Lymphoid Malignancies Think Tank
Therapeutic Opportunities in Lymphomas

Wyndham H. Wilson, MD, PhD
Bethesda, Maryland
Distribution of Lymphoma Subtypes

% of Total Cases

- Diffuse Large B-cell: 30.6%
- Follicular Lymphoma: 22.1%
- Marginal zone B-cell lymphoma, MALT: 13.7%
- Peripheral T-cell lymphomas: 6.7%
- CLL/SLL: 6.0%
- Mantle Cell Lymphoma: 6.7%
- Mediastinal Large B-cell Lymphoma: 7.6%
- Anaplastic Large Cell Lymphoma/T-null: 7.0%
Historical Outcome of Lymphoma Subtypes

- Aggressive “B” Cell Lymphomas
  - Diffuse Large B-cell Lymphoma (DLBCL)
  - Mantle cell lymphoma (MCL)
- Indolent “B” Cell Lymphomas
  - Follicular center cell (FCC)
Aggressive Lymphoma (DLBCL)

\[ P = 0.35 \]

Cure \( \approx 33\% \)

Fisher Intergroup Trial (PFS)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Patients at Risk</th>
<th>Relapses or Deaths</th>
<th>3-Year Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOP</td>
<td>225</td>
<td>114</td>
<td>41%</td>
</tr>
<tr>
<td>m-BACOD</td>
<td>223</td>
<td>109</td>
<td>46%</td>
</tr>
<tr>
<td>ProMACE-CytaBOM</td>
<td>233</td>
<td>115</td>
<td>46%</td>
</tr>
<tr>
<td>MACOP-B</td>
<td>218</td>
<td>119</td>
<td>41%</td>
</tr>
</tbody>
</table>
Mantle Cell Lymphoma

Median survival $\cong 3$ years

Indolent Lymphoma (FCC)

Patient Survival With Indolent NHL

Median Survival 10 years

1987-1996 (n=668)
1976-1987 (n=513)
1960-1976 (n=195)

Courtesy of Sandra J. Horning, MD.
Strategies in DLBCL

- Empiric
- Targeted
  - Identify targets and reagents
    - Clinically assess and validate
  - Clinical trials
    - Evaluate molecular mechanisms of treatment failure
    - Build molecular prognostic models
    - Assess tumor biology ↔ treatment regimens
  - \textit{Rationally} develop new-generation treatments
Rituximab: An Anti-CD20 MAb
An Empiric Success

- Chimeric murine/human MAb
  - Variable light- and heavy-chain regions from murine anti-CD20 antibody
  - Linked to human IgGκ constant regions
LNH-98.5
Phase III CHOP v CHOP plus Rituximab

Cyclophosphamide 750 mg/m²
Doxorubicine 50 mg/m²
Vincristine 1.4 mg/m²
Prednisone 40 mg/m²/d x 5 d

3 weeks                               8 cycles

CHOP
Rituximab 375 mg/m²
GELA study – Median follow-up 5 y

Event-Free Survival

R-CHOP

CHOP

p = .00002

Survival

R-CHOP

CHOP

p = .007

48%
Diffuse Large B-Cell Lymphoma
Molecular Prognostic Model of “CHOP” Failure
Proliferation Signature

Rosenwald et al. NEJM 346:1937, 2002
Ki-67 Analysis of Tumor Proliferation Survival Outcome With CHOP bolus

Ki-67 < 80%
Median survival 39 months

Ki-67 > 80%
Median survival 7 months

Blood 83:1460, 1994
Hypothesis

- Drug schedule increases tumor drug sensitivity

Doxorubicin

- Schedule dependent drugs
  - Doxorubicin
  - Etoposide
  - Vincristine
Hypothesis

- Drug schedule impacts drug sensitivity and mechanism
  - Cell cycle ↔ apoptotic threshold
  - Cell cycle ↔ Drug targets

