Short Telomeres Predict Poor Prognosis in Chronic Lymphocytic Leukemia

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Prognostic factors

- Clinical course is unpredictable with median survival ranging from 2 to 10 years.
- Closer monitoring with early treatment in high-risk subgroups.
- Good risk subgroups may require less therapy.

<table>
<thead>
<tr>
<th>CLINICAL</th>
<th>MOLECULAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher Rai staging</td>
<td><em>IgHv unmutated</em> status</td>
</tr>
<tr>
<td>Shorter lymphocyte doubling time</td>
<td>β2-microglobulin (+)</td>
</tr>
<tr>
<td>Shorter time to first treatment</td>
<td>CD 38 (+)</td>
</tr>
<tr>
<td>Age &gt; 70</td>
<td>ZAP70 (+)</td>
</tr>
<tr>
<td>FISH: del(17p), del(11q), trisomy (12q)</td>
<td>Absence of del(13q)</td>
</tr>
</tbody>
</table>
Limitations with existing factors

• Majority of patients present with early stage disease, but an easy to measure and reliable predictive marker is presently not available.

• Standard predictive markers may be less useful in patients >75 years.
What are telomeres?


Telomeres shorten with age

Shorter telomere lengths are associated with increased mortality.
Cawthon R. Lancet. 2003. 361; 393.
CLL patients have shorter telomeres

- CLL cell telomeres are short.
- Short CLL telomeres associated with molecular markers of poor prognosis and short survival.
Our study is unique

• Investigates the telomere length (TL) in non-CLL (buccal) cells in CLL patients to determine:
  o Whether TL in buccal cells (BC) predicts for co-morbidities and patient outcome.
  o Whether the predictive value of CLL cell TL is influenced by adjusting for changes in BC – TLs.
## Definition

<table>
<thead>
<tr>
<th></th>
<th>CLL</th>
<th>SLL</th>
<th>MBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>B cell count in blood</td>
<td>&gt;5 x 10⁹/L</td>
<td>&lt;5 x 10⁹/L</td>
<td>&lt;5 x 10⁹/L</td>
</tr>
<tr>
<td>Lymphadenopathy/splenomegaly</td>
<td>Maybe</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

60% mutated \( IgHv \)

40% unmutated \( IgHv \)

**MBL:** Monoclonal B cell lymphocytosis  
**CLL:** Chronic lymphocytic leukemia  
**SLL:** Small lymphocytic lymphoma

# Patient Characteristics

## (CCMB CLL Roche Cohort Database)

### Clinical

<table>
<thead>
<tr>
<th>Clinical</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean = 65.5, Median = 66</td>
</tr>
<tr>
<td>Gender</td>
<td>F: 73, M: 122</td>
</tr>
<tr>
<td>Rai stage</td>
<td>MBL: 28, 14%</td>
</tr>
<tr>
<td></td>
<td>Rai 0: 90, 46%</td>
</tr>
<tr>
<td></td>
<td>Rai 1: 30, 15%</td>
</tr>
<tr>
<td></td>
<td>Rai 2: 9, 5%</td>
</tr>
<tr>
<td></td>
<td>Rai 3: 6, 3%</td>
</tr>
<tr>
<td></td>
<td>Rai 4: 8, 4%</td>
</tr>
<tr>
<td>SLL</td>
<td>23, 12%</td>
</tr>
<tr>
<td>Treatment</td>
<td>Treated: 77, 39%</td>
</tr>
<tr>
<td></td>
<td>Untreated: 118, 61%</td>
</tr>
<tr>
<td>Survival</td>
<td>77%</td>
</tr>
</tbody>
</table>

