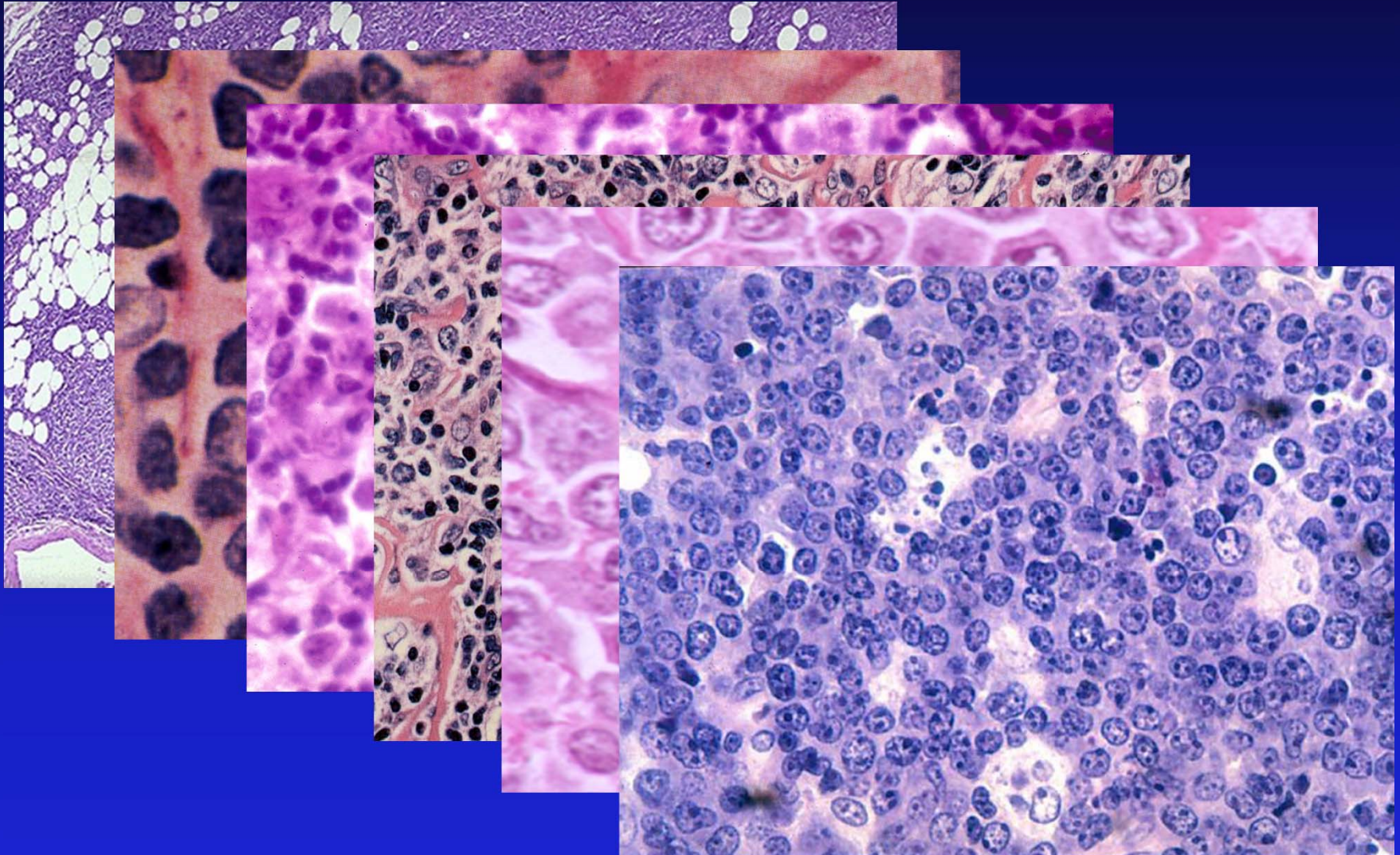


# The Diversity of Human Lymphomas





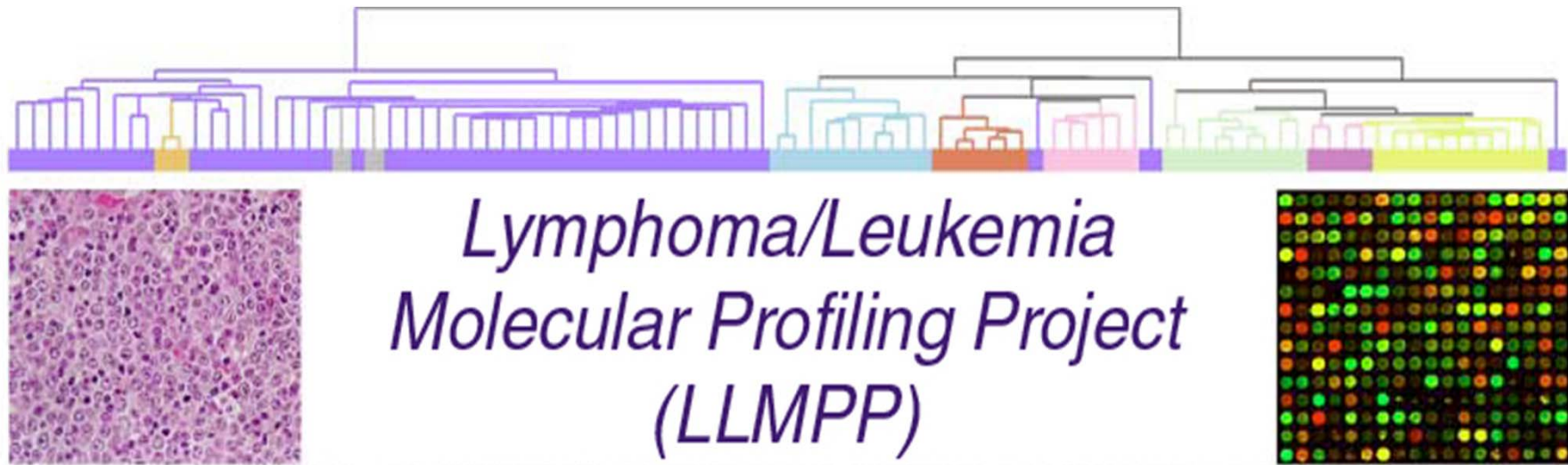
# The Diversity of Human Lymphomas



# Lymphoma Subtype Diagnosis Alters Treatment Choice

<u>Lymphoma subtype</u>	<u>Curable?</u>	<u>Therapy</u>
Diffuse Large B Cell Lymphoma	Yes	CHOP chemotherapy + Rituximab
Primary Mediastinal B Cell Lymphoma	Yes	CHOP chemotherapy + Rituximab +/-radiation
Burkitt Lymphoma	Yes	High dose chemotherapy CNS prophylaxis
Follicular Lymphoma	No	Watchful waiting Rituximab Symptomatic chemotherapy
Mantle Cell Lymphoma	No	Watchful waiting Symptomatic chemotherapy Responsive to: --Bortezomib --rapamycin analogues





- 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.

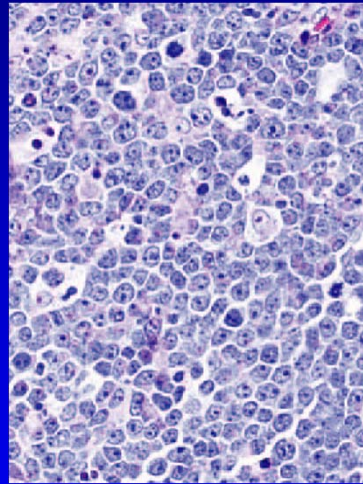
Collaborating Institutions

- |  |                     |
|--|---------------------|
| Univ. of Nebraska Medical Center                     | Univ. of Würzburg   |
| British Columbia Cancer Agency                       | Univ. of Barcelona  |
| Southwest Oncology Group                             | Univ. of Arizona    |
| Cleveland Clinic                                     | Univ. of Rochester  |
| Norwegian Radium Hospital                            | St. Bart's Hospital |
| National Cancer Institute Center for Cancer Research |                     |

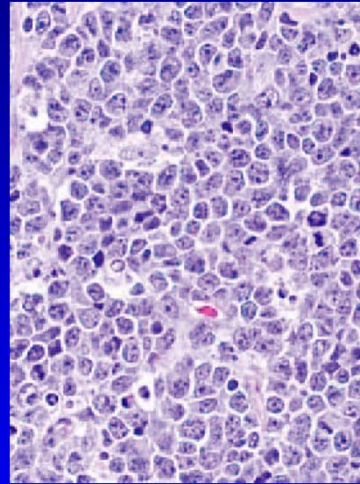
Improving the Accuracy and  
Reproducibility of Diagnosis  
Using Gene Expression Profiling

