Chronic Lymphocytic Leukemia
- Disease and Current Therapies from a Medical Perspective

C. Reinhardt
Medical Clinic I, Hematology/Oncology
CECAD, Research Area C
Clinical and Molecular Oncology
CLL – an introduction

- What is CLL?
- Diagnostic tools
- Prognostic scores
- The biology of CLL
  - the cell of origin dilemma
  - cytogenetic aberrations
  - mutational landscape in CLL
  - clonal evolution and therapy resistance
- Science becomes medicine (novel therapeutic approaches)
R. Virchow coins the term leukemia (white blood) in 1847
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The blood smear reveals essential hints leading to the diagnosis.
CLL is a disease of the elderly → a minority of patients qualifies for toxic therapy

- Most frequent leukemia in the Western hemisphere.
- Median age at diagnosis: 72 years
- Elderly patients may have comorbidities

<table>
<thead>
<tr>
<th>Age at CLL diagnosis (years)</th>
<th>Patients (%)</th>
<th>Mean comorbidities (all cancer types, n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 54</td>
<td>11</td>
<td>n/a</td>
</tr>
<tr>
<td>55–64</td>
<td>19</td>
<td>2.9</td>
</tr>
<tr>
<td>65–74</td>
<td>27</td>
<td>3.6</td>
</tr>
<tr>
<td>75+</td>
<td>43</td>
<td>4.2</td>
</tr>
</tbody>
</table>

The Rai classification allows risk stratification

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>Median survival (months)</th>
<th>Risk status (Modified Rai)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Lymphocytosis, lymphocytes in blood &gt;15,000/mcL and &gt;40% lymphocytes in the bone marrow</td>
<td>140</td>
<td>Low</td>
</tr>
<tr>
<td>I</td>
<td>Stage 0 with enlarged node(s)</td>
<td>100</td>
<td>Intermediate</td>
</tr>
<tr>
<td>II</td>
<td>Stage 0–1 with splenomegaly, hepatomegaly, or both</td>
<td>70</td>
<td>Intermediate</td>
</tr>
<tr>
<td>III</td>
<td>Stage 0–II with hemoglobin &lt;11.0 g/dL or hematocrit &lt;33%</td>
<td>20</td>
<td>High</td>
</tr>
<tr>
<td>IV</td>
<td>Stage 0–III with platelets &lt;100,000/mcL</td>
<td>20</td>
<td>High</td>
</tr>
</tbody>
</table>

* Adapted from the 2008 NCI guidelines; BC Cancer Agency 2008 guidelines.
The Rai classification allows risk stratification.
The Binet classification allows risk stratification

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Hemoglobin $\geq 10 \text{ g/dL}$ and platelets $\geq 100,000/\text{mm}^3$ and $&lt;3$ involved nodal areas</td>
</tr>
<tr>
<td>B</td>
<td>Hemoglobin $\geq 10 \text{ g/dL}$ and platelets $\geq 100,000/\text{mm}^3$ and $\geq 3$ involved nodal areas</td>
</tr>
<tr>
<td>C</td>
<td>Hemoglobin $&lt;10 \text{ g/dL}$ and or platelets $&lt;100,000/\text{mm}^3$ and any number of involved nodal areas</td>
</tr>
</tbody>
</table>

*Adapted from the 2008 NCI guidelines.\(^3\)

\(^\dagger\)Areas of involvement considered for staging are as follows: (1) Head and neck, including the Waldeyer ring (this counts as one area, even if more than one group of nodes is enlarged). (2) Axillae (involvement of both axillae counts as one area). (3) Groins, including superficial femorals (involvement of both groins counts as one area). (4) Palpable spleen. (5) Palpable liver (clinically enlarged).
The Binet classification allows risk stratification
A novel, molecularly-guided risk score allows more detailed risk stratification