![Graph showing correlation between Proliferation and Topoisomerase 2 Alpha with a correlation coefficient of 0.58](image)
Tumor Proliferation
Survival Outcome CHOP v DA-EPOCH

CHOP Bolus

DA-EPOCH Infusional

High Proliferation (Ki67 > 80%)
Low Proliferation (Ki67 < 80%)

Survival Probability

Years on study

P = 0.0004
P = 0.11
DA-EPOCH-R Infusional Survival Outcomes

PFS and OS: 83%

Untreated DLBCL (n=90)

Median Follow-up: 3 years
Diffuse Large B-Cell Lymphoma
Biology of “CHOP” Failure

Gene Expression Outcome Predictor

Overall Survival (years)

Probability

Proliferation

MHC Class II

GC B cell

Lymph Node

Signature

GCB DLBCL  Type 3 DLBCL  ABC DLBCL

Genes (n=4128)
Effect of GCB v ABC Subtypes on Outcome

CHOP *Bolus* Treatment

Diffuse Large B Cell Lymphoma Subgroups are Clinically Distinct

5-yr Survival

<table>
<thead>
<tr>
<th></th>
<th>GCB DLBCL</th>
<th>ABC DLBCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival</td>
<td>60%</td>
<td>31%</td>
</tr>
</tbody>
</table>

$p = 7.9 \times 10^{-6}$
**GCB v ABC DLBCL**

- **BCL-2 impacts survival**
  - 25% v. 59% @ 8 yrs
  - (P < 0.001) CHOP-based

- **BCL-2 Expression**
  - Associated with ABC
  - ABC v GCB (p = 0.003)

(Blood 83:1460, 1994)
Effect of Rituximab on BCL-2

Clin Cancer Res 2001
Mar;7(3):709-23

BCL-2 Expression

CD20+

CD20−

BCL-2 Expression
Effect of Rituximab in BCL-2+ Tumors
NCI EPOCH v EPOCH-R

DA-EPOCH
- BCL-2+ (82%)
- BCL-2+ (50%)
Median Follow-up 60 mos
\[ p_2 = 0.04 \]

DA-EPOCH-R
- BCL-2+ (83%)
- BCL-2− (71%)
Median Follow-up 23 mos
Effect of Rituximab in BCL-2+ Tumors
GELA CHOP v R-CHOP

CHOP
- bcl2 – (45)
- bcl2 + (92)
OS, P=0.05

R-CHOP
- bcl2 – (54)
- bcl2 + (101)
OS, P=0.71
GCB v ABC Survival Outcome
EPOCH-R Infusional

GCB: 89%
ABC: 69%

\[ p_2 = 0.15 \]

Median Follow-up: 36 months
CALGB Phase III Randomized Study of R-CHOP v. DA-EPOCH-R with Microarray

ARM A: R-CHOP

ARM B: DA-EPOCH-R

Randomization

Tumor Biopsy

Blood Samples

Stage

Stage

Stage

Treatment completed if no change C5 to C7 staging

Repeat Blood Samples at Staging Proteomics/Pharmacogenomics

Time Line (weeks)
Targeting NF-κB in the ABC Subtype

Selective Expression of NF-kB Target Genes in Activated B-like DLBCL

<table>
<thead>
<tr>
<th>Gene</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclin D2</td>
<td>0.00001</td>
</tr>
<tr>
<td>IRF-4</td>
<td>0.00003</td>
</tr>
<tr>
<td>c-FLIP</td>
<td>0.00065</td>
</tr>
<tr>
<td>BCL-2</td>
<td>0.00308</td>
</tr>
<tr>
<td>CCR7</td>
<td>0.00665</td>
</tr>
<tr>
<td>IκB alpha</td>
<td>0.03110</td>
</tr>
</tbody>
</table>

Fold Relative Expression: 0.33, 0.67, 1.0, 1.5, 3.0
Bortezomib and NF-κB

- Bortezomib inhibits release of NF-κB dimers through inhibiting proteosome clearance of IκB