# Diagnosis of Burkitt Lymphoma vs. Diffuse Large B Cell Lymphoma Alters Treatment Choice

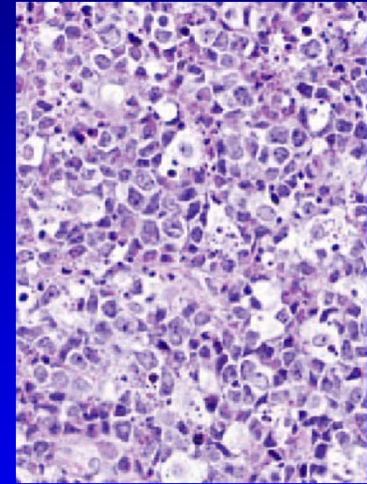
Classic  
Burkitt Lymphoma



Atypical  
Burkitt Lymphoma



Diffuse Large  
B Cell Lymphoma



Recommended  
Treatment

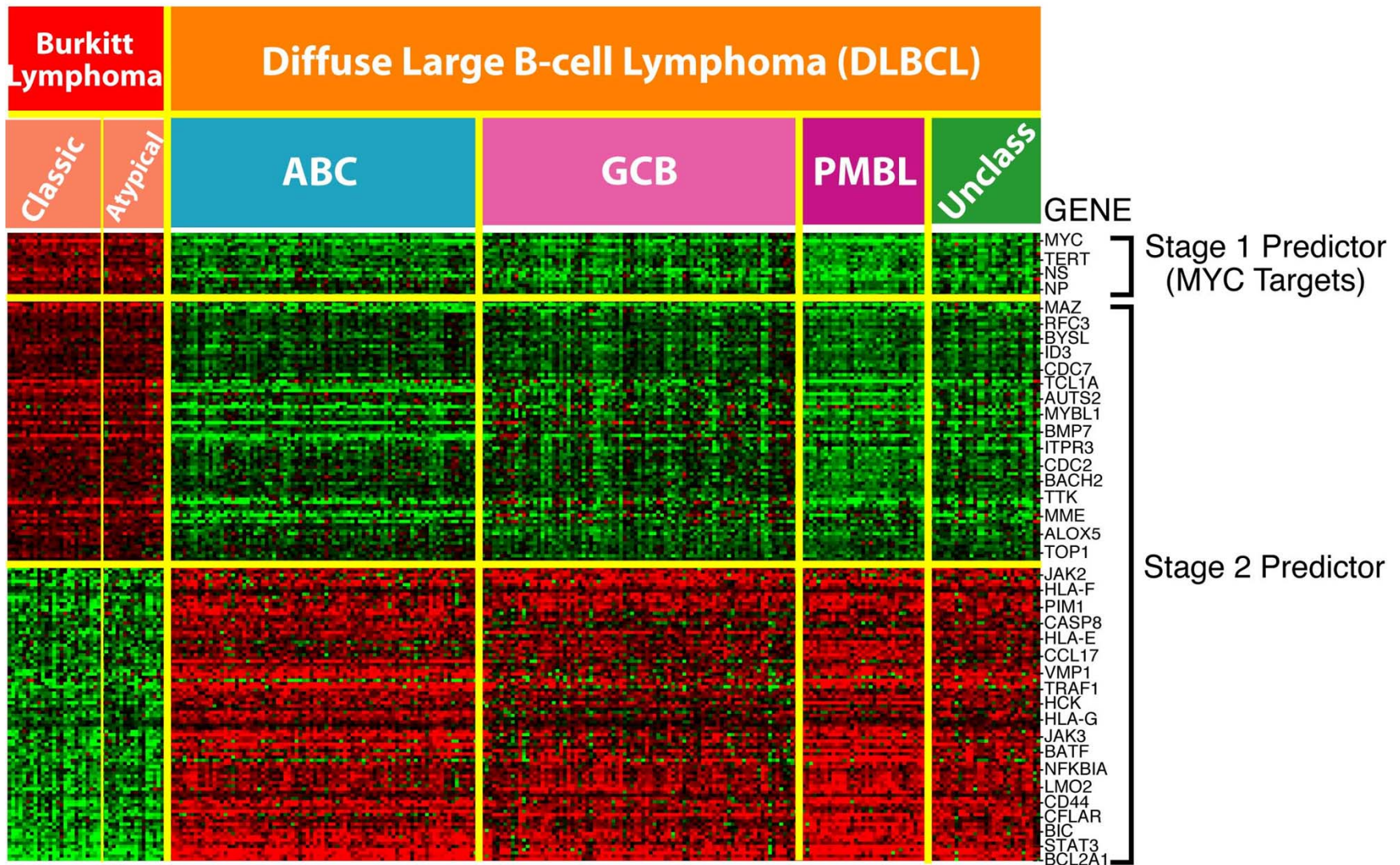
Intensive  
chemotherapy

Intensive  
chemotherapy

CHOP-like  
chemotherapy

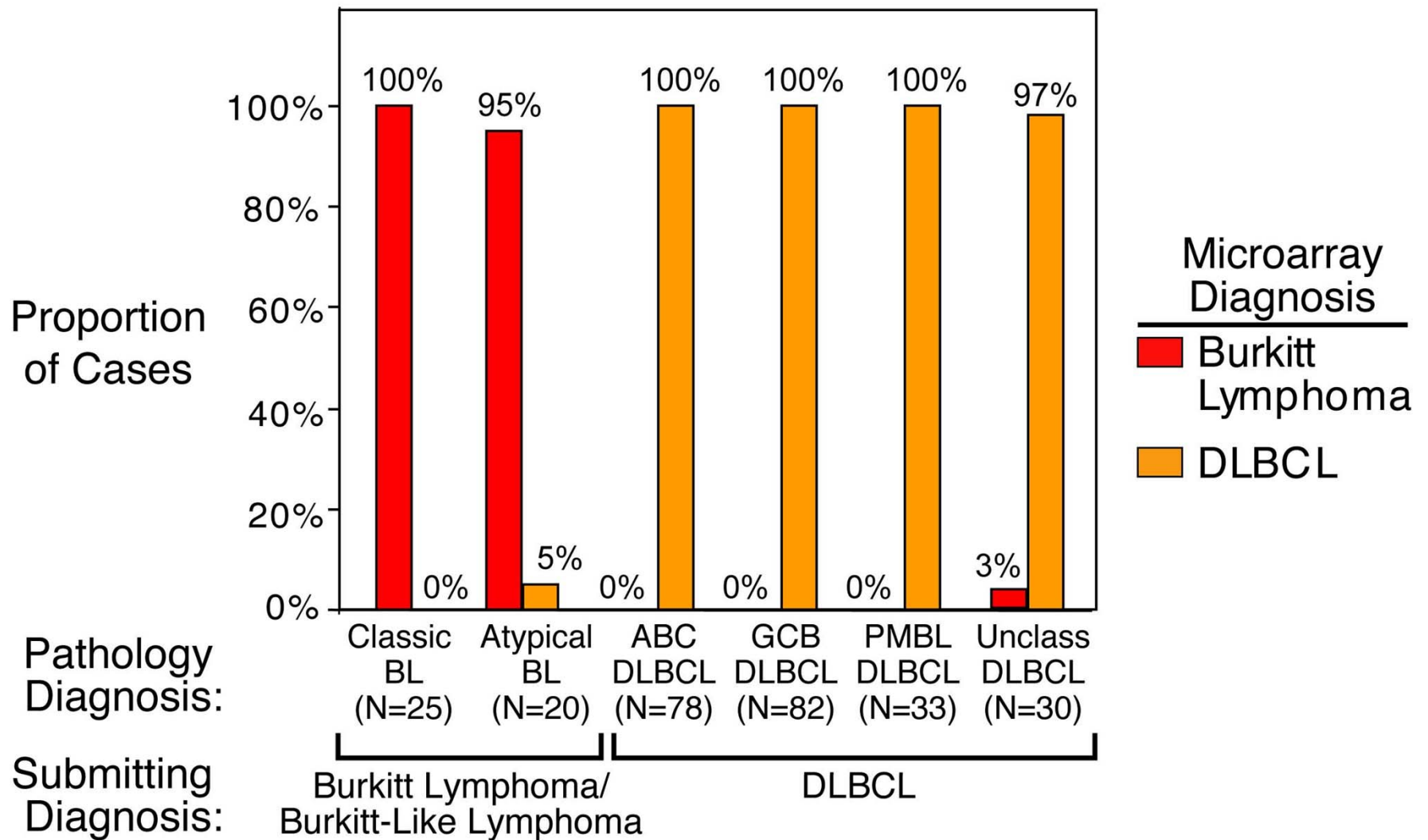


# Gene Expression Differentiates Burkitt Lymphoma from all Subgroups of Diffuse Large B Cell Lymphoma

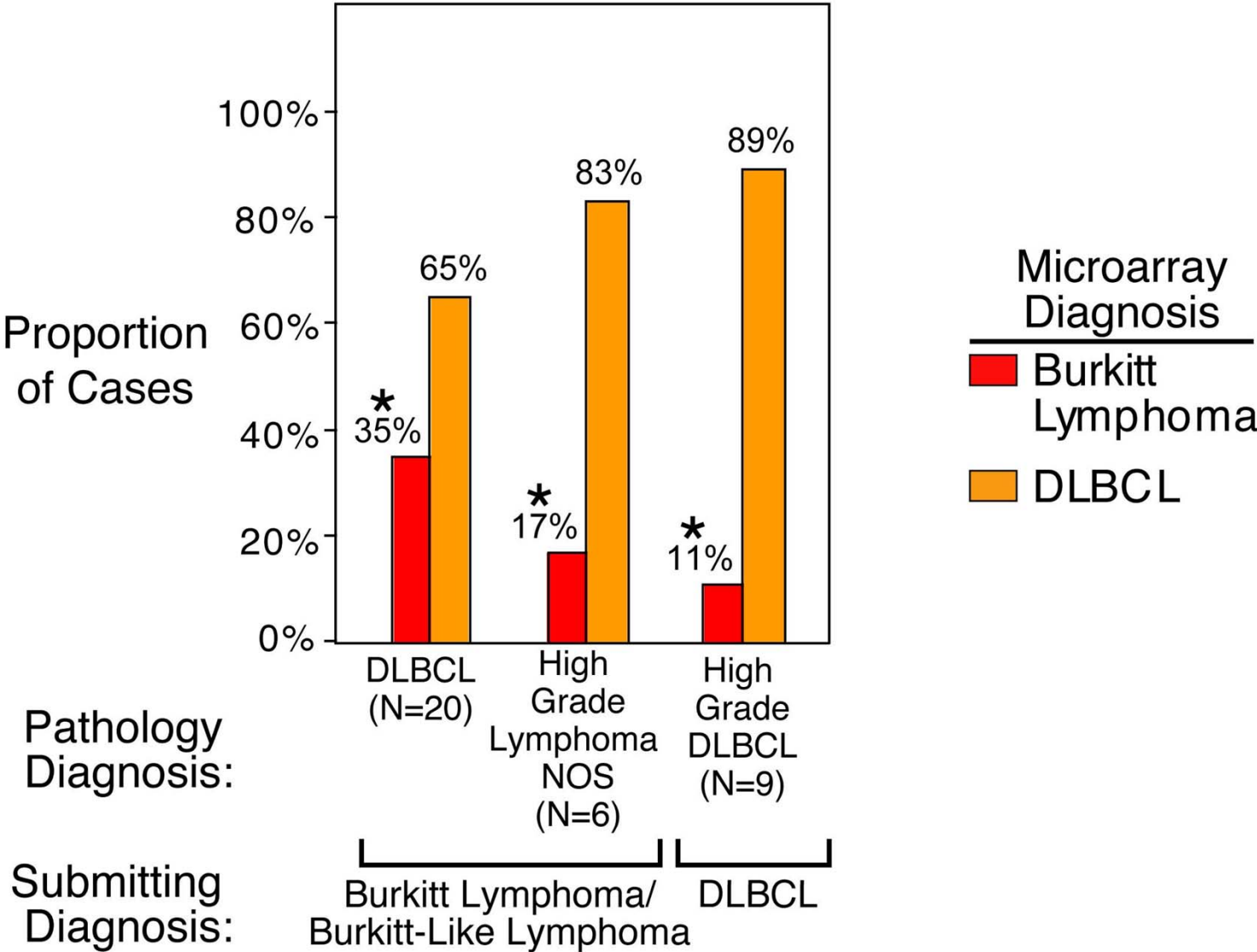




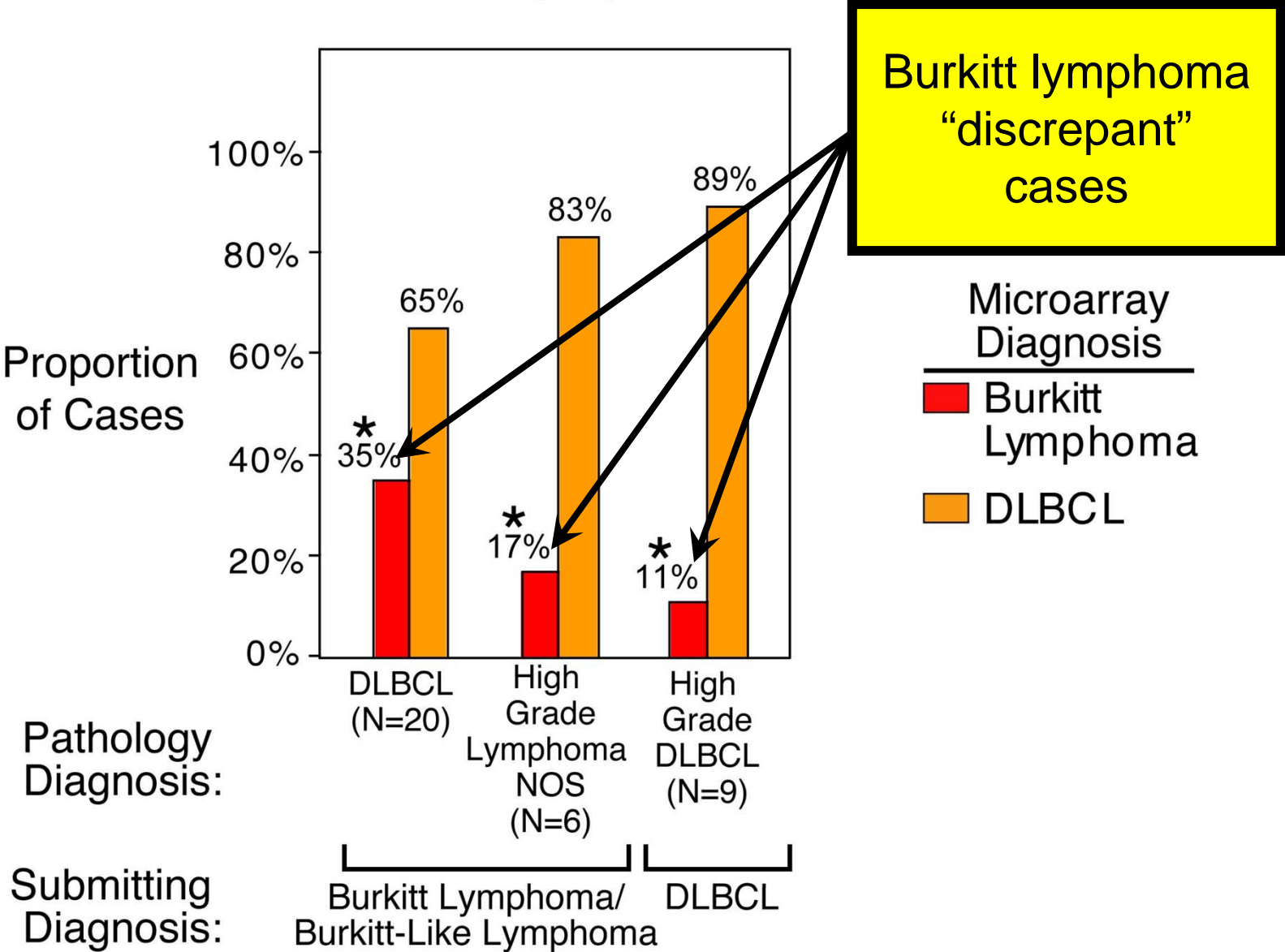
# Performance of a Gene Expression-based Predictor of Burkitt Lymphoma



