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Clinical History

- 61 year-old asymptomatic male with no significant prior clinical history
- Slowly progressing lymphocytosis first diagnosed in 2014
- Current CBC:
  - WBC 16.1 k/ul with absolute lymphocyte count of 10.7 k/ul
  - hemoglobin 14.7 gm/dL
  - MCV 90 fL
  - platelet count 219 k/ul
  - Differential count: neutrophils 25%, lymphocytes 67%, monocyte 6%, eosinophils 1%, basophils 1%
- No lymphadenopathy or FDG avid lesions on skull base to mid-thigh PET/CT
- No skin lesions
- Peripheral blood smear review, flow cytometry and fluorescence in situ hybridization, and bone marrow exam with flow cytometry, cytogenetic and molecular genetic testing have been performed
Peripheral Blood Flow Cytometry
Bone marrow biopsy
Bone marrow biopsy
Bone marrow aspirate smear
Cytogenetic and Molecular Genetic Studies

- 46,XY[20] (bone marrow)
- Fluorescence in situ hybridization performed on blood sample:
  - rearrangement involving TCL1A (14q32) in 29% nuclei
  - 17p13.1 deletion in 64% nuclei
  - deletion of chromosome 11 centromere in 60% nuclei
  - monosomy 13 in 60% of nuclei
  - 6q23 deletion in 21% of nuclei
- TCRG@ – clonally rearranged
- Mutation TP53 G334V with allelic frequency of 55% (blood and bone marrow)
  - Negative for mutations of JAK-STAT pathway, cell cycle and epigenetic regulators in T-PLL
- P53 and STAT5B (phosphorylated) are not expressed by immunohistochemistry
Proposed diagnosis

T-cell prolymphocytic leukemia in indolent phase
T-cell Prolymphocytic Leukemia (T-PLL)

Formulating a diagnosis

• Morphology
• Immunophenotype
• TCR gene rearrangement
• Genetic alterations
  – Cytogenetics
  – Molecular
T-PLL: Morphology

- Irregular nuclear membrane; mature, condensed chromatin, prominent nucleolus; cytoplasm-basophilic with protrusions

- Prolymphocytic morphology

- Small cell variant - chronic lymphocytic leukemia-like morphology (6%-19% with much higher frequency 38% in Japanese population)

- Sézary cell-like - cerebriform morphology (2%-7%)

- Multilobated morphology- Adult T-cell leukemia like (5%)
T-PLL: Immunophenotype

- Pan-T-cell antigens: CD2+, CD3+(dim, surface), CD5+, CD7+ (bright)
- CD4/CD8 variable
  - CD4+ CD8- (60-65%)
  - CD4+ CD8+ (17-21%)
  - CD4- CD8+ (13-15%)
  - CD4- CD8- (1-8%)
- Others:
  - CD52+, CD26+, TCL1 +
  - TdT -, CD1a-, CD25- (rare positive)
- Postulated normal counterpart: Post-thymic mature T-cell

**T-PLL: Cytogenetic abnormalities**

- **TCL1A/TCL1B** (14q32.1) (80%)
- **MTCP1** (Xq28)

**TCL1 family**  +  **TCR α/δ (14q11)**  =  **PI3K-AKT-mTOR pathway activation**

**Diagram:**
- **TCL1** as Coactivator
  - **PI3K** → **AKT** → **M-TOR**
  - **IKKα - nF-κB**
  - **BAD - BCL2**
  - **MDM2 - p53**
  - **FOXO - Cyclin D1**
  - **GSK3B - B-catenin**
  - **ACL - Fatty acid synthesis**
  - **Survival**
  - **Proliferation**
  - **Cell growth**

Are rearrangements of *TCL1* family genes sufficient for leukemogenesis?

- *TCL1* or *MTCP1* transgenic mice develop mature T-PLL like leukemia (15-20 months):
  - *TCRB*@ monoclonal
  - CD3+/CD8+ immunophenotype predominant in most mouse models
  - Both prolymphocytic and small cell morphology were identified
- “Pre-leukemic phase” characterized by:
  - Lymphocytosis (75-90% blood lymphocytes vs. normal ~60%)
  - *TCL1* positive T cell aggregates in spleen – which may be polyclonal by *TCRB*@

- p13\(^{MTCP1}\) oncoprotein showed a dose - response relation with leukemia incidence
- Early and aggressive presentation in a transgenic line with intermediate level p13\(^{MTCP1}\) expression- potential role of other genetic factors
- Double transgenic mice *MTCP1* + *TEL-JAK2* die significantly faster

T-PLL: Cytogenetic abnormalities

- **ATM** (11q22.3): deletions, loss of heterozygosity/biallelic deletions
  - Ataxia telangiectasia patients may have circulating T lymphocytes with chromosome 14 abnormalities without clonal TCR gene rearrangements
  - Narducci et al. described ataxia telangiectasia patient with a persistent T-cell population with inv14 and expansion of this population from 4% to 60% over nearly 8 years without leukemic manifestations

