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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1. To provide reproducible, quantitative diagnoses for all cancer patients.
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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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<table>
<thead>
<tr>
<th>Lymphoma subtype</th>
<th>Curable?</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse Large B Cell Lymphoma</td>
<td>Yes</td>
<td>CHOP chemotherapy + Rituximab</td>
</tr>
<tr>
<td>Primary Mediastinal B Cell Lymphoma</td>
<td>Yes</td>
<td>CHOP chemotherapy + Rituximab +/- radiation</td>
</tr>
<tr>
<td>Burkitt Lymphoma</td>
<td>Yes</td>
<td>High dose chemotherapy CNS prophylaxis</td>
</tr>
<tr>
<td>Follicular Lymphoma</td>
<td>No</td>
<td>Watchful waiting</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rituximab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Symptomatic chemotherapy</td>
</tr>
<tr>
<td>Mantle Cell Lymphoma</td>
<td>No</td>
<td>Watchful waiting</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Symptomatic chemotherapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Responsive to:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>--Bortezomib</td>
</tr>
<tr>
<td></td>
<td></td>
<td>--rapamycin analogues</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th></th>
<th>Classic Burkitt Lymphoma</th>
<th>Atypical Burkitt Lymphoma</th>
<th>Diffuse Large B Cell Lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recommended Treatment</td>
<td>Intensive chemotherapy</td>
<td>Intensive chemotherapy</td>
<td>CHOP-like chemotherapy</td>
</tr>
</tbody>
</table>

Recommended Treatment choice depends on the type of Burkitt Lymphoma.
Gene Expression Differentiates Burkitt Lymphoma from all Subgroups of Diffuse Large B Cell Lymphoma

<table>
<thead>
<tr>
<th>Burkitt Lymphoma</th>
<th>Diffuse Large B-cell Lymphoma (DLBCL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classic</td>
<td>ABC</td>
</tr>
<tr>
<td></td>
<td>GCB</td>
</tr>
<tr>
<td></td>
<td>PMBL</td>
</tr>
<tr>
<td></td>
<td>Unclass</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage 1 Predictor (MYC Targets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYC</td>
</tr>
<tr>
<td>TERT</td>
</tr>
<tr>
<td>NS</td>
</tr>
<tr>
<td>NP</td>
</tr>
<tr>
<td>MAZ</td>
</tr>
<tr>
<td>RFC3</td>
</tr>
<tr>
<td>BLYSL</td>
</tr>
<tr>
<td>BID3</td>
</tr>
<tr>
<td>CDC7</td>
</tr>
<tr>
<td>TOLL1A</td>
</tr>
<tr>
<td>AUTS2</td>
</tr>
<tr>
<td>MYBL1</td>
</tr>
<tr>
<td>BMP7</td>
</tr>
<tr>
<td>ITPR3</td>
</tr>
<tr>
<td>CDC2</td>
</tr>
<tr>
<td>BACH2</td>
</tr>
<tr>
<td>TTK</td>
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<tr>
<td>MME</td>
</tr>
<tr>
<td>ALOX5</td>
</tr>
<tr>
<td>TOP1</td>
</tr>
<tr>
<td>JAK2</td>
</tr>
<tr>
<td>HLA-F</td>
</tr>
<tr>
<td>PIM1</td>
</tr>
<tr>
<td>CASP8</td>
</tr>
<tr>
<td>HLA-E</td>
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<tr>
<td>CCL17</td>
</tr>
<tr>
<td>VMP1</td>
</tr>
<tr>
<td>TRAF1</td>
</tr>
<tr>
<td>HCK</td>
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<tr>
<td>HLA-G</td>
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<tr>
<td>JAK3</td>
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<tr>
<td>BATF</td>
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<tr>
<td>NFkBIA</td>
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<tr>
<td>LMO2</td>
</tr>
<tr>
<td>CD44</td>
</tr>
<tr>
<td>CFLAR</td>
</tr>
<tr>
<td>BLC</td>
</tr>
<tr>
<td>STAT3</td>
</tr>
<tr>
<td>BCL2A1</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Stage 2 Predictor</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK2</td>
</tr>
<tr>
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</tr>
<tr>
<td>PIM1</td>
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Performance of a Gene Expression-based Predictor of Burkitt Lymphoma

- Classic BL (N=25): 100% Burkitt Lymphoma
- Atypical BL (N=20): 95% Burkitt Lymphoma, 5% DLBCL
- ABC DLBCL (N=78): 100% Burkitt Lymphoma
- GCB DLBCL (N=82): 100% Burkitt Lymphoma
- PMBL (N=33): 100% Burkitt Lymphoma
- Unclass DLBCL (N=30): 97% Burkitt Lymphoma, 3% DLBCL

Microarray Diagnosis:
- Red: Burkitt Lymphoma
- Orange: DLBCL

Pathology Diagnosis:
- Submitting Diagnosis: Burkitt Lymphoma/Burkitt-Like Lymphoma
- DLBCL
Discrepancies Between Molecular and Pathological Diagnoses of Burkitt Lymphoma