# Discrepancies Between Molecular and Pathological Diagnoses of Burkitt Lymphoma



# Discrepancies Between Molecular and Pathological Diagnoses of Burkitt Lymphoma



# Effect of Treatment on Overall Survival in Burkitt Lymphoma

## Classification of Treatments

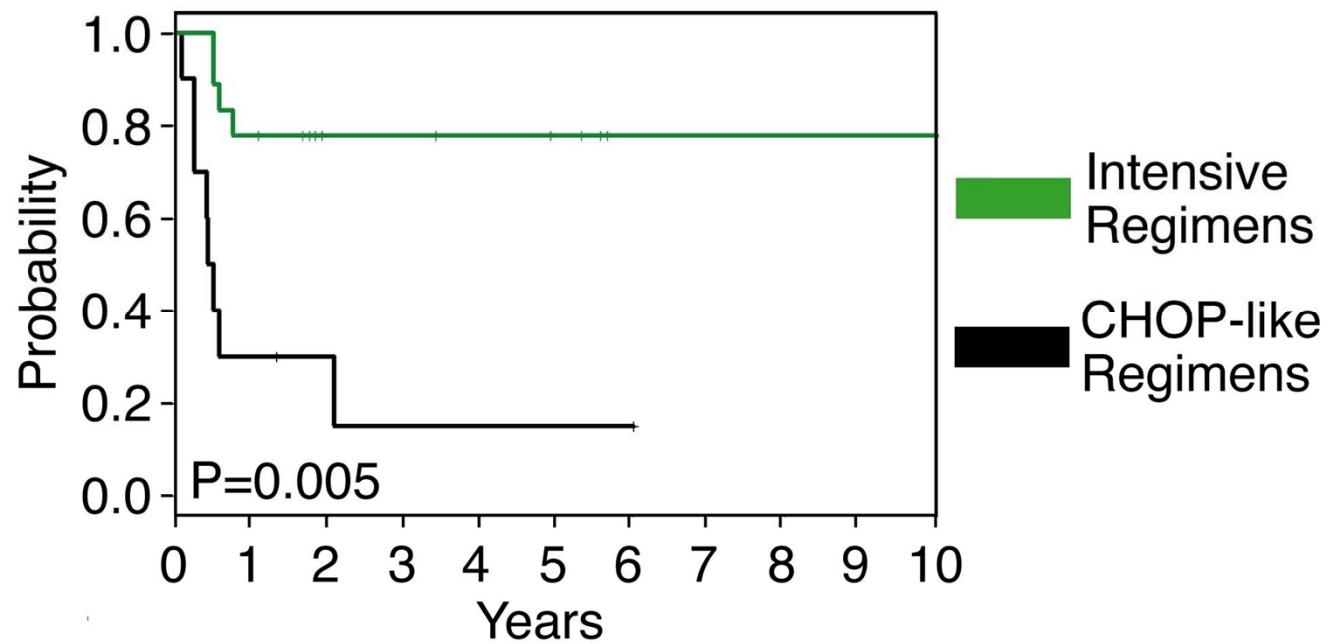
### CHOP-Like

CHOP  
CNOP

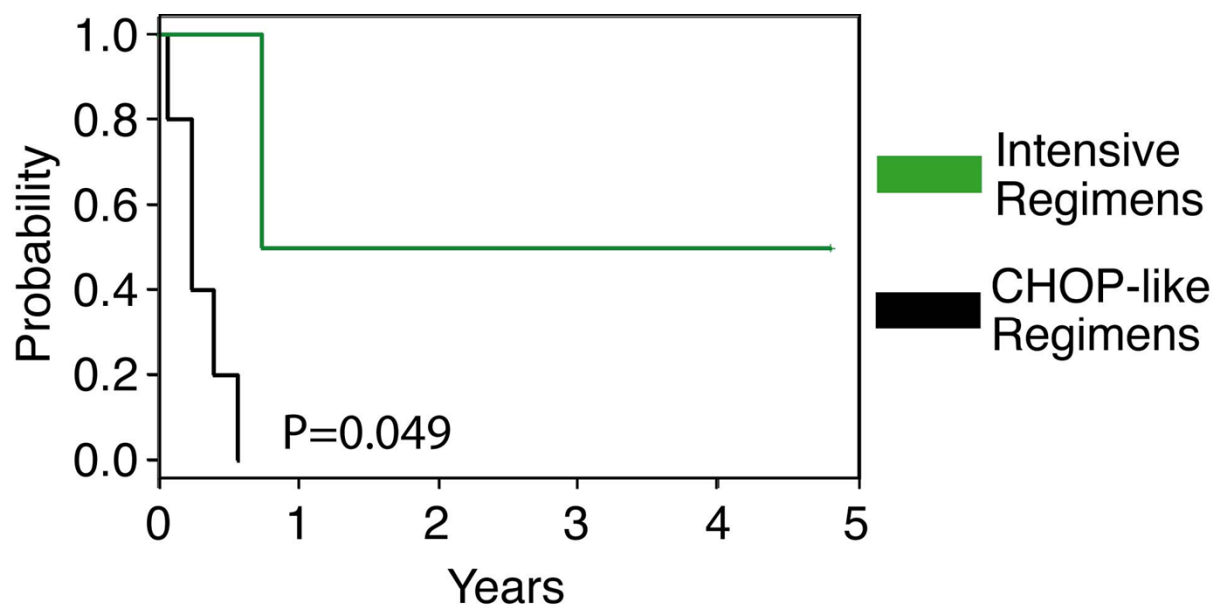
### Intensive

BFM  
CODOX-M/IVAC  
Regimens requiring  
autologous stem cell  
rescue

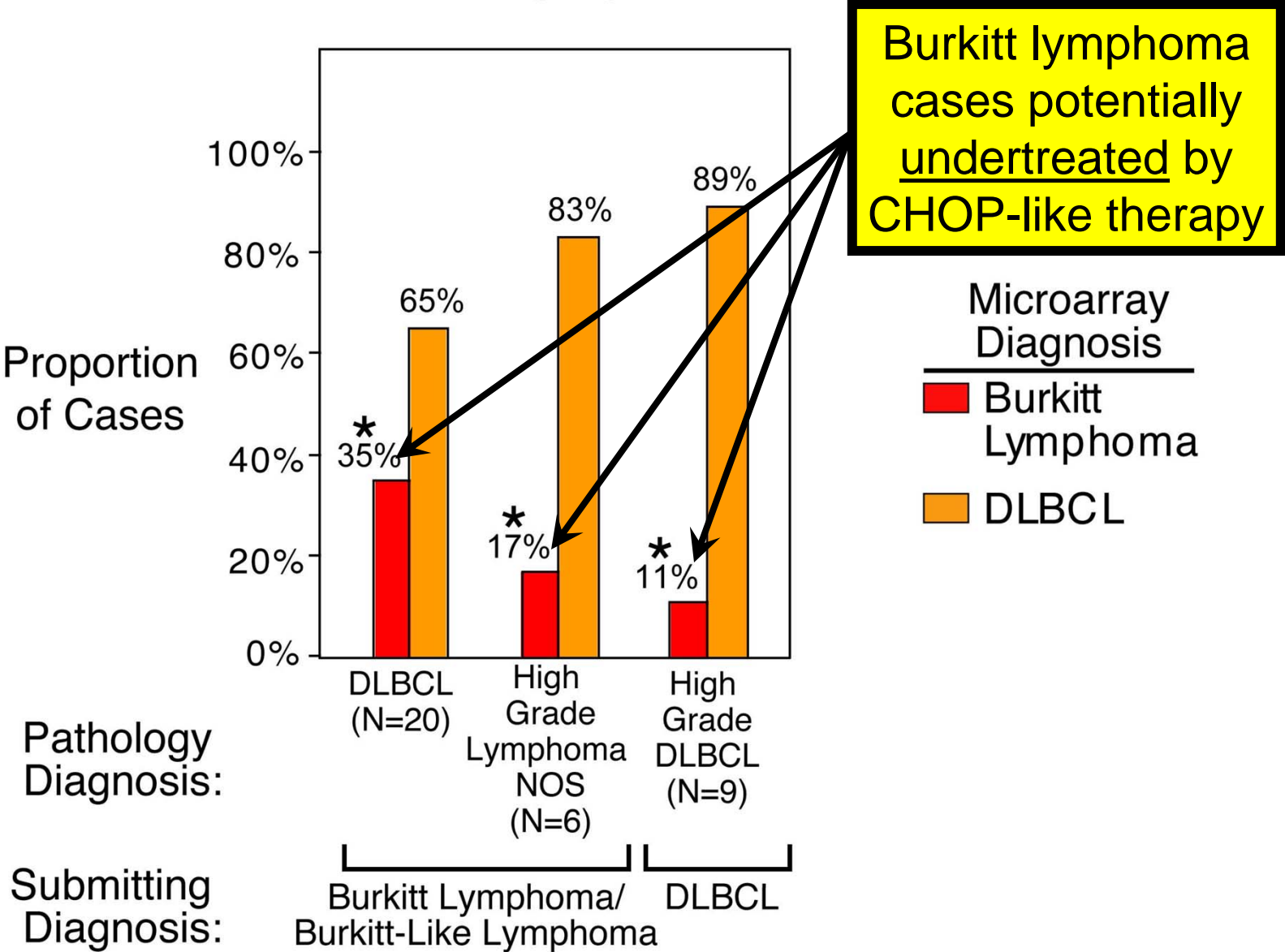
# High Cure Rates in Burkitt Lymphoma Treated With Intensive Regimens But Not CHOP-like Regimens



## Burkitt Lymphoma Discrepant Cases are Not Curable With CHOP-like Regimens



# Discrepancies Between Molecular and Pathological Diagnoses of Burkitt Lymphoma



# Conclusions

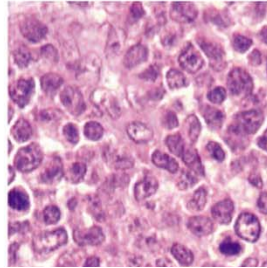
- Burkitt lymphoma has a distinct molecular profile that can reliably distinguish it from all forms of diffuse large B-cell lymphoma.
- Current means used for the diagnosis of Burkitt lymphoma disagree with the molecular diagnosis of Burkitt lymphoma in 17% of cases.
- The distinction between Burkitt lymphoma and DLBCL is critical because of significant differences in treatment.

