Mature B-cell lymphoproliferative disorders

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DISCLOSURES OF COMMERCIAL SUPPORT

<table>
<thead>
<tr>
<th>Name of Company</th>
<th>Research support</th>
<th>Employee</th>
<th>Consultant</th>
<th>Stockholder</th>
<th>Speaker’s Bureau</th>
<th>Advisory Board</th>
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Chronic Lymphocytic Leukaemia / Small Lymphocytic Lymphoma

- Incidence 7.1 per 100K per year
- Abnormal B-cells in the blood (>5 x 10^9/L), bone marrow and or tissues
- B-cells co-express CD19, CD5 and CD23 with weak sIg, CD79b, CD20, CD22.
- Treatment depends on clinical features: cytopenia, rate of progression, lymphadenopathy, ~85% do not require treatment at presentation in UK
- Precursor syndrome MBL (<5 x 10^9/L) incidence 2.6/100K/year: ~1% progression to CLL per year
- “low-count” MBL (<0.5 x 10^9/L) – no known clinical consequences

The genomic landscape in CLL

13q14 deletion is the most common abnormality in CLL but also other in disorders, e.g., ~10% of mantle cell lymphoma

Pathway analysis of inherited susceptibility loci show a role for GC-transition & apoptosis

IGHV mutation status and BCR signalling

~30% of patients with CLL carry immunoglobulin receptors with highly similar primary sequence
**The Unique IgH Gene Sequence**

Bone Marrow: 
IGHV re-arrangement

- P-looping of complimentary nucleotides onto ss signal joint
- N-3’ insertion of 1-20 nucleotides by TdT
- Exonuclease trimming of unpaired nucleotides

Lymph nodes: 
Affinity Maturation

**Features associated with oncogenesis vs. disease progression**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>CLL-type MBL (clinic MBL)</th>
<th>CLL-like B-cells (low-count MBL)</th>
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<tbody>
<tr>
<td>Phenotype</td>
<td>CD19+CD5−CD20+</td>
<td>CD19+CD5−CD20+</td>
</tr>
<tr>
<td>Inherited pre-disposition SNP</td>
<td>As CLL</td>
<td>As CLL</td>
</tr>
<tr>
<td>Chromosomal Abnormalities</td>
<td>13q14 deletion common; 11q23/17p deletions rare</td>
<td>13q14 deletion in some cases; 11q23/17p deletions v. rare</td>
</tr>
<tr>
<td>IGHV repertoire</td>
<td>Similar to CLL (most common: IGHV 3-07, 1-69, 4-34, 3-23)</td>
<td>Different to CLL</td>
</tr>
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</table>

**BCR pathway inhibitors in CLL:**
high response rates & durable remissions

Simplified overview of potential B-cell-receptor-mediated events related to antigen- and superantigen-binding in CLL.

**Chronic lymphocytic leukaemia is driven by antigen-independent cell-autonomous signalling**

**Distinct homotypic B-cell receptor interactions in CLL**

(a) The Fab molecules in both the CLL183 and CLL240 crystals interact through VH CDR3-dominated contacts. The heavy and light chains of the Fab acting as ‘receptor’ are coloured light blue and pink, with the VH CDR3 loop highlighted in red. The chains in the ‘antigen’ molecule are coloured blue and cyan (VH and CH1 domains), and violet and magenta (VK and CL), respectively. (b) Molecular surface of the epitope, with the amino acids from VH CDR3 involved in the binding interactions shown as sticks coloured as in the previous panel.
Mechanisms and clinical impact of Bcl-2 dysregulation in CLL

- **13q14 deletion:**
  - miR-15/miR-16 family
  - Downregulate BCL2 gene expression → del13q14 leads to Bcl-2 over-expression.
- Inherited Susceptibility
  - Genetic polymorphisms that impact on BCL2 family
  - Trisomy 12
  - Lower bax/bcl-2 ratio
- BLC2-IGH translocation