![Activation of the NF-kB Signaling Pathway](image)
Phase I/II Study of Bortezomib + EPOCH

- **Part A**
  - Bortezomib single agent

- **Part B**
  - DA-EPOCH + Bortezomib days 1 and 4
  - **Bortezomib Dose Escalated:**
    - Level I  0.5 mg/m²
    - Level II 1.0 mg/m²
    - Level III 1.5 mg/m²
    - Level IV  1.7 mg/m²
Phase I/II Study of Bortezomib + EPOCH

• Part A
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    - Level III 1.5 mg/m²
    - Level IV  1.7 mg/m²

ORR

6% (1/16)

23% (6/26)
Strategies in Mantle Cell Lymphoma

- Chemotherapy sensitive but persistent MRD
- Characterized by cell-cycle dysregulation

**Proliferative Signal**
- Cyclin D1 / cdk4
- Rb
- E2F → p14 ARF → mdm2
- Cyclin E / cdk2
- p27
- p21
- PROLIFERATION

**DNA DS Breaks**
- ATM 50%
- p53
- p16
- Methylated promoter
- More common in blastic

- Sequestration
- Deleted
- Deleted

**APOPTOSIS**
- BAX

Courtesy O. O’Connor
Tumor Proliferation

Variable Expression of Proliferation Signature Genes in Mantle Cell Lymphoma

Proliferation Signature Average

Mantle Cell Lymphoma Biopsies (n = 92)

CDC2
FLJ10517
tubulin-α
CENP-F
RAN
LC34790
FLJ10858
CIP2
HPRT
UHRF1
MCM2
HMG-2
DNA Pol E2
p55CDC
TFIIB
LC26191
Topoisomerase II α
PCNA
NF-IL6
DNA helicase PIF1
Tumor Proliferation Predicts Outcome

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

- Quartile 1: 6.7 yrs
- Quartile 2: 3.3 yrs
- Quartile 3: 2.3 yrs
- Quartile 4: 0.8 yrs

Overall Survival (years)
PFS Not Dependent on Proliferation Signature

EPOCH-R *Infusional*
Immune Eradication of MRD

• Idiotype Vaccine

Protocol Schema

- LN Bx
- EPOCH-R Begins
- EPOCH-R 6 cycles
- EPOCH-R Ends
- Id/KLH + GM-CSF s.c.
- Weeks
- n = 26

- EPOCH-R – Rituximab iv Day 1
  Continuous infusion Etoposide, Doxorubicin, Vincristine Days 1-5
  Cyclophosphamide iv Day 5
  Prednisone po Days 1-5
  Dose Adjustment

- Id-KLH+GM-CSF – 0.5 mg autologous Id + 0.5 mg KLH + 100 μg/m² GM-CSF
PFS and OS With Idiotype Vaccine

**Progression-Free Survival**

- Median follow-up 46 months
- Median PFS 22 months
- PFS 19% @ 4 years

**Overall Survival**

- OS 89% @ 4 years

- Median follow-up 46 months
### Inhibition of Cell-Cycle Targets

- **Proteosome inhibitor**

#### Known Proteosome Substrates

<table>
<thead>
<tr>
<th>CLASS</th>
<th>PROTEIN</th>
<th>PROTEIN FUNCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclins</td>
<td>Cyclins A, B, D E</td>
<td>Cell cycle</td>
</tr>
<tr>
<td></td>
<td><strong>Cdk Inhibitors</strong> <em>(p21 and p27)</em></td>
<td>Cyclin regulation</td>
</tr>
<tr>
<td>Tumor Suppressor</td>
<td>P53</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>Oncogenes</td>
<td>C-fos/c-jun</td>
<td>Transcription factor</td>
</tr>
<tr>
<td></td>
<td>C-myc</td>
<td>Transcription factor</td>
</tr>
<tr>
<td></td>
<td>N-myc</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>Inhibitory Proteins</td>
<td><strong>IkB</strong></td>
<td><strong>Inhibitor of NF-kB</strong></td>
</tr>
<tr>
<td></td>
<td>P130 / bcl-2</td>
<td><strong>Inhibitor of E2F-1</strong></td>
</tr>
<tr>
<td>Enzymes</td>
<td>Cdc25 phosphatase</td>
<td>CDK1/cyclin B phosphatase</td>
</tr>
<tr>
<td></td>
<td>Tyrosine aminotransferase</td>
<td>Tyrosine metabolism</td>
</tr>
</tbody>
</table>
## Summary of Recent Data
### Mantle Cell Lymphoma