=>Molecular diagnosis of Burkitt lymphoma will improve patient outcome.



Defining New Molecular Subgroups  
of Cancer  
by Gene Expression Profiling

# Dissecting a Cancer into Molecularly and Clinically Distinct Subgroups by Gene Expression Profiling



## Diffuse large B cell lymphoma

40% of Non-Hodgkin lymphomas

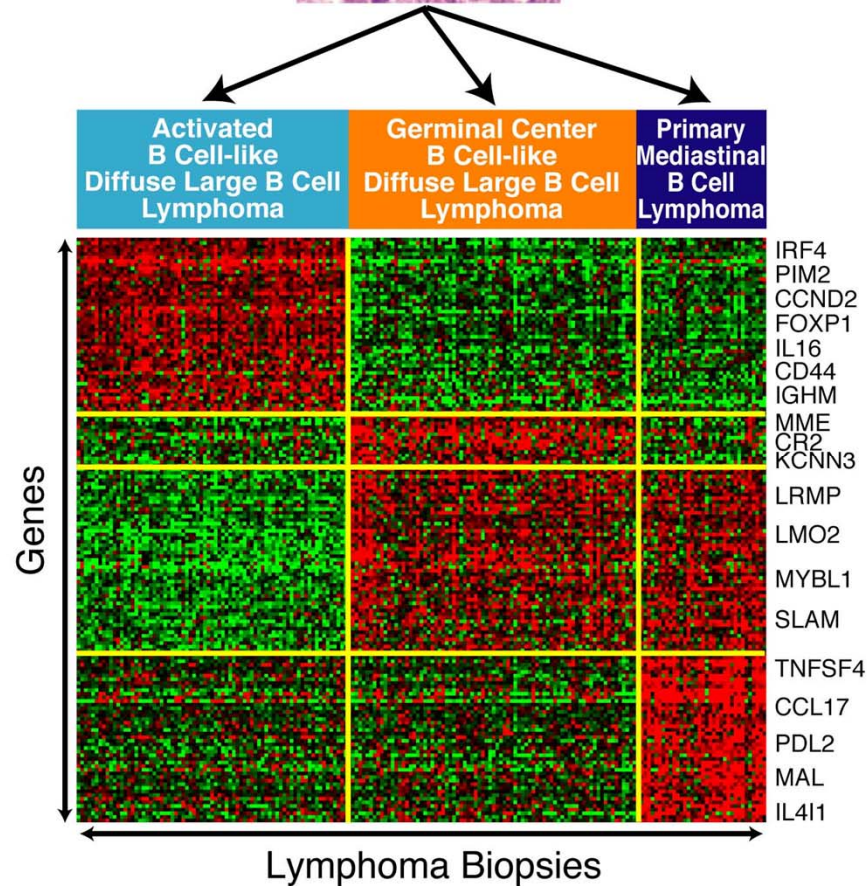
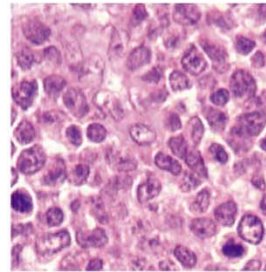
~23,000 new diagnoses/yr

~40% cure rate

~10,000 deaths/yr

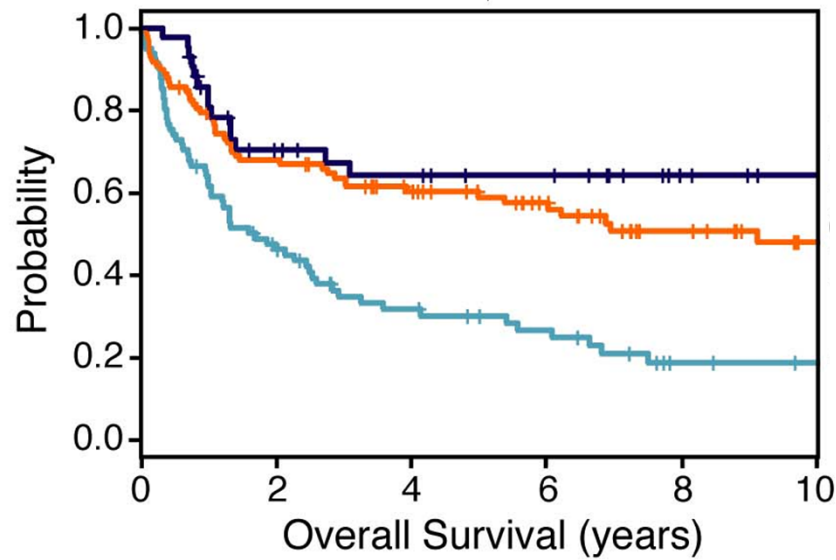
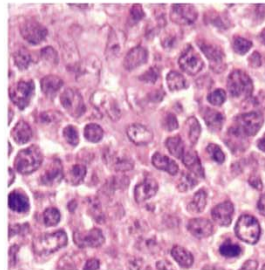
# Dissecting a Cancer into Molecularly and Clinically Distinct Subgroups by Gene Expression Profiling

Diffuse Large B Cell Lymphoma



# Dissecting a Cancer into Molecularly and Clinically Distinct Subgroups by Gene Expression Profiling

Diffuse Large B Cell  
Lymphoma

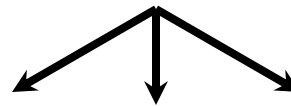
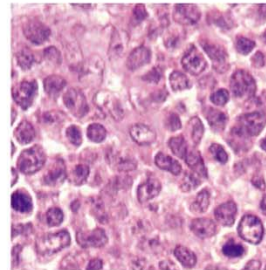


PMBL  
GCB  
DLBCL  
ABC  
DLBCL

5-year survival  
64%  
59%  
30%

# Dissecting a Cancer into Molecularly and Clinically Distinct Subgroups by Gene Expression Profiling

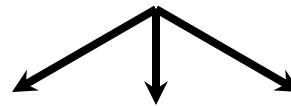
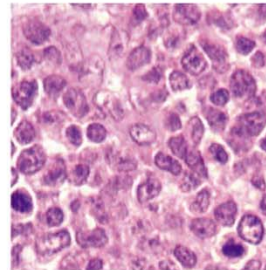
Diffuse Large B Cell Lymphoma