Venetoclax (BH3-mimetic) can effect MRD-negative remissions: treatment cessation is a possibility

Summary

- CLL: no driver mutation, expansion driven by BCR-signalling (possibly autonomous) coupled with reduced propensity for apoptosis

MRD is an independent predictor of outcome

CR with MRD >1% equivalent to PR

<table>
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<tr>
<th>MRD Level</th>
<th>Equivalent to</th>
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<tbody>
<tr>
<td>MRD 1 = &lt;10%</td>
<td>1 in 10 (&lt;10^-1) MRD</td>
</tr>
<tr>
<td>MRD 2 = &lt;1%</td>
<td>1 in 100 (&lt;10^-2) MRD</td>
</tr>
<tr>
<td>MRD 3 = &lt;0.1%</td>
<td>1 in 1000 (&lt;10^-3) MRD</td>
</tr>
<tr>
<td>MRD 4 = &lt;0.01%</td>
<td>1 in 10000 (&lt;10^-4) MRD</td>
</tr>
<tr>
<td>MRD 5 = &lt;0.001%</td>
<td>1 in 100000 (&lt;10^-5) MRD</td>
</tr>
<tr>
<td>MRD 6 = &lt;0.0001%</td>
<td>1 in 1000000 (&lt;10^-6) MRD</td>
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</table>

Impact of MRD on long-term survival
MRD in CLL as an intermediate endpoint for licensure: requires a quantitative method that is not influenced by the polyclonal background with prospective validation

Development of 'MRD' as a regulatory endpoint:

1. Identify key features and clinical trials
2. Develop assay
3. Standardisation of assay
4. Apply standardised assay prospectively
5. Apply to regulatory action

Typical Leukemia Positive (>20%)

Stop MRD4 (<0.01%) confirmed in BM.

MRD as an endpoint needs a highly validated quantitative assay

- Requires a quantitative method that is not influenced by the polyclonal B-cell background
- PFS/GS benefit is detectable with a 1-2 log depletion
- MRD assay is ideally quantitative to ±0.3 log
- Prospective validation: multi-parameter cytometry and qPCR
- Validated assays require “limit of detection” for each result stated on the report
  - Cellular assays: number of events acquired
  - Molecular assays: total DNA, patient-specific IGH effects
- Combination of both ≥6-CLR flow cytometry and HTS is optimal
- Should MRD assessment be performed in PB or BM?

MRD as an intermediate endpoint

Improving Flow Cytometry MRD Detection

Leads routine: CD19 gating + core + ROR1 & CD23

MRD in CLL as an intermediate endpoint for licensure: requires a quantitative method that is not influenced by the polyclonal background with prospective validation

Stop Rx 6M after confirmed MRD <0.01%

Ibrutinib costs £55K/patient/yr

UK NHS accepts up to ~£30K/yr

Funded for relapsed/refractory or 17p del/mut TN but concerns about side effects, selection of resistant clones and high cost

UK trials aimed at stopping therapy

PB monitoring until 6M sustained MRD4 (<0.01%) confirmed in BM.

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- Should MRD assessment be performed in PB or BM?
The Outcome for Patients with MRD Detectable in BM but Not in PB (PB- / BM +) Differs According to Type of Therapy

- **FCR-based therapy**
  - 3 yrs median follow-up
  - Log-Rank \( z = 17.1020, p < 0.0001 \)
  - PFS similar to PB-BM+ in the short term

- **Alemtuzumab therapy**
  - Log-Rank \( z = 53.2562, p < 0.0001 \)
  - PFS similar to PB+BMB+ in the short term


The Outcome for Patients with MRD Detectable in BM but Not in PB (PB- / BM +) Differs According to Type of Therapy

- **Ibrutinib + Venetoclax**
  - 4 yrs median follow-up
  - Log-Rank \( z = 53.2562, p < 0.0001 \)
  - PFS similar to PB+BMB+ in the short term