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adverse factor</th>
<th>Hazard ratio for death</th>
<th>Factor - grading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosomal aberration</td>
<td>del(17p)</td>
<td>6.0</td>
<td>6</td>
</tr>
<tr>
<td>s-TK</td>
<td>&gt; 10.0 U/L</td>
<td>2.8</td>
<td>2</td>
</tr>
<tr>
<td>s-β2m</td>
<td>&gt; 3.5 mg/L</td>
<td>2.3</td>
<td>2</td>
</tr>
<tr>
<td>IgHV mutational status</td>
<td>unmutated</td>
<td>1.9</td>
<td>1</td>
</tr>
<tr>
<td>s-β2m</td>
<td>&gt; 1.7 mg/L - ≤ 3.5 mg/L</td>
<td>1.7</td>
<td>1</td>
</tr>
<tr>
<td>ECOG</td>
<td>&gt; 0</td>
<td>1.7</td>
<td>1</td>
</tr>
<tr>
<td>Chromosomal aberration</td>
<td>del(11q)</td>
<td>1.4</td>
<td>1</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>1.3</td>
<td>1</td>
</tr>
<tr>
<td>Age</td>
<td>&gt; 60 years</td>
<td>1.3</td>
<td>1</td>
</tr>
</tbody>
</table>
A novel, molecularly-guided risk score allows more detailed risk stratification

Overall Survival and Risk Groups Using a Weighted Score of Clinical, Biological and Gene Variables (n=1223)

- Low Risk, 0-2 points (N=300)
  Survival after 5 years: 95.2%

- Intermediate risk, 3-5 points (N=460)
  Survival after 5 years: 86.9%

- High risk, 6-10 points (N=410)
  Survival after 5 years: 67.7%

- Very high risk, > 6 points (N=53)
  Survival after 5 years: 18.7%

p < 0.0001
CLL is the most common leukemia in the Western world.
What is the cell of origin???
B-cell development can go awry at each step along the way

**Bone marrow**
- Pre-BCR
- VpreB
- V\(_{\text{L}5}\)
- Large pre-B cell
- Small pre-B cell
- Immature B cell

**Spleen or lymph node**
- BCR
- Mature B cell
- Follicle
  - B-CLL
  - MCL
  - Mutations in RUNX1, PBX1, MLL, PTPN11 and/or RAS
  - B-ALL
  - BCR–ABL1 translocation
  - Mutations in RAG

**Marginal zone**
- SMZL
  - Mutations in NOTCH2
  - MALT lymphoma
  - Mutations in MALT1 or BCL10
- Follicular lymphoma
  - Mutations in BCL2
  - DLBCL
  - Mutations in BCL6
- Burkitt’s lymphoma
  - Mutations in MYC
- Germinal centre B cell
- Memory B cell
- Plasma cell
- Multiple myeloma
  - Mutations in CCND1, MAF, FGFR3 or IRF4

**Follicle**
- Marginal zone B cell
- Germinall centre B cell

**Marginal zone (secondary follicle)**
- Germinall centre
- Memory B cell
- Plasma cell

**Germinal centre**
- Mutated B-CLL

**Follicular lymphoma**
- Mutations in BCL2
- DLBCL
- Mutations in BCL6
- Burkitt’s lymphoma
- Mutations in MYC

**CLL clones can emerge before and after Ig hypermutation**
There is something special about CLL
CLL cells are microenvironment-addicted
CLL cells are microenvironment-addicted
Summary I

- CLL is the most common leukemia in the Western World
- CLL is characterized by the accumulation of mature lymphocytes
- Multiple risk scores exist and allow patient stratification
- CLL cells are addicted to micro-environmental stimuli
- Transformation can occur before or after somatic hypermutation
Cancer is a genetic disease
Boveri hypothesizes that chromosomes carry the genetic information (1902!)
Boveri postulates that chromosomal imbalances are the underlying cause of malignant disease (1914!)
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The result is a malignant transformation
P. Rous discovers a chicken sarcoma virus – and postulates that cancer is a transmittable disease (1911!)
The transforming viral genetic material is also present in non-transformed eucaryotic cells!
The SRC gene encodes for an oncogenic tyrosine kinase
B. Weinberg identifies H-Ras$^{V12}$ – the first human oncogene (1980)
A. Levine findet p53 - das Onkogen, das keines war (1979–89)
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Cancer is a collection of genetic diseases
The biology of EGFR signaling
The human EGFR L858R mutant is oncogenic in mice

EGFR\textsuperscript{L858R} lung adenocarcinomas are oncogene-addicted!

Politi et al., 2006
EGFR-specific inhibitors are available

Politi et al., 2006
First line gefitinib outperforms standard carboplatin/paclitaxel in EGFR mutant non-small cell lung cancer

Hazard ratio <1 implies a lower risk of progression in the M+ group than in the M- group

M+, mutation positive; M-, mutation negative

Mok et al., 2009
CLL is a genetic disease
CLL is a genetic disease

Döhner et al NEJM 2000 343:1910
were eventually treated after sample collection and again confirmed this finding \((p = 0.02)\). In these 42 patients, a higher number of subclonal mutations was not correlated with a shorter time to treatment \((\text{correlation coefficient} = 0.03; p = 0.87)\). Thus, therapy prior to sample was associated with a higher number of subclonal mutations, and furthermore, the number of subclonal sSNVs detected increased with the number of prior therapies \((p = 0.011, \text{Table S1})\).