	GCB DLBCL	ABC DLBCL	PMBL
c-rel amplification	16%	0	25%
BCL-2 translocation	45%	0	18%
Gain Chromosome 3q	0	24%	5%
Gain/amp Chromosome 9p24	0	6%	43%
Constitutive NF- $\kappa$ B Activation	-	+	+

# Dissecting a Cancer into Molecularly and Clinically Distinct Subgroups by Gene Expression Profiling

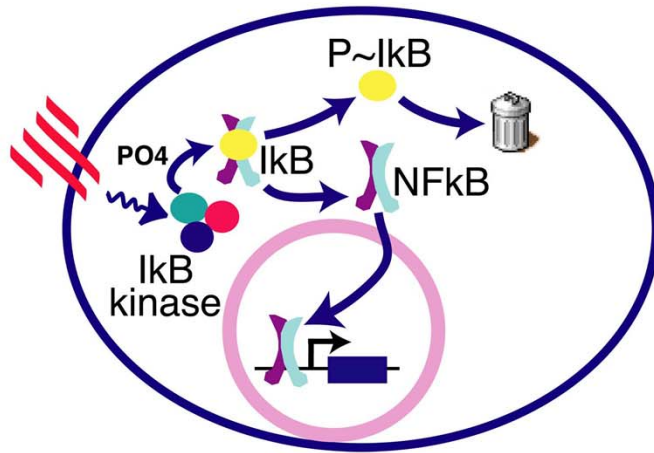
Diffuse Large B Cell  
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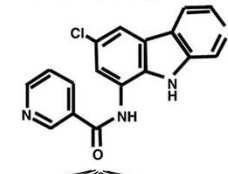


# Validation of the NF- $\kappa$ B Pathway as a Therapeutic Target in Activated B Cell-like Diffuse Large B Cell Lymphoma



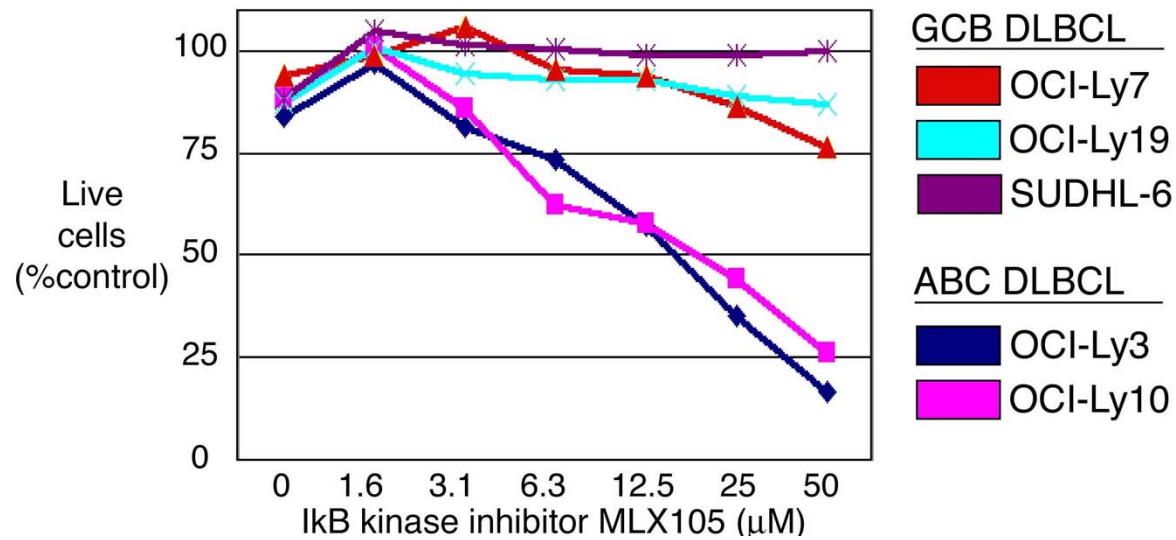
The  $\beta$ -carboline class of I $\kappa$ B kinase inhibitors

PS-1145



Structural analogues

MLX105

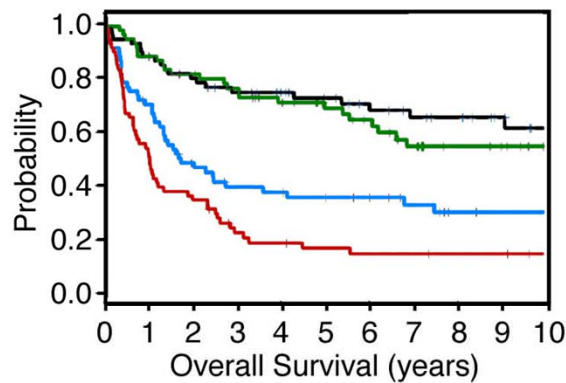


Molecular Predictors of Outcome  
In Cancer  
Using Gene Expression Profiling

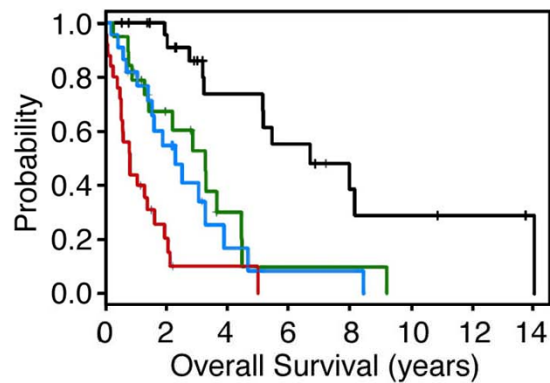


# Survival Prediction Based on the Gene Expression Profile of the Diagnostic Biopsy

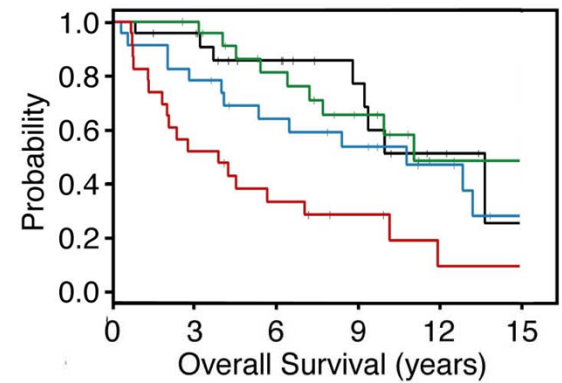
Diffuse Large B Cell Lymphoma



Mantle Cell Lymphoma

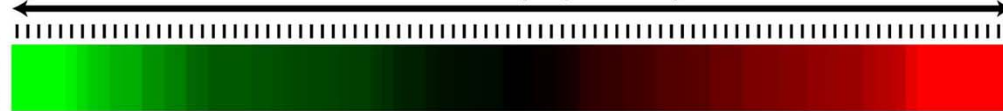


Follicular Lymphoma



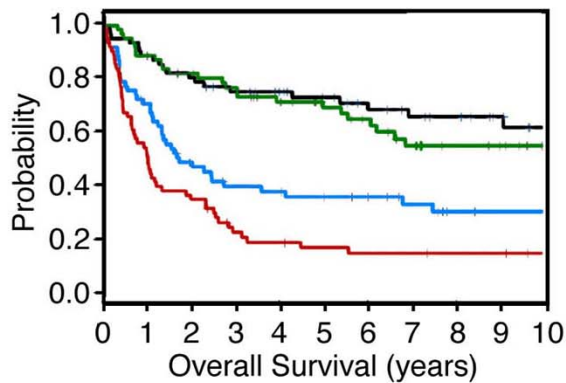
← Patient Biopsy Samples →

Gene Expression-based  
Survival Predictor Score

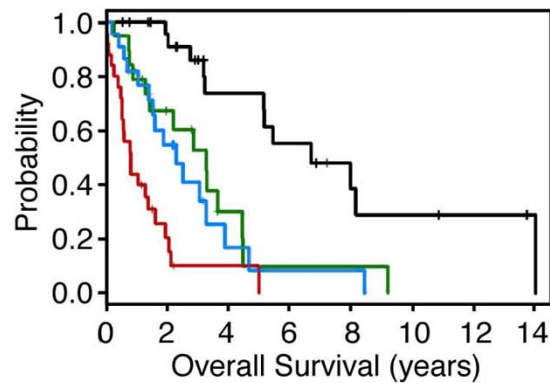


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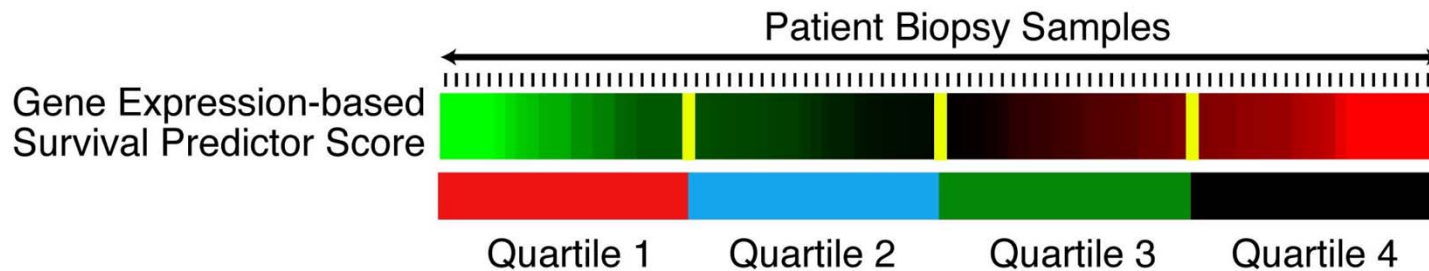
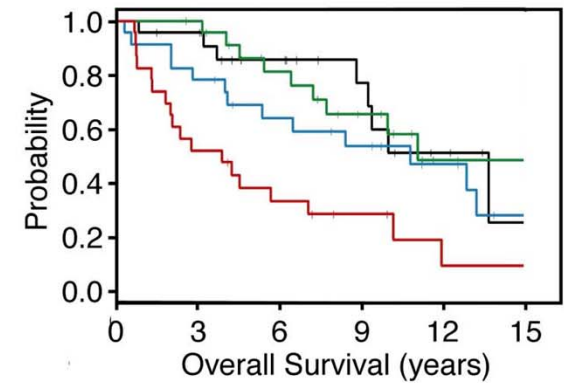
Diffuse Large B Cell Lymphoma



Mantle Cell Lymphoma



Follicular Lymphoma



# Why Can Gene Expression Profiling Predict Outcome in Cancer?

Within a current diagnostic category, gene expression profiling can identify:

1. Heterogeneity in cell of origin.
2. Heterogeneity in oncogenic pathways.
3. Heterogeneity in common cellular functions e.g. proliferation, survival, cell-cell interactions.