- **Ibrutinib/Idelalisib**
  - Log-Rank \( z = 17.1020, p < 0.0001 \)
  - PFS similar to PB+BMB+ in the short term


Compartment effect

- PB MRD <0.01%: patient may have extensive disease during or shortly after antibody therapy
- It is possible to assess the compartment effect per treatment
  - Alemtuzumab: BM ≥2.4 log higher than PB, 52% BM+PB-
  - FC×Rituximab: BM ≥0.9 log higher than PB, 22% BM+PB-
  - Ibrutinib/Idelalisib: BM ≥0.2 log higher than PB, 2% BM+PB-
  - Ibr+Obinutuzumab: BM ≥0.5 log higher than PB, 5% BM+PB-
  - Ibr+Venetoclax: BM ≥0.3 log higher than PB, 10% BM+PB-
- PB is acceptable for monitoring (during and after therapy) but BM may be required if there is a significant compartment effect
- PB >1% BM uninformative

Summary

- CLL: no driver mutation, expansion driven by BCR-signalling (possibly autonomous) coupled with reduced propensity for apoptosis
- BCR/BCL2-pathway inhibitor combinations are effecting deep remissions and MRD monitoring is being used to guide treatment duration

Kinetics of response to BCR-pathway inhibitors

- Rapid (4-24 hrs) entry of proliferating cells into blood. Peripheral counts peak at week 1 as proliferation starts to decline

Loss of normal proliferating CLL cell expression profile during ibrutinib therapy

- CKCR4 requires BTK for internalisation and signalling. Ibrutinib → rapid loss of homing to proliferation centres
The dynamic cellular kinetics of chronic lymphocytic leukemia B cells

The birth and death rates of CLL cells vary between patients.

Measuring the kinetics of response to BCR-pathway inhibitors: MRD analysis to determine "CLL halving-time"

Exponential depletion unless plateau reached.

Clonal evolution leading to ibrutinib resistance in chronic lymphocytic leukemia

The rate of fall is rapid in all patients with a median of 3 log reduction in CLL level after 8 weeks of combined therapy.

Hillmen et al., Poster, IWCLL, 2017, Board 421

Summary

• CLL: no driver mutation, expansion driven by BCR-signalling (possibly autonomous) coupled with reduced propensity for apoptosis
• BCR/BCL2-pathway inhibitor combinations are effecting deep remissions and MRD monitoring is being used to guide treatment duration
• CLL cells have a birth rate, potentially abrogated by BCR-pathway inhibition, and a death rate, potentially enhanced by BCL2-pathway inhibition → exponential decline in disease until plateau

Inhibiting BCR signalling

Analysis of CLL Syk phosphorylation: ? Unsuitable even as exploratory endpoint?

<table>
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<tr>
<th></th>
<th>CLL cell %</th>
<th>CLL medium</th>
<th>CLL fold change</th>
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<tr>
<td>No inhibitor</td>
<td>2.42</td>
<td>0.31</td>
<td>1.00</td>
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<tr>
<td>Inhibitor</td>
<td>1.46</td>
<td>0.22</td>
<td>0.71</td>
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<tr>
<td>IgM/D</td>
<td>34.08</td>
<td>1.04</td>
<td>3.35</td>
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<tr>
<td>Inhibitor + IgM/D</td>
<td>24.18</td>
<td>0.72</td>
<td>3.32</td>
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</table>
Analysis of CLL and T-cell Syk phosphorylation:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CLL cell</th>
<th>CLL fold change</th>
<th>T-cell fold change</th>
<th>CLL/T-cell MFI ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>No stimulus</td>
<td>2.42</td>
<td>0.31</td>
<td>1.00</td>
<td>0.30</td>
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<tr>
<td>Inhibitor</td>
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<td>2.32</td>
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Fold change in phospho-Syk/Akt after Ig stimulation in CLL cells from patients on ibrutinib

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- CLL cells have a birth rate, potentially abrogated by BCR-pathway inhibition, and a death rate, potentially enhanced by BCL2-pathway inhibition → exponential decline in disease until plateau
- Potential efficacy of inhibitor therapies readily & rapidly assessable using Phosflow in vitro for drug development and in vivo for clinical trials

Lymphoplasmacytic lymphoma / Waldenstrom Macroglobulinaemia – simple model.