Cancer therapy has been theorized to be an evolutionary bottleneck, in which a massive reduction in malignant cell numbers results in reduced genetic variation in the cell population \((\text{Gerlinger and Swanton, 2010})\). It is likely that the overall diversity in CLL is diminished after therapeutic bottlenecks as well. Because most of the genetic heterogeneity within a cancer is present at very low frequencies \((\text{Gerstung et al., 2012})\)—below the level of detection afforded by the sequence coverage we generated—we were unable to directly assess reduction in overall genetic variation.

However, in the range of larger subclones that were observable by our methods \((>10\% \text{ of malignant cells})\), we witnessed increased diversity after therapy \((\text{Figure 2 D})\). Although the available data cannot definitively rule out extensive diversification following therapy, this increase likely results, at least in part, from outgrowth of pre-existing minor subclones \((\text{Schuh et al., 2012}; \text{Wu, 2012})\). This may result from the removal of dominant clones by cytotoxic treatment, eliminating competition for growth and allowing the expansion of one or more fit subclones to frequencies above our detection threshold. Further supporting our interpretation that fitter clones grow more effectively and become detectable after treatment, we observed an increased frequency of subclonal driver events \((\text{which are presumably fitter})\) in treated relative to untreated patients \((\text{Figure 2 D, bottom})\) \((\text{note that driver events include CLL driver mutations}\ [\text{Figure 1 A}])\).

Inferring the Order of Genetic Changes Underlying CLL

While general aspects of temporal evolution could not be completely resolved in single time point WES samples, the order of driver mutation acquisition could be partially inferred from the aggregate frequencies at which they are found to be clonal or subclonal. We considered the 149 samples as a series of "snapshots" taken along a temporal axis. Clonal status in all or most mutations affecting a specific gene or chromosomal lesion would suggest that this alteration was acquired at or prior to the most recent selective sweep before sampling and hence could be defined as a stereotypically early event. Conversely, predominantly subclonal status in a specific genetic alteration implies a likely later event that is tolerated and selected for only in the presence of an additional mutation.

This strategy was used to infer temporal ordering of the recurrent sSNVs and sCNAs \((\text{Figures 3 A and S4})\). We focused on alterations found in at least three samples within the cohort of 149 CLL samples. We found that three driver mutations—\text{MYD88} \((n = 12)\), trisomy 12 \((n = 24)\), and hemizygous del \((13q) \((n = 70)\)—were clonal in \(80\%–100\%\) of samples harboring these alterations, a significantly higher level than for other driver events \((q < 0.1, \text{Fisher exact test with Benjamini-Hochberg FDR}) [\text{Benjamini and Hochberg, 1995}]\), implying that they arise earlier in typical CLL development. Mutations in \text{HIST1H1E}, although...
CLL is a dynamic disease and clonal evolution represents a clinical challenge
CLL clones can acquire additional genetic aberrations

CLL at presentation
- del13q14 (50%)
- +12 (15%)
- ATM (15%)
- TP53 (5–10%)
- NOTCH1 (10%)
- SF3B1 (5–10%)
- BIRC3 (4%)
- MYD88 (3–5%)

Chemorefractoriness
- TP53 (40%)
- BIRC3 (25%)
- SF3B1 (25%)
- ATM (25%)
- NOTCH1 (25%)
- del13q14 (50%)

RS
- TP53 (60%)
- NOTCH1 (30%)
- MYC (28%)
- del13q14 (20%)
- +12 (15%)
- ATM (12%)
- SF3B1 (5%)
- BIRC3 (0%)
Therapeutic interventions shift the selective pressure.
The therapy is often directed at a particular genetic context which may not be shared by all subclones. This relationship between therapy and genetic adaptation is likely to result in convergent evolution, in which a mutation that confers resistance will become highly prevalent in relapsed disease. Indeed, this process has been reported in relapsed T-cell ALL after treatment with nucleoside-analog chemotherapy drugs.