This heterogeneity is present in the tumor at the time of diagnosis.

Routine Molecular Diagnosis of  
Cancer in Clinical Oncology:

Development of a Lymphoma  
Diagnostic Microarray

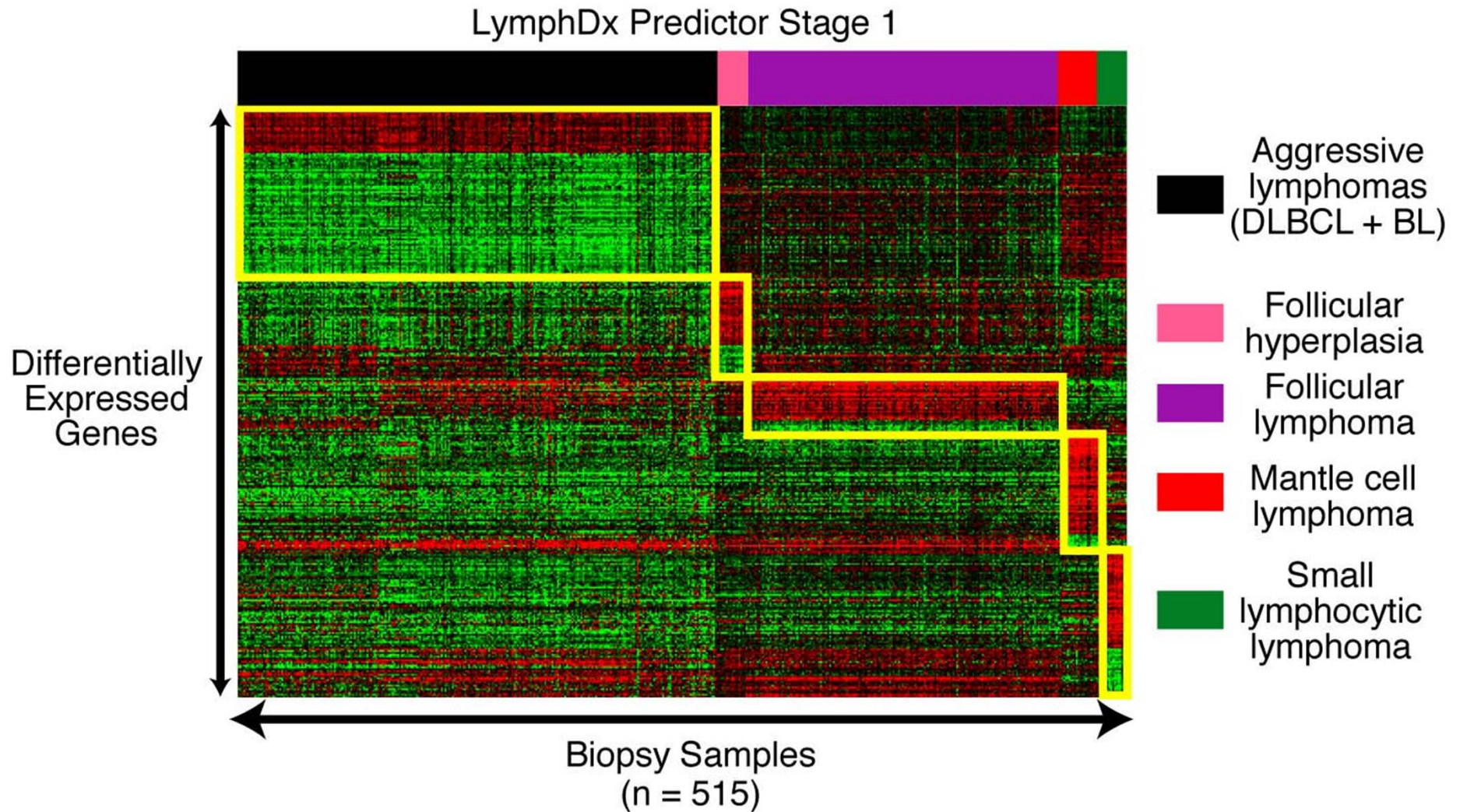
# The LymphDx Project Team



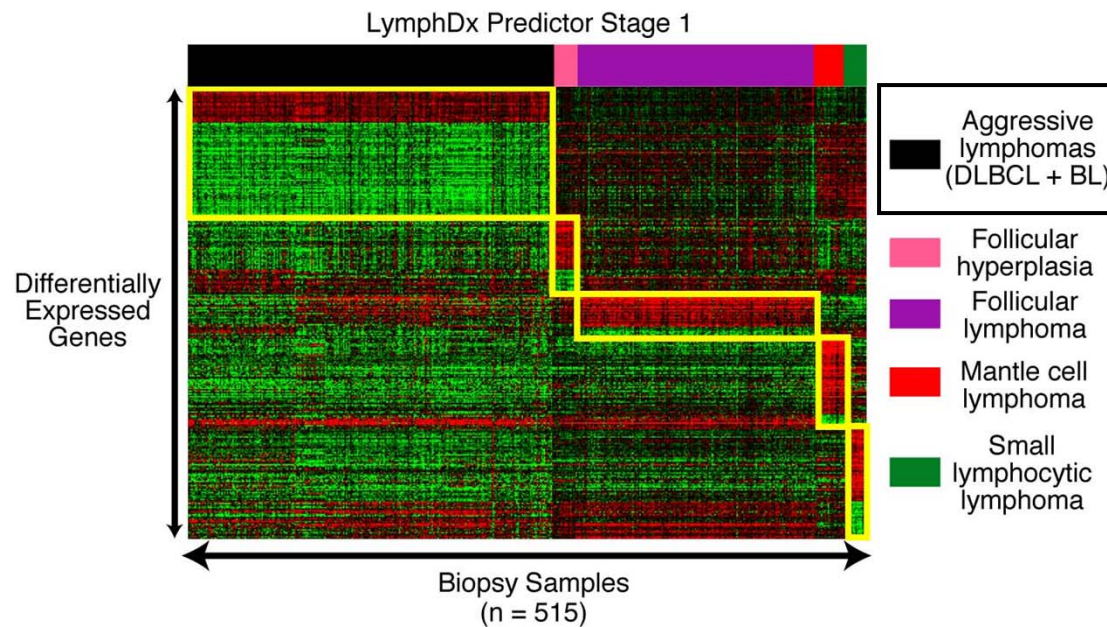
- Metabolism Branch, CCR, NCI
  - George Wright
  - Sandeep Dave
  - Bruce Tan
  - Andreas Rosenwald
  - Michael Chiorazzi
  - Hong Zhao
  - Liming Yang
  - Louis Staudt
- Members of the LLMPP
- Affymetrix
  - John Palma
  - Janet Warrington