### Molecular

<table>
<thead>
<tr>
<th>Molecular</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>(IgHv)</td>
<td>Mutated: 120, 63%</td>
</tr>
<tr>
<td></td>
<td>Unmutated: 72, 38%</td>
</tr>
<tr>
<td>(CD38)</td>
<td>Positive: 48, 27%</td>
</tr>
<tr>
<td></td>
<td>Negative: 132, 73%</td>
</tr>
<tr>
<td>(ZAP70)</td>
<td>Positive: 46, 25%</td>
</tr>
<tr>
<td></td>
<td>Negative: 140, 75%</td>
</tr>
<tr>
<td>(\beta2) micro</td>
<td>&lt;mean: 144, 85%</td>
</tr>
<tr>
<td></td>
<td>&gt;mean: 26, 15%</td>
</tr>
<tr>
<td>LDT (mo)</td>
<td>&lt;6: 9, 5%</td>
</tr>
<tr>
<td></td>
<td>6-12: 36, 21%</td>
</tr>
<tr>
<td></td>
<td>&gt;12: 125, 74%</td>
</tr>
<tr>
<td>Comorbidity</td>
<td>&lt;2: 129, 66%</td>
</tr>
<tr>
<td></td>
<td>≥2: 67, 34%</td>
</tr>
<tr>
<td>Second cancer</td>
<td>Yes: 51, 26%</td>
</tr>
<tr>
<td></td>
<td>No: 144, 74%</td>
</tr>
</tbody>
</table>
Methods

• CLL Roche Cohort database:
  o 195 CLL/SLL patients from time of diagnosis.
  o Recruited 2007-2011.

• Isolated genomic DNA from:
  o CLL cells
  o Buccal cells
  o Control lymphocytes from volunteers.

• Relative telomere length determined by multiplex real-time PCR.
  o Telomere and control β-globin gene specific primers.
  o Results expressed as telomere/standard (t/s) ratio.

• Statistical analysis with SAS and Prism softwares
  o Median t/s ratio as cut-off for normal and short telomeres.
Telomeres in **CLL cells** of CLL Patients
Shorter CLL-TLs are associated with poorer prognostic factors

LDT

- Relative Telomere Length (t/s)
- >12 months
- <12 months

P = 0.004

Rai stage

- Relative Telomere Length (t/s)
- Early Stage
- Late Stage

P = 0.02

Treatment

- Relative Telomere Length (t/s)
- Treatment Naive
- Treated

P < 0.0001

ZAP70

- Relative Telomere Length (t/s)
- ZAP70 (-)
- ZAP70 (+)

P = 0.05

CD38

- Relative Telomere Length (t/s)
- (-)
- (+)

P = 0.003

ß2 Microglobulin

- Relative Telomere Length (t/s)
- <mean
- >mean

P = 0.008

OR 3.67 95%CI (1.9-7.1)

OR 2.56 95%CI (1.3-5.2)

OR 5.3 95%CI (2.4-11.6)

OR 2.46 95%CI (1.2-5.2)

OR 3.43 95%CI (1.6-7.3)

OR 2.34 95%CI (0.90-6.34)
Shorter CLL-TLs associate with unmutated \textit{IgHv} status

Unmutated \textit{IgHv} is the ONLY prognostic factor that independently predicts short telomeres in multivariable regression analysis.
Shorter CLL-TLs are associated with poor survival

**Vital Stats**
- Relative Telomere Length (t/s)
  - Alive
  - Deceased
  - P=0.05

**Survival Based on Telomere Length**
- Normal: N=82
- Short: N=85
- P=0.04

<table>
<thead>
<tr>
<th>Outcome</th>
<th>P-value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progression</td>
<td>&lt;0.0001</td>
<td>3.5</td>
<td>1.9-7.1</td>
</tr>
<tr>
<td>LDT &lt;12 mths</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>&lt;0.001</td>
<td>4.8</td>
<td>2.5-9.2</td>
</tr>
<tr>
<td>Sec. Cancer</td>
<td>0.31</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Medial survival:**
- Normal = 69 mths
- Short = 59 mths
Telomeres in **buccal cells** of CLL Patients
Telomere lengths are shortest in CLL cells

Mean: 1.23 2.27 0.61
Median: 0.98 2.06 0.53

**P=0.009**

P=0.27  **P<0.0001**
Only BC telomeres shorten with age

- Buccal telomere: P = 0.011
- CLL telomere: P = 0.47
## Telomere length in buccal cells

<table>
<thead>
<tr>
<th></th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmutated <em>IgHv</em></td>
<td>0.19</td>
</tr>
<tr>
<td>ZAP70+</td>
<td>0.73</td>
</tr>
<tr>
<td>CD38+</td>
<td>0.10</td>
</tr>
<tr>
<td>β2-microglobulin</td>
<td>0.32</td>
</tr>
<tr>
<td>Rai staging</td>
<td>0.97</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.70</td>
</tr>
<tr>
<td>LDT</td>
<td>0.60</td>
</tr>
<tr>
<td>Co-morbidities</td>
<td>0.73</td>
</tr>
<tr>
<td>Second malignancies</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>OR 2.12  95%CI (0.95-4.97)</td>
</tr>
<tr>
<td>Survival</td>
<td>0.44</td>
</tr>
</tbody>
</table>
Do BC-TLs increase prognostic utility of CLL-TL?