- Other anomalies
  - Gains of 8q and losses of 8p (70-80%)
  - Deletions of 12p13 (43%)
  - Chromosome 6 (33%)
  - Chromosome 17 alterations (33%)
    - P53 over-expressed in all cases with allele deletion or any chromosome 17 anomaly
  - 22q alterations (6%)

# T-PLL: JAK-STAT pathway

JAK-STAT pathway mutations involve 76% T-PLL cases

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Cases involved</th>
<th>Molecular alterations</th>
<th>Protein Domain Involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL2RG</td>
<td>X</td>
<td>2%</td>
<td>G268_M270del*, K315E*</td>
<td>Transmembrane domain, other regions</td>
</tr>
<tr>
<td>JAK1</td>
<td>1</td>
<td>8%</td>
<td>R629_D630del*, V658F, S703I, T901R*</td>
<td>Pseudokinase domain</td>
</tr>
<tr>
<td>JAK3</td>
<td>19p</td>
<td>30%-42%</td>
<td>M511I, A573V, R657W*, Q507P, K563_C565del*</td>
<td>SH2- Pseudokinase junction, Pseudokinase domain</td>
</tr>
<tr>
<td>STAT5B</td>
<td>17</td>
<td>7%-36%</td>
<td>T628S*, N642H, R659C*, Y665H, Q706L*</td>
<td>SH2 domain</td>
</tr>
</tbody>
</table>

* Novel mutation

Targeting JAK-STAT pathway


Extracellular space
Cell Membrane
Cytoplasm
Nuclear membrane

IL2

IL2R

JAK1

JAK3

Pimozide

Ruxolitinib (JAK1/2)
Tofacitinib (JAK1/3)

Higher sensitivity to ruxolitinib than to tofacitinib
High resistance of STAT5B (p.N642H) to JAK inhibitors

Reduce phosphorylated STAT5 levels
Reduce STAT5 transcriptional activity
Induce apoptosis in T-PLL cells

Transcriptional activation

T-PLL: Additional Molecular Abnormalities

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Cases involved</th>
<th>Alteration</th>
<th>Domain/Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>11q22</td>
<td>70%</td>
<td>Frameshift, nonsense and missense</td>
<td>FAT and PI3K domains</td>
</tr>
<tr>
<td>EZH2</td>
<td>7</td>
<td>13%</td>
<td>Frameshift, nonsense and missense</td>
<td>Transcriptional repressor</td>
</tr>
<tr>
<td>CHEK2</td>
<td>22</td>
<td>5%</td>
<td>Frameshift, Missense</td>
<td>Protein kinase -DNA repair</td>
</tr>
<tr>
<td>FBWX10</td>
<td>17</td>
<td>7.5%</td>
<td>Frameshift, nonsense and missense</td>
<td>Ubiquitin ligase</td>
</tr>
<tr>
<td>TET2</td>
<td>4</td>
<td>17%</td>
<td>Missense mutations</td>
<td>DNA methylation</td>
</tr>
<tr>
<td>BCOR</td>
<td>X</td>
<td>8%-9%</td>
<td>Missense mutations</td>
<td>Histone deacetylation</td>
</tr>
<tr>
<td>TP53</td>
<td>17p</td>
<td>14%</td>
<td>Missense mutation</td>
<td>DNA repair</td>
</tr>
</tbody>
</table>

High selective drug sensitivity scores:
- CDK inhibitor
- P53 activator
- MDM2 inhibitors

From Wagner et al. (Eds.) Cancer Signalling
T-PLL: Clinical - Molecular correlation

<table>
<thead>
<tr>
<th>TCL1 family Cytogenetic anomaly</th>
<th>JAK-STAT pathway mutation</th>
<th>Case% (number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>Present or absent</td>
<td>20% (10)</td>
</tr>
<tr>
<td>Present or absent</td>
<td>Absent</td>
<td>24% (12)</td>
</tr>
<tr>
<td>Absent</td>
<td>Absent</td>
<td>6% (3)</td>
</tr>
</tbody>
</table>

(Kiel et al. 2014)

- In general, JAK-STAT pathway mutation status did not correlate with overall survival

- However, JAK3 mutation carry a poor prognosis on median overall survival (OS)
  - $JAK3^{mut}$ vs. $JAK3^{wt}$ OS: 11 months (n=7) vs. 37 months (n=33); P=0.018 (Stengel et al. 2015)
  - $JAK3^{mut}$ vs. $JAK3^{wt}$ OS: 15 months (n=14) vs. 48 months (n=17); P=0.008 (Andersson et al. 2017)
  - $JAK3^{p.M511I}_{mut}$ vs. all patients median OS: 15.1 months vs. 27.1 months, trend noted (Kiel et al. 2014)

Molecular evolution in T-PLL

Random mutations
Non-neoplastic cells

1st hit (transforming)
Chromosome 14 and X rearrangements

Random mutations
Chromosome abn. such as 8q and 11q

2nd Hit
Subclone with aggressive mutations

Phase 1 (indolent)

Common precursor with Aggressive mutation

(Rashidi et al. Eur J Haematol. 2015)
Final Panel Diagnosis

T-cell prolymphocytic leukemia