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>CR/CRu</th>
<th>PR</th>
<th>ORR</th>
</tr>
</thead>
<tbody>
<tr>
<td>O’Connor et al (MSKCC) (ASH 2004)</td>
<td>26</td>
<td>3/2 (19%)</td>
<td>9 (38%)</td>
<td>54%</td>
</tr>
<tr>
<td>Strauss et al (St. Barts) (ASH 2004)</td>
<td>18</td>
<td>1 (6%)</td>
<td>6 (33%)</td>
<td>39%</td>
</tr>
<tr>
<td>Belch et al (NCIC) (ASH 2004)</td>
<td>28</td>
<td>1 (4%)</td>
<td>12 (43%)</td>
<td>46%</td>
</tr>
<tr>
<td>Goy et al (MDACC) (JCO 2005, ahead of print Dec 21, 2004)</td>
<td>29</td>
<td>6 (21%)</td>
<td>6 (21%)</td>
<td>41%</td>
</tr>
</tbody>
</table>
# Summary of Recent Data

## Indolent Lymphoma

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>CR/CRu</th>
<th>PR</th>
<th>ORR</th>
</tr>
</thead>
<tbody>
<tr>
<td>O’Connor (MSKCC) (ASH 2004)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>15</td>
<td>1/1 (13%)</td>
<td>7 (47%)</td>
<td>60%</td>
</tr>
<tr>
<td>SLL</td>
<td>5</td>
<td>0</td>
<td>1 (20%)</td>
<td>20%</td>
</tr>
<tr>
<td>MZL</td>
<td>6</td>
<td>0</td>
<td>3 (50%)</td>
<td>50%</td>
</tr>
<tr>
<td>Strauss et al (St. Barts)* (ASH 2004)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>11</td>
<td>0</td>
<td>2 (18%)</td>
<td>18%</td>
</tr>
<tr>
<td>Follicular</td>
<td>NA*</td>
<td>0/1</td>
<td>-</td>
<td>NA*</td>
</tr>
<tr>
<td>SLL</td>
<td>NA*</td>
<td>1/0</td>
<td>-</td>
<td>NA*</td>
</tr>
</tbody>
</table>
Inhibition of mTOR

Growth Factor

Cell Membrane

PI3K

Cell Growth

Regulate Translation of cell cycle regulatory proteins

PTEN

CCI-779/RAD001

mTOR

P70 S6K1

4E-BP1

Courtesy of O’Connor
# Phase II Study CCI-779 (Temsirolimus) Relapsed Mantle Cell Lymphoma

<table>
<thead>
<tr>
<th>Arm A: N = 34</th>
<th>Response: <strong>ORR: 38%</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CR – 1</td>
</tr>
<tr>
<td></td>
<td>PR – 12</td>
</tr>
<tr>
<td></td>
<td><strong>PFS Median (range)</strong></td>
</tr>
<tr>
<td></td>
<td>6.5 (2.9-8.3)</td>
</tr>
<tr>
<td></td>
<td><strong>Toxicity (Grade 3):</strong></td>
</tr>
<tr>
<td></td>
<td>Thrombocytopenia: 63%</td>
</tr>
<tr>
<td></td>
<td>Neutropenia: 23%</td>
</tr>
<tr>
<td>Arm B: N = 13</td>
<td>Response: <strong>ORR: 54%</strong></td>
</tr>
<tr>
<td></td>
<td>CR – 1</td>
</tr>
<tr>
<td></td>
<td>PR – 6</td>
</tr>
<tr>
<td></td>
<td><strong>PFS too early</strong></td>
</tr>
<tr>
<td></td>
<td>6/7 responses ≥ 2 months</td>
</tr>
<tr>
<td></td>
<td><strong>Toxicity (Grade 3):</strong></td>
</tr>
<tr>
<td></td>
<td>Thrombocytopenia: 23%</td>
</tr>
<tr>
<td></td>
<td>Neutropenia: 23%</td>
</tr>
</tbody>
</table>

