Detection of Burkitt Lymphoma immunoglobulin rearrangements in blood may have prognostic value

Katharine Lombardo
Outline

• B cell receptor in normal cells and in B cell malignancies

• B cell receptor in Burkitt Lymphoma
Outline

• B cell receptor in normal cells and in B cell malignancies

• B cell receptor in Burkitt Lymphoma
All B cells express a unique B cell receptor (BCR)

V gene segments

D gene segments

J gene segments

Immunoglobulin
Heavy chain (IGH)
All B cells express a unique B cell receptor (BCR)

V gene segments  D gene segments  J gene segments

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V gene segments  D gene segments  J gene segments

Immunoglobulin
Heavy chain (IGH)

V gene segments  J gene segments

Immunoglobulin
Light chain
The BCR sends survival, differentiation and proliferation signals
The BCR sends survival, differentiation and proliferation signals

Adapted from Young & Staudt. *Nature Reviews*. 2013
# BCR signaling in B cell lymphomas

<table>
<thead>
<tr>
<th>Role of BCR Signaling</th>
<th>Malignancy</th>
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BL patient cohort

- 19 BL tumor samples from Uganda Cancer Institute with 14 diagnostic patient-matched blood samples (13 post-treatment)
BL patient cohort

- 19 BL tumor samples from Uganda Cancer Institute with 14 diagnostic patient-matched blood samples (13 post-treatment)

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</tr>
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<td>37%</td>
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<td>Male</td>
<td>63%</td>
</tr>
<tr>
<td><strong>Age at enrollment</strong></td>
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<td><strong>HIV Status</strong></td>
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<td>95%</td>
</tr>
<tr>
<td>Positive</td>
<td>5%</td>
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<td><strong>Ziegler Disease Stage</strong></td>
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<td>A</td>
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- 9 BL tumor samples from NCI Ghana BL Study with 6 patient-matched serum samples and 9 patient-matched CSF samples
Long-term BL patient survival is 37%

Standard treatment regimen: 6 cycles of cyclophosphamide, vincristine and methotrexate chemotherapy
Long-term BL patient survival is 37%

Standard treatment regimen: 6 cycles of cyclophosphamide, vincristine and methotrexate chemotherapy
Deep sequencing of BL immunoglobulin genes

1. Cryopreserved BL tumor, blood, serum or CSF sample
2. Digested and lysed to extract gDNA
3. Amplification of Ig genes
4. High-throughput sequencing of the Ig repertoire in the tumor or blood sample
Clonal *IGH* rearrangements identified in 24/28 BL tumors

Top 50 unique sequences from representative BL patient tumors
Clonal *IGH* rearrangements identified in 24/28 BL tumors

Top 50 unique sequences from representative BL patient tumors

<table>
<thead>
<tr>
<th>Clonal</th>
<th>009-0184</th>
<th>009-232</th>
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<td>24/28</td>
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<table>
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<tr>
<th>Polyclonal</th>
<th>009-0192</th>
<th>H057520</th>
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BL BCR as a patient-specific biomarker

1. Identify unique *IGH* rearrangement

2. Probe for clonal circulating tumor-DNA (ct-DNA) sequence
BL BCR as a patient-specific biomarker

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<td>4/14 patients</td>
<td>10/14 patients</td>
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<tr>
<td>Serum</td>
<td>2/5 patients*</td>
<td>3/5 patients*</td>
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*Serum data from 1 patient still pending*
**BL BCR as a patient-specific biomarker**

1. Identify unique *IGH* rearrangement

2. Probe for clonal circulating tumor-DNA (ct-DNA) sequence

---

**Source** | **Negative for ct-DNA** | **Positive for ct-DNA**
---|---|---
Blood | 4/14 patients | 10/14 patients
Serum | 2/5 patients* | 3/5 patients*
CSF | 7/9 patients | 2/9 patients**

*Serum data from 1 patient still pending
**1 positive CSF sample did not match patient
BL BCR as a patient-specific biomarker

1. Identify unique \( IGH \) rearrangement

2. Probe for clonal circulating tumor-DNA (ct-DNA) sequence
1. Identify unique *IGH* rearrangement

2. Probe for clonal circulating tumor-DNA (ct-DNA) sequence

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<tbody>
<tr>
<td>Blood</td>
<td>11/13 patients</td>
<td>2/13 patients</td>
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BL BCR as a prognostic indicator at diagnosis