- B cell component
  - Symptoms related to tumour bulk
  - Anaemia
  - B symptoms
  - Lymph nodes
  - Spleen
- Plasma cell component
  - Symptoms attributable to M-protein
  - Hyperviscosity syndrome
  - Neuropathy
  - Haemolytic anaemia
  - Cryoglobulinaemia
  - Immunodeficiency

MYD88 L265P: driver mutation in the majority of LPL/WM and IgM MGUS

- MYD88 mutation:
  - Spontaneous homodimerization and recruitment of IRAK1/4
  - Promotes NF-κB also by activating Bruton’s tyrosine kinase (BTK)
Mantle cell lymphoma – first line treatment

MCL – second line treatment over time

LYMPHOID NEOPLASIA

The genomic landscape of Waldenström macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell lymphomagenesis

Treon et al, NEJM; 2015

Response to ibrutinib associated with CXCR4 mutation status

<table>
<thead>
<tr>
<th>Response Rate</th>
<th>Mutated MYD88 and Wild-type CXCR4 (N = 10)</th>
<th>Mutated MYD88 and CXCR4 WT (N = 8)</th>
<th>Wild-type MYD88 and CXCR4 WT (N = 8)</th>
<th>P Value</th>
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<tr>
<td>Overall</td>
<td>100</td>
<td>85.7</td>
<td>40</td>
<td>&lt;0.001</td>
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<tr>
<td>Major</td>
<td>91.7</td>
<td>61.9</td>
<td>0</td>
<td>&lt;0.001</td>
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</table>

Treon et al; NEJM, 2015

Acquired mutations associated with ibrutinib resistance in Waldenstrom Macroglobulinemia

Key points

- BTK (exons 20, 22) mutations, including multiple mutated variants within individual patients are common in ibrutinib-progressing WM patients.
- BTK (exon 22) mutations were associated with mutated CXCR4 in WM patients progressing on ibrutinib.

HMRN real world data
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- Potential efficacy of inhibitor therapies readily & rapidly assessable using Phosflow in vitro for drug development and in vivo for clinical trials
- Btk-inhibition also effective in MYD88-mutated disorders, potentially modulated by capacity for developing resistance mutations, and has promising activity in mantle cell lymphoma

CD22 vs. CD20: B-cell CD20 is lost but CD22 expression persists during anti-CD20 therapy

High-Throughput Sequencing Shows Good Linearity to One CLL Cell in One Million Leukocytes

Issues to be resolved for quantitative HTS using universal primers

Correction factors
- Calculations based on addition of reference DNA
- Estimation of total leukocytes,
- Amplification bias → probably requires calculation of quantitative range per patient as qPCR
- Replica amplicons and non-functional rearrangements

False positive results
- ERIC: no "CLL-associated" sequences present in an unrelated sample at the level of 0.010% or greater;
- Four CLL-associated sequences were detected in one or more unrelated cases, representing a median 0.00080% (range 0.00046-0.00199%)

Prospective Validation

Developing a Mathematical Model Based on Partitions of Residual Disease Assuming a Lognormal Distribution: "Cure" Target <=1 CLL Cell in 10 Million Leucocytes?


Design and timing of assay is critical

Duration of stimulus may have significant impact

Viability of cells is critical

IgM+IgD stimulation → optimal 2 minutes

Day 0 analysis not possible → all analyses on Day 1

TAP trial development: Talha Munir


Manne BK, J Biol Chem. 2015 May 1;290(18):11557-68. Syk protein activation downstream of ITAM/hemITAM receptors in platelets