- **Microarray Diagnosis**
  - Burkitt Lymphoma
  - DLBCL

- **Pathology Diagnosis**
  - DLBCL (N=20)
  - High Grade Lymphoma NOS (N=6)
  - High Grade DLBCL (N=9)

- **Submitting Diagnosis**
  - Burkitt Lymphoma/Burkitt-Like Lymphoma
  - DLBCL

- **Proportion of Cases**
  - DLBCL: 35% (N=20)
  - High Grade Lymphoma NOS: 17% (N=6)
  - High Grade DLBCL: 11% (N=9)
  - Burkitt Lymphoma: 65% (N=20)
  - DLBCL: 89% (N=20)
  - High Grade Lymphoma NOS: 83% (N=6)
Discrepancies Between Molecular and Pathological Diagnoses of Burkitt Lymphoma

Microarray Diagnosis

- **Burkitt lymphoma**
- **“discrepant” cases**

<table>
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<th>Submitting Diagnosis</th>
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<tr>
<td></td>
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Proportion of Cases

- DLBCL: 35%
- High Grade Lymphoma NOS: 17%
- High Grade DLBCL: 11%
Effect of Treatment on Overall Survival in Burkitt Lymphoma

**Classification of Treatments**

<table>
<thead>
<tr>
<th>CHOP-Like</th>
<th>Intensive</th>
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<tr>
<td>CHOP</td>
<td>BFM</td>
</tr>
<tr>
<td>CNOP</td>
<td>CODOX-M/IVAC</td>
</tr>
<tr>
<td></td>
<td>Regimens requiring autologous stem cell rescue</td>
</tr>
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High Cure Rates in Burkitt Lymphoma Treated With Intensive Regimens But Not CHOP-like Regimens

![Graph showing cure rates over years with different regimens](image)

- **Probability**
- **Years**
- **P=0.005**
Burkitt Lymphoma Discrepant Cases are Not Curable With CHOP-like Regimens

\[ P = 0.049 \]
Discrepancies Between Molecular and Pathological Diagnoses of Burkitt Lymphoma

Burkitt lymphoma cases potentially undertreated by CHOP-like therapy
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
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
Dissecting a Cancer into Molecularly and Clinically Distinct Subgroups by Gene Expression Profiling

Diffuse Large B Cell Lymphoma

5-year survival

PMBL: 64%
GCB: 59%
DLBCL: 30%

Overall Survival (years)

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

Diffuse Large B Cell Lymphoma

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<td>16%</td>
<td>0</td>
<td>25%</td>
</tr>
<tr>
<td>BCL-2 translocation</td>
<td>45%</td>
<td>0</td>
<td>18%</td>
</tr>
<tr>
<td>Gain Chromosome 3q</td>
<td>0</td>
<td>24%</td>
<td>5%</td>
</tr>
<tr>
<td>Gain/amp Chromosome 9p24</td>
<td>0</td>
<td>6%</td>
<td>43%</td>
</tr>
<tr>
<td>Constitutive NF-κB Activation</td>
<td>−</td>
<td>+</td>
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Dissecting a Cancer into Molecularly and Clinically Distinct Subgroups by Gene Expression Profiling

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Validation of the NF-κB Pathway as a Therapeutic Target in Activated B Cell-like Diffuse Large B Cell Lymphoma

The β-carboline class of IkB kinase inhibitors

PS-1145

Structural analogues

MLX105

Live cells (% control)

0  25  50  75  100

0  1.6  3.1  6.3  12.5  25  50

IkB kinase inhibitor MLX105 (μM)

GCB DLBCL

- OCI-Ly7
- OCI-Ly19
- SUDHL-6

ABC DLBCL

- OCI-Ly3
- OCI-Ly10
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
Survival Prediction Based on the Gene Expression Profile of the Diagnostic Biopsy

![Graphs showing survival prediction for different types of lymphoma (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

Molecular Prognosis

LymphDx microarray

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British Columbia Cancer Center  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
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
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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

**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

Clinical Trial

Rx Arm A

Rx Arm B

Genomic-scale gene expression profiling

Build a molecular predictor of response

Create a diagnostic test for routine clinical use

New Therapy
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

Test previous survival predictors:
ABC vs GCB DLBCL
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Create new survival predictor
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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.
“I feel fine, but I thought there may be something amiss on the molecular level”
Collaborating Institution

Univ. of Nebraska Medical Center  John Chan
British Columbia Cancer Agency  Randy Gascoyne
Southwest Oncology Group  Rich Fisher
Univ. of Würzburg  Konrad Muller-Hermelink
Univ. of Barcelona  Elias Campo
Norwegian Radium Hospital  Erlend Smeland
St. Bart’s Hospital, London  Andrew Lister

Center for Cancer Research, National Cancer Institute  Lou Staudt
Sandeep Dave
George Wright