Uganda BL Patient Survival by Ziegler Stage

- Zeigler A
- Zeigler B
- Zeigler C
- Zeigler D

n=19
BL BCR as a prognostic indicator at diagnosis

Uganda BL Patient Survival by Ziegler Stage

- Zeigler A
- Zeigler B
- Zeigler C
- Zeigler D

n=19

BL Patient Survival by Tumor-Specific Ig Detection in Blood at Diagnosis

- No Evidence of Tumor in Peripheral Blood
- Tumor-Specific Ig Detected in Blood

p=.06

n=14
BCR as a therapeutic target

- RAF
- MEK1/2 (MAPK pathway)
- mTOR (PI3K pathway)
- Calcineurin (NFAT pathway)
- NF-kB (NF-kB pathway)

- BTK
- CBM (NF-kB pathway)
BCR as a therapeutic target

**PI3Kδ Inhibition by Idelalisib in Patients with Relapsed Indolent Lymphoma**

Ajay K. Gopal, M.D., Brad S. Kahl, M.D., Sven de Vos, M.D., Ph.D., Nina D. Wagner-Johnston, M.D., Stephen J. Schuster, M.D., Wojciech J. Jurczak, M.D., Ph.D., Ian W. Flinn, M.D., Ph.D., Christopher R. Flowers, M.D., Peter Martin, M.D., Andreas Viardot, M.D., Kristie A. Blum, M.D., Andre H. Goy, M.D., Andrew J. Davies, M.R.C.P., Ph.D., Pier Luigi Zinzani, M.D., Ph.D., Martin Dreyling, M.D., Dave Johnson, B.S., Langdon L. Miller, M.D., Leanne Holes, M.B.A., Daniel Li, Ph.D., Roger D. Dansey, M.D., Wayne R. Godfrey, M.D., and Gilles A. Salles, M.D., Ph.D.

**Targeting BTK with Ibrutinib in Relapsed or Refractory Mantle-Cell Lymphoma**

Michael L. Wang, M.D., Simon Rule, M.D., Peter Martin, M.D., Andre Goy, M.D., Rebecca Auer, M.D., Ph.D., Brad S. Kahl, M.D., Wojciech Jurczak, M.D., Ph.D., Ranjana H. Advani, M.D., Jorge E. Romaguera, M.D., Michael E. Williams, M.D., Jacqueline C. Barrientos, M.D., Ewa Chmielewska, M.D., John Radford, M.D., Stephan Stilgenbauer, M.D., Martin Dreyling, M.D., Wieslaw Wiktor Jedrzejczak, M.D., Peter Johnson, M.D., Stephen E. Spurgeon, M.D., Lei Li, Ph.D., Liang Zhang, M.D., Ph.D., Kate Newberry, Ph.D., Zhishuo Ou, M.D., Nancy Cheng, M.S., Bingliang Fang, Ph.D., Jesse McGreivy, M.D., Fong Clow, Sc.D., Joseph J. Buggy, Ph.D., Betty Y. Chang, Ph.D., Darrin M. Beaupre, M.D., Ph.D., Lori A. Kunkel, M.D., and Kristie A. Blum, M.D.
Conclusions & future directions

• The BCR in BL:
  • Patient-specific prognostic biomarker
  • Oncogene
  • Therapeutic target

• Measurement of residual disease to identify patients for salvage therapy
Acknowledgements

• BL patients and their families

• Warren Lab
  Edus H. Warren
  Andrea Towleron
  David Coffey
  Yuexin Xu
  Alicia Morales
  Scott Tykodi
  Sharon Ornales

• Fred Hutch
  Chris Carlson

• NCI Ghana BL Repository
  Robert Biggars
  Sam Mbulaiteye

• Fred Hutch Global Oncology
  Sarah Gerdts
  Corey Casper
THANK YOU
Top 50 unique $IGH$ sequences from all BL tumors

- 009-0184
- 009-232
- 009-0103
- 009-0109
- 009-0122
- 009-0128
- 009-0182
- 009-0186
- 009-0209
- 009-210
- 009-0249
- 009-0202
- H057518
- 009-0112
- 009-0148
- 009-0171
- HU14423
- H057519
- 009-0231
- 009-0174
- H058001
- H057521
- H003840
- H058003
- 009-0203
- 009-0192
- H032101
- H057520

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### A.

#### IGH Expression

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<thead>
<tr>
<th>Isotype</th>
<th>BL Sample Number (N=16)</th>
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<tbody>
<tr>
<td>IgM⁺ IgD⁺</td>
<td>13</td>
</tr>
<tr>
<td>IgG⁺</td>
<td>2</td>
</tr>
<tr>
<td>No Expression</td>
<td>1</td>
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### B.

#### IG κ/λ Expression

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<tbody>
<tr>
<td>IG κ⁺</td>
<td>12</td>
</tr>
<tr>
<td>IG λ⁺</td>
<td>3</td>
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