# Gene Expression-based Diagnosis of Lymphoma

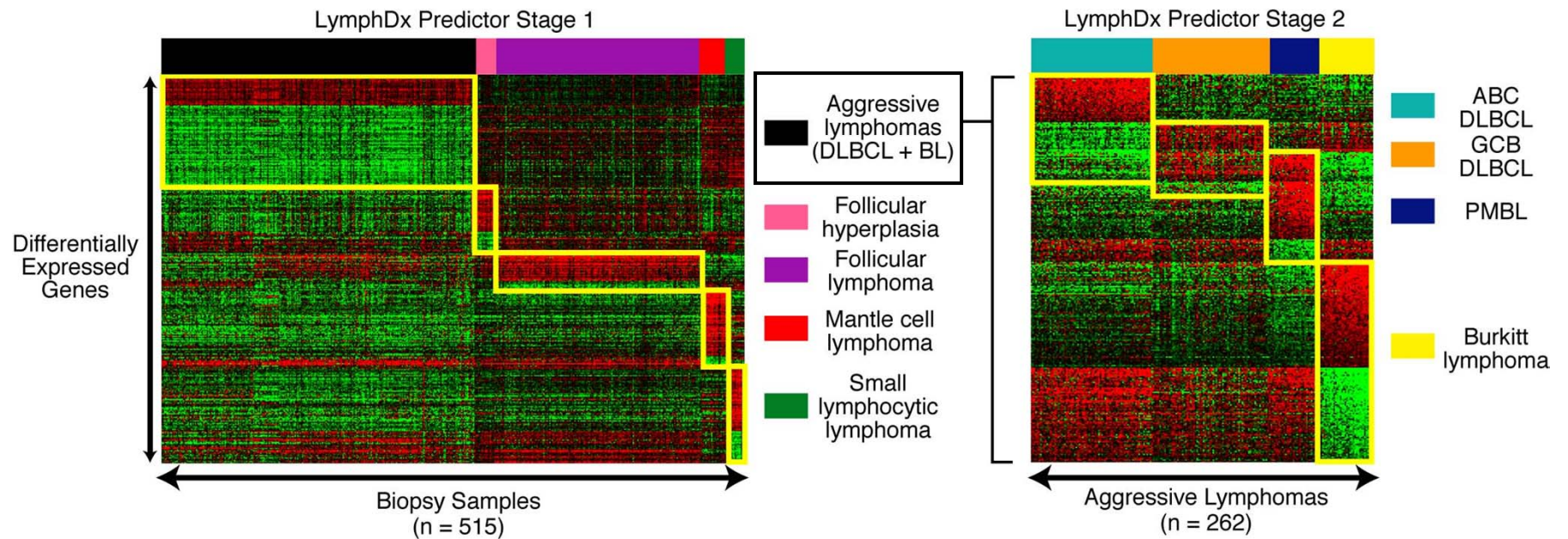


# Gene Expression-based Diagnosis of Lymphoma

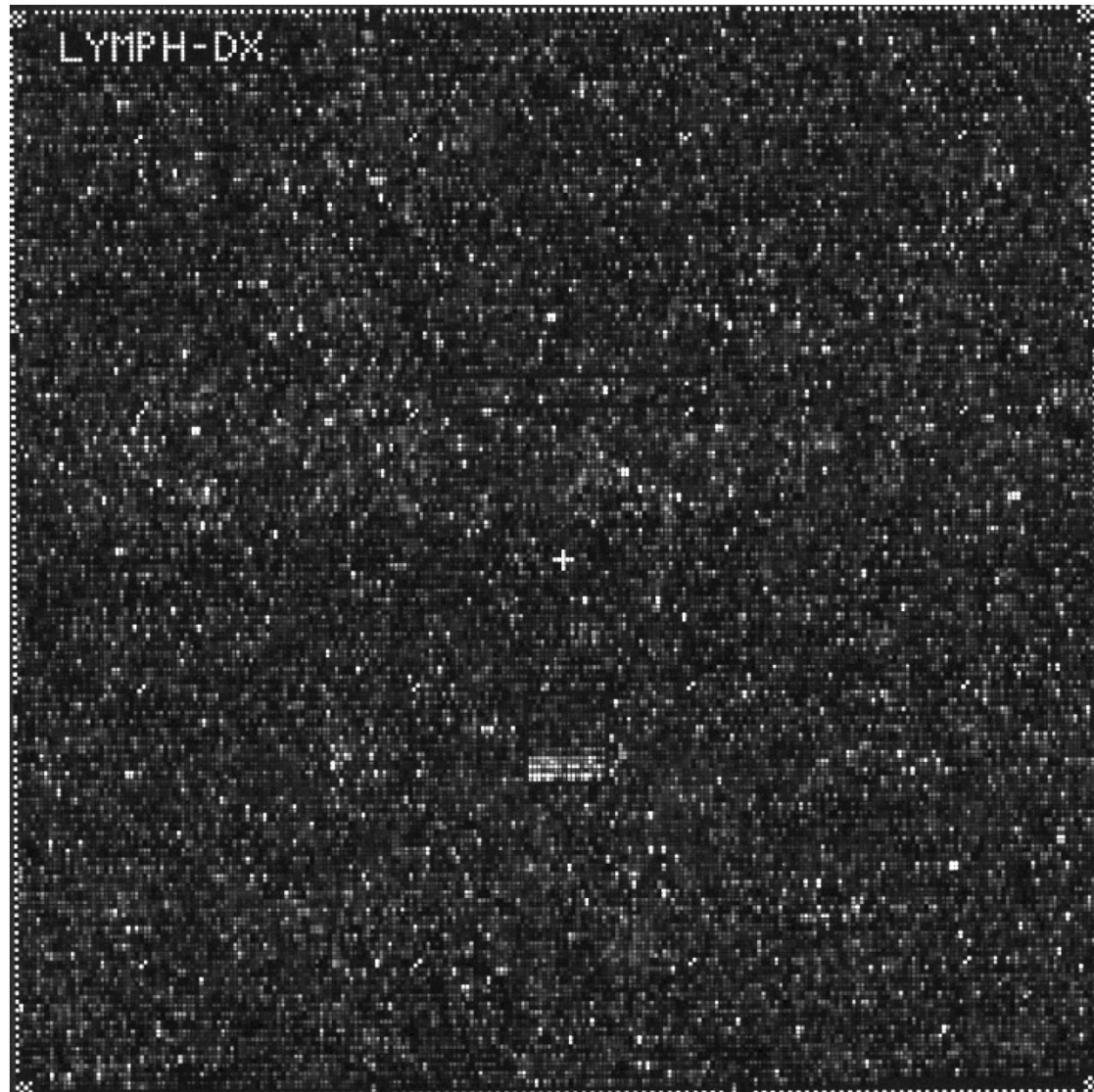




# Gene Expression-based Diagnosis of Lymphoma



# The LymphDx Custom DNA Microarray



~2643 human genes:

Lymphoma diagnostic genes

Lymphoma prognostic genes

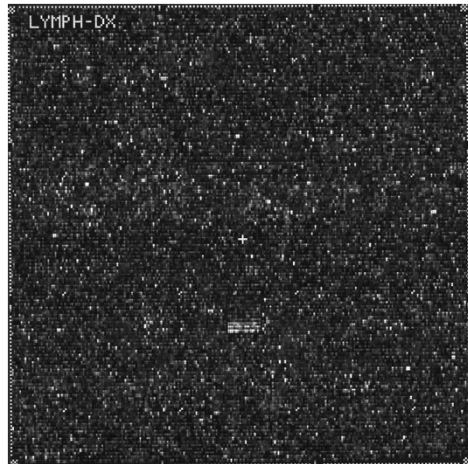
EBV, HHV8, HTLV1  
viral genes

Genes encoding all human  
kinases  
cytokines  
chemokines  
cytokine receptors  
chemokine receptors

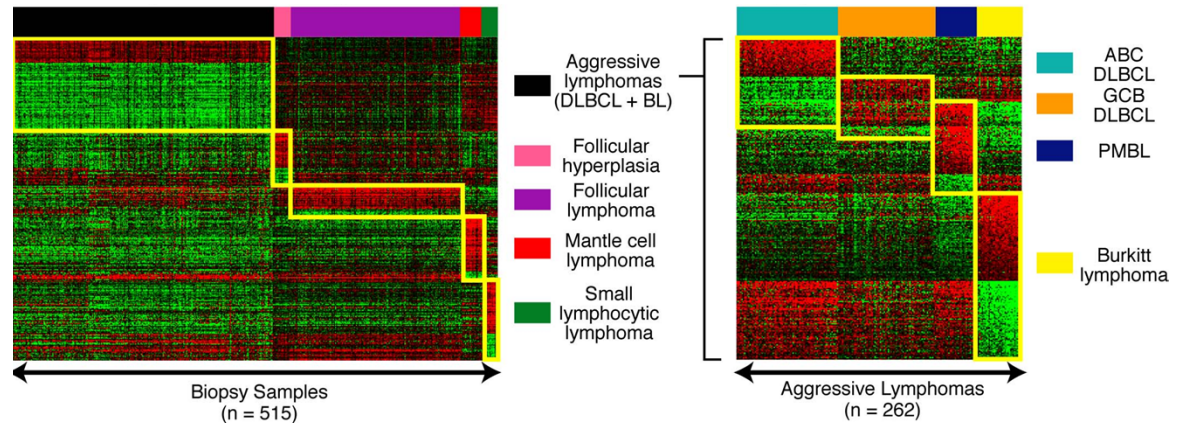
Invariantly expressed  
control genes

# LymphDx: The One-stop Shopping Approach to Lymphoma Diagnosis

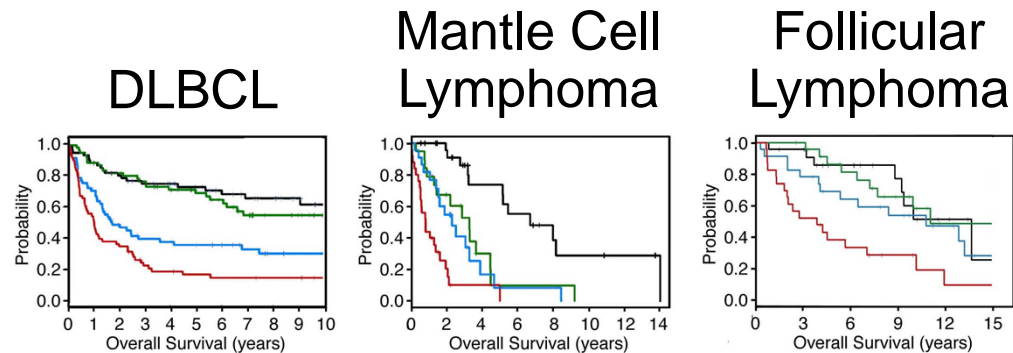
## Molecular Diagnosis



LymphDx  
microarray



## Molecular Prognosis







GOAL: Implementation of a gene expression-based molecular diagnosis of lymphoma in routine clinical practice.

Collaborating LLMPP Institutions

Univ. of Nebraska Medical Center  
British Columbia Cancer Center  
Southwest Oncology Group  
Cleveland Clinic  
Norwegian Radium Hospital

Univ. of Würzburg  
Univ. of Barcelona  
Univ. of Arizona  
Univ. of Rochester  
St. Bart's Hospital

National Cancer Institute Center for Cancer Research



## PHASE 1

Affymetrix whole genome profiles of retrospectively ascertained lymph node biopsies  
(non-Hodgkin and Hodgkin lymphoma, other cancers, benign conditions)  
(n= 2000)

Design custom diagnostic microarray



*SPECS: Specialized Program for the Evaluation of Cancer Signatures*

PHASE 1

Affymetrix whole genome profiles of retrospectively ascertained lymph node biopsies (non-Hodgkin and Hodgkin lymphoma, other cancers, benign conditions)  
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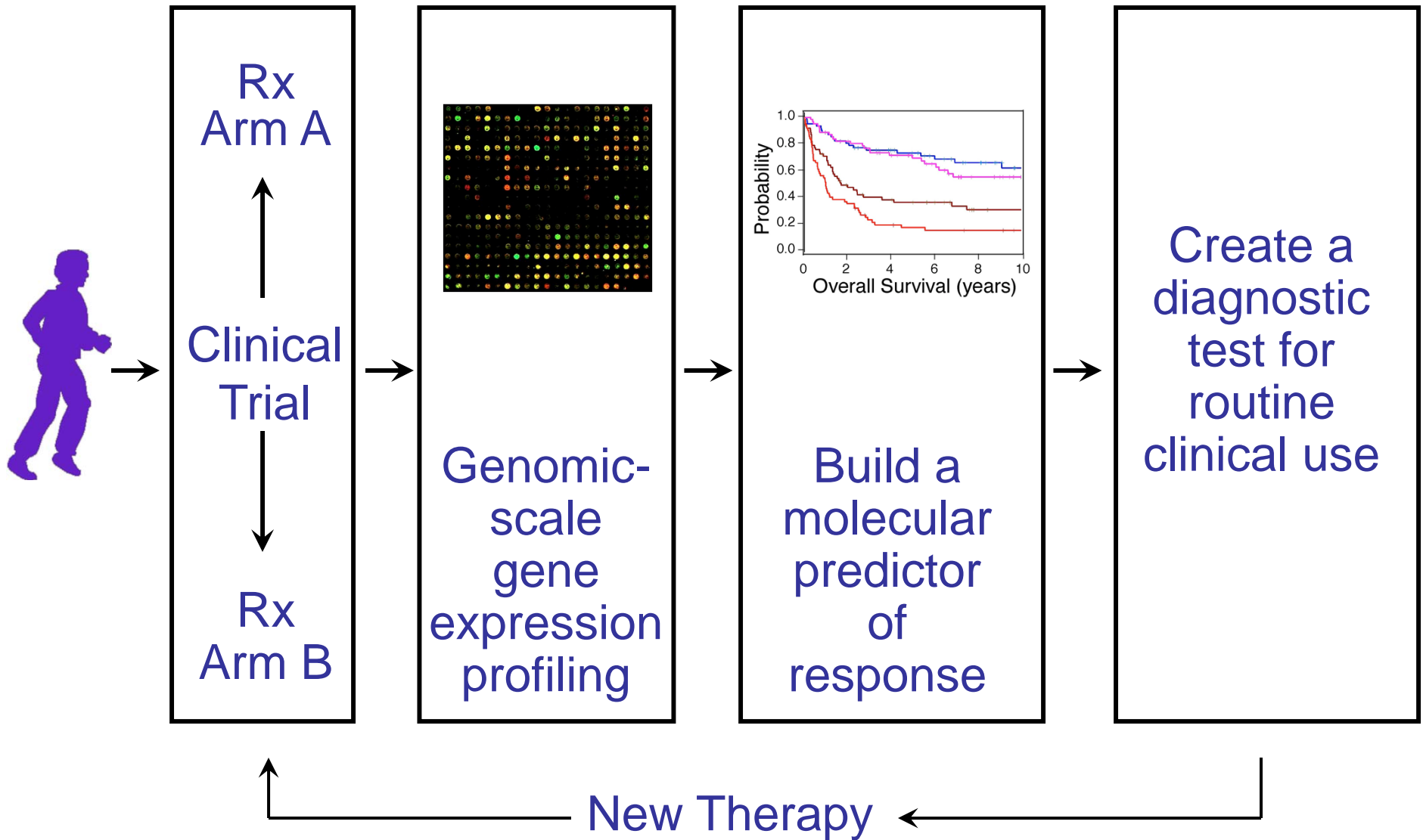
PHASE 2