Inhibition of Histone Deacetylation

Deacetylated Histones
  ↓
Transcriptional Repression of Pre-Programmed Set of Genes
  ↓
Cell Growth
  ↓
Tumor Growth

Hyperacetylated Histone
  ↓
Transcriptional Activation of Pre-Programmed Set of Genes
  ↓
Cell Growth Arrest, Differentiation and/or Apoptosis
  ↓
Inhibition of Tumor Growth
Phase I Study of Suberoylanilide Hydroxamic Acid (SAHA) Histone Deacetylase (HDAC) Inhibitor

- Inhibition of Histone H3 Deacetylation by SAHA in PBMC

Cyclin-Dependent Kinase Inhibitor

- Flavopiridol

Derived by synthesis from *Dysoxylum binectariferum*, plant indigenous to India

**CDK inhibitor**: Directly binds to the ATP binding site at nanomolar concentrations (e.g. cyclin D1)

Flavopiridol in Mantle Cell Lymphoma

<table>
<thead>
<tr>
<th></th>
<th>No Prior Therapy</th>
<th>Prior Therapy</th>
<th>Total # Pts. (n=28)</th>
<th>Duration</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete Remission</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Partial Remission</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>3.3</td>
<td>2.8-13.2</td>
</tr>
<tr>
<td>Stable Disease</td>
<td>7</td>
<td>13</td>
<td>20</td>
<td>3.4</td>
<td>1.4-10.3</td>
</tr>
<tr>
<td>POD</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Overall Response</td>
<td>2 / 11 [18%]</td>
<td>1 / 17 [6%]</td>
<td>24%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Kouroukis et al., 2003
Immunomodulatory Drugs (IMiD)

- Inhibition of TNF-α Production in Monocytes
  - Mediator of inflammation
  - Pro-angiogenic
  - Induces cellular proliferation

- T-cell Costimulatory Activity
  - Increased activation/proliferation
  - Increased cytokine production (IL-2, IL-12 and TNF-α)
  - Shift toward Th1 (helper) phenotype
  - Enhance NK and LAK activity
Survival Outcomes in Mantle Cell Lymphoma Thalidomide and Rituximab


PFS; Median 20.4 months

Overall Survival

Prior Therapy

PFS Thalidomide + Rituximab

Lenalidomide (CC-5013, Revlimid™)

New-Generation ImiD

Thalidomide

Lenalidomide

Courtesy of O’Connor

Stirling D. Semin Oncol. 2001;28:602
Protocol Construction

Opportunity window
For activity biomarkers

Maximize cytotoxicity and therapeutic index

Targeted Agent
Obtain pre and post Bx after one cycle

Chemotherapy + Targeted agent

Post-CT+ TA Randomize

Targeted Agent

Observation

Neoadjuvant Eradicate RTC
Protocol Construction

Bortezomib
Obtain pre and post Bx after one cycle

DA-EPOCH-R +
Bortezomib

Post-CT+ TA
Randomize

Bortezomib
X 18 months

Observation

Biomarkers
Microarray
Proteomics

Endpoints
EFS
OS
MRD
Correlate bortezomib biomarkers with outcome

MRD Detection
Acknowledgements

Clinical Studies

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Metabolism Branch

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