An alternative process contributing to the emergence of continuously more aggressive clones may be entirely independent of differential sensitivity to therapy (Figure 2c). We recently observed a higher number of large subclones (4% of cancer cells) in 149 CLL cases that were exposed to treatment before sampling compared with patients who received therapy after the sample was obtained. This finding of increased clonal diversity with treatment held true even after accounting for potential confounders, such as longer follow-up time. We interpret this observation to result, at least in part, from the outgrowth of many diverse pre-existing minor but fit subclones. This latter interpretation is further supported by our observation of an increased frequency of subclonal-driver events (presumably fitter) in treated relative to untreated patients. Overall, our data support the idea that CLL therapy, by markedly reducing disease bulk, may act as a classic evolutionary restriction point and reset interclonal dynamics.

Within this conceptual framework, when subclones with high fitness already exist within a tumor population, treatment could favor the development of more aggressive clones, potentially reducing post-relapse survival. In this context, cytotoxic therapy would effectively remove the incumbent clone—acting like a 'mass extinction' event—and thereby shift the evolutionary landscape in favor of one or more aggressive subclones. Thus, highly fit subclones probably benefit from treatment and exhibit rapid outgrowth. These data provide mechanistic support to the observation that the 'watch and wait' strategy for...
Multiple competing clones might exist in the same patient.
How do these mutations re-wire the intracellular signaling networks in CLL?
Genetic evidence for re-wired signaling in CLL

- DNA damage
- ATM
- p53
- Puma
- Noxa
- apoptosis
- miR-15A
- miR-16-1
- UCH-L1
- UBL?
- NK cell
- NKp30L
- NKp46L
- NKG2DL
- MCL1
- BCL2
- proliferation
- AKT
- IKK
- NFkB
- MDM2
- 13q deletion
- 11q deletion
- 17p deletion
- 17q trisomy
- Normal
- BAFFR
- CXCR
- CCR
- CD40
- BCR
- TLR
- Myd88
- CCND1
- CCNE
- Notch
- MYC
- FBXW7
- CCNE
- FBXW7
- MYC
- FBXW7
- Notch
- NKp30L
- NKp46L
- NKG2DL
- UBL?
- UCH-L1
- NK cell

Döhner et al. NEJM 2000 343:1910
Genetic evidence for re-wired signaling in CLL

DNA damage
- ATM
- p53
- Puma
- Noxa

Oncogenic stress
- CHD2
- Pot1

Apoptosis
- UCH-L1

Proliferation
- AKT
- NFκB
- IKK
- IKK
- MCL1
- BCL2

Notch
- TLR
- Myd88
- CCND1
- BTK
- LCK
- LYN

CD40
- BCR

BCR
- CXCR
- CCR

BAFFR

NK cell
- NKp30L
- NKp46L
- NKG2DL

p300
- CBP

mRNA
- miR-15A
- miR-16-1

NFκB
- Myc
- CCNE

FBXW7

CHD2
- Pot1

CDN
- CD3

UBL?

Genetic evidence for re-wired signaling in CLL

NK cell
- NKp30L
- NKp46L
- NKG2DL

p300
- CBP

mRNA
- miR-15A
- miR-16-1

NFκB
- Myc
- CCNE

FBXW7

CHD2
- Pot1

CDN
- CD3

UBL?
Genetic evidence for rewired signaling in CLL
Imbalance of two competing groups of pathways

- apoptosis
- proliferation
The efforts of the CRU-286 are focused on developing novel, personalized therapeutic approaches for CLL patients.
Three fundamentally distinct approaches have to be pursued to overcome this challenge!

restoration of mutant tumor suppressor genes is currently not a viable option in CLL therapy.

Approach I

Thus, molecular liabilities associated with these genetic lesions have to be identified and therapeutically exploited. (RP1, RP2, RP3)
Three fundamentally distinct approaches have to be pursued to overcome this challenge!

Approach II

Restoration of the pro-apoptotic DDR might be possible through pharmacologic intervention, if ATM and p53 are not mutated.

Thus, apoptosis repressive signaling cascades have to be identified and inhibited. (RP5, RP6)
Three fundamentally distinct approaches have to be pursued to overcome this challenge!

Approach III

DDR-mediated recognition of malignant cells through the immune system needs to be exploited for CLL treatment in the framework of combination immuno-therapies to overcome uncontrolled survival signals (RP4, RP6).
Unique strengths of this CRU

strong basic science

- DNA
- γH2AX
- AATF

strong core support

- CECAD

robust model systems

University Hospital

active clinical study group
Summary II

- CLL is the most common leukemia in the Western World
CLL clones can emerge before and after Ig hypermutation.