Diagnostic microarray profiles of prospectively ascertained lymph node biopsies  
(n= 2000)

Generate data for regulatory approval

# Evolving Molecular Diagnosis To Match Changes in Cancer Treatment

# The Molecular Diagnosis Cycle





Phase III Randomized Clinical Trial of  
CHOP-Rituximab  
vs. Dose-adjusted EPOCH-Rituximab  
with Gene Expression Profiling Analysis  
in Untreated Diffuse Large B cell Lymphoma

CALGB Study 50303  
Opened for accrual: May 2005

Study Chairs:

Wyndham H. Wilson, Center for Cancer Research, NCI  
Bruce Cheson, CALGB Lymphoma Committee  
Andrew D. Zelenetz, CALGB Lymphoma Committee  
Richard Fisher, Chair, SWOG Lymphoma Committee  
Louis M. Staudt, Center for Cancer Research, NCI

# Phase III Randomized DLBCL Trial Design CALGB Study 50303

Untreated DLBCL patients  
(n= 430)

CHOP-R  
(n= 215)

DA-EPOCH-R  
(n= 215)

Test previous survival  
predictors:

ABC vs GCB DLBCL  
GC B cell Signature  
Proliferation Signature  
Lymph Node Signature  
MHC Class II Signature

Create  
new  
survival  
predictor

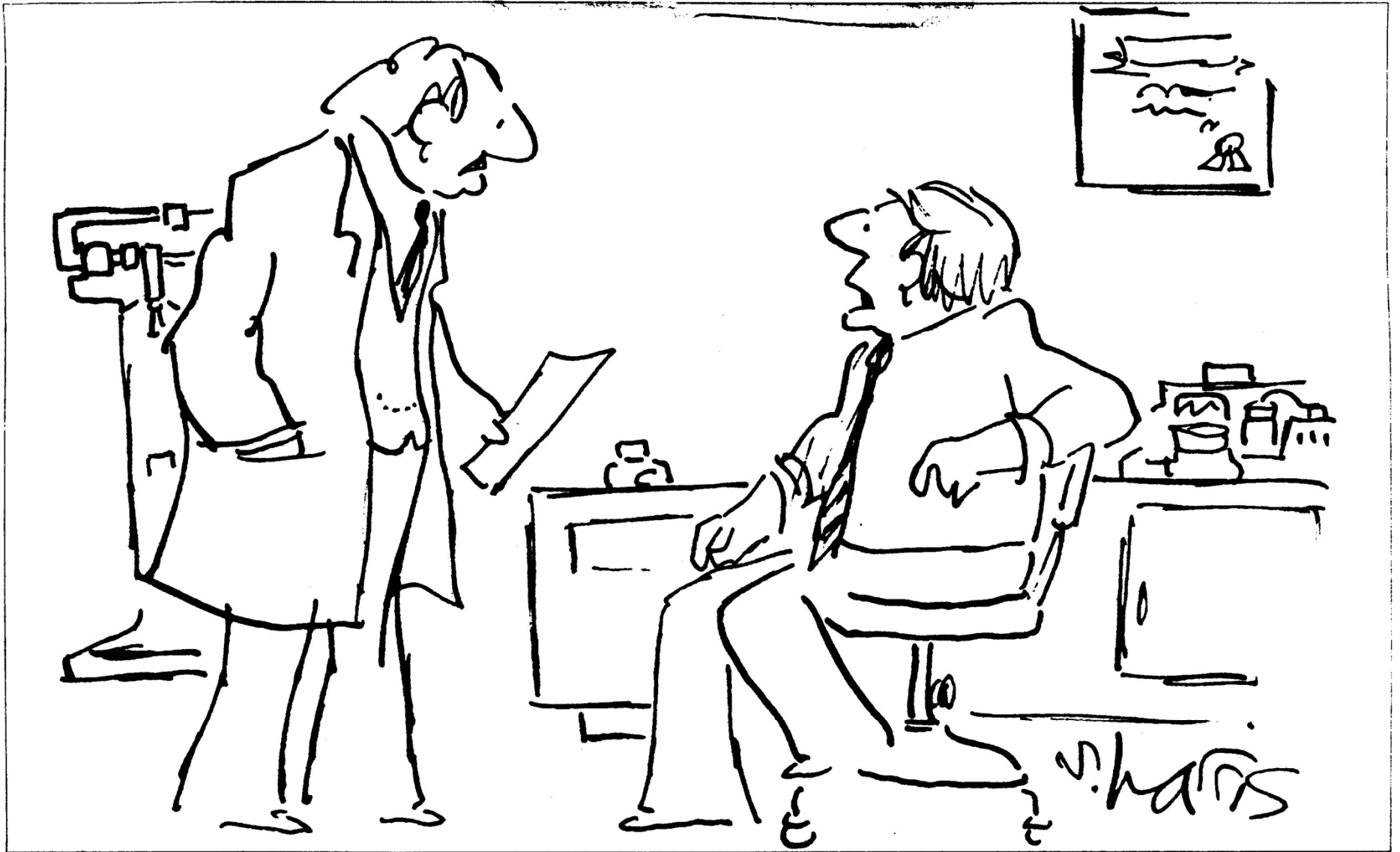
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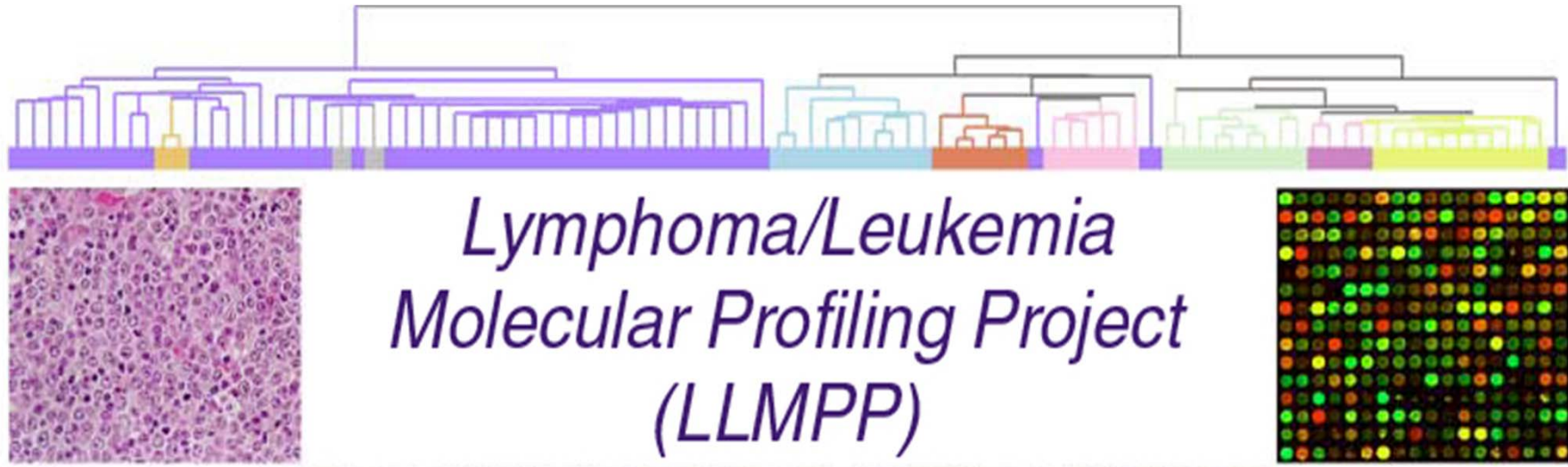
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1. To provide reproducible, quantitative diagnoses for all cancer patients.
2. To clarify diagnostic distinctions that are problematic using current methods.
3. To deliver newly defined molecular diagnoses that influence treatment choice and/or prognosis.
4. To translate insights from therapeutic trials that incorporate molecular profiling.
5. To promote excellence in clinical science.



*"I feel fine, but I thought there may be something amiss on the molecular level"*



*Lymphoma/Leukemia  
Molecular Profiling Project  
(LLMPP)*

Collaborating Institution

Univ. of Nebraska Medical Center  
British Columbia Cancer Agency  
Southwest Oncology Group  
Univ. of Würzburg  
Univ. of Barcelona  
Norwegian Radium Hospital  
St. Bart's Hospital, London

John Chan  
Randy Gascoyne  
Rich Fisher  
Konrad Muller-Hermelink  
Elias Campo  
Erlend Smeland  
Andrew Lister

Center for Cancer Research,  
National Cancer Institute

Lou Staudt  
Sandeep Dave  
George Wright