**Rai stage**
- P = 0.46
- OR 1.49 95%CI (0.74-3.04)

**ZAP70**
- P = 0.09
- OR 1.9 95%CI (0.9-4.0)

**CD38**
- P = 0.20
- OR 1.6 95%CI (0.8-3.4)

**β2-microglobulin**
- P = 0.08

**Relative Telomere Length (t/s)**
- P = 0.02
- OR 2.2 95%CI (1.1-4.2)
- P = 0.004
- OR 2.75 95%CI (1.34-6)
- p<0.0001
- OR 5.04 95%CI (2.5-10.2)
Discussion

• BC telomeres in CLL patients shorten with aging, but does not correlate with total comorbidities.
  o Determine subtypes of comorbidities.

• Short CLL-TLs is associated with unmutated IgHv and can identify a subgroup of patients with poorer prognosis.
  o Is this a dynamic process? Does telomere length attrition rate change with worsening prognosis?

• Short CLL-TLs can predict disease progression and poor survival.
  o Whether the predictive value of CLL-TLs can be enhanced by combining BC-TLs.
THANK YOU

- Dr. James Johnston
- Dr. Spencer Gibson and lab
- Dr. Versha Banerji and lab
- Dr. Yunli Zhang
- Dr. Sara Beiggi
- Liz Henson
- Robert Schmidt
- Manitoba Tumor Bank (Mandy Squires, Donna Hewitt, Michelle Brown)
Can telomere length predict disease progression in MBL patients

- P = 0.44
- Survival: p = 0.54
- LDT: p = 0.54
- Treated: p = 0.54
- Sec cancer: p = 1.0
2008 NCI guidelines

- Absolute B lymphocyte count in the **peripheral blood $\geq 5 \times 10^9$/L**.

- Demonstration of **clonality** of the circulating B lymphocytes by flow cytometry of the peripheral blood.

- A majority should express these monoclonal B cell markers:
  - extremely low levels of SmIg and kappa or lambda light chains; and
  - expression of B cell associated antigens (**CD19, CD20, and CD23**); and
  - expression of the T cell associated antigen **CD5**.

*Peripheral blood smear of a CLL patient showing presence of smudge cells*

Reference: uptodate.com
Can MBL-TL predict progression to CLL?

MBL patients with short telomeres

MBL patients with normal telomeres

Telomere length p=0.54

IgHv mutational status p=insufficient data

#pt number
(IgHv mutational status)

→ alive

→ deceased
Can MBL-TL predict progression in MBL?

<table>
<thead>
<tr>
<th>Telomere length</th>
<th>Normal</th>
<th>Short</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>7</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Yes</td>
<td>5</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>12</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity: 55%
Specificity: 58%
PPV: 0.54
NPV: 0.58
LR(+): 1.3
LR(-): 0.78

Can MBL-TL predict progression in MBL?
Do BC telomere lengths increase prognostic utility of CLL telomere lengths?

**NO LONGER SIGNIFICANT**
- ZAP70
  - P = 0.11
  - OR 1.76 95% CI (0.87-3.54)
- CD 38
  - P = 0.31
  - OR 1.44 95% CI (0.72-2.89)
- Rai stage
  - OR 1.49 95% CI (0.74-3.04)
  - P = 0.26
- B2microglobulin
  - P = 0.07

**STILL SIGNIFICANT**
- treatment
  - P = 0.004
  - OR 2.75 95% CI (1.34-6)
- LDT
  - OR 1.91 95% CI (1.03-3.54)
  - P = 0.04
- Mutational status
  - p < 0.0001
  - OR 4.22 95% CI (2.19-8.15)
  - Still maintains multi-variable regression analysis.