T-Lymphoproliferative Diseases

WHO 2016 update: recognition of indolent "Lymphoma/LPD"

- Pediatric-type FL
- Duodenal-type FL
- In situ follicular/mantle cell neoplasia
- EBV+ monocytoid B-cell

* Breast implant-associated ALCL
* Primary cutaneous CD4+ small/medium T-cell LPD
* Primary cutaneous CD8+ T-cell lymphoma

Recurrent genetic alterations in mature T/NK-cell leukemias

<table>
<thead>
<tr>
<th>Disease</th>
<th>Genetic/molecular alteration</th>
<th>Frequency</th>
<th>Effect</th>
</tr>
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<tbody>
<tr>
<td>T-PLL</td>
<td>TRADD-TGFBBI fusion, TRADD-MYC fusion</td>
<td>20%</td>
<td>Aberrant TGFbeta expression and activation of MYC pathway</td>
</tr>
<tr>
<td>T-PLL</td>
<td>BRAFV600E, ATM1043S, ATM1043D</td>
<td>70%</td>
<td>Aberrant ATM1043S expression and activation of ATM pathway</td>
</tr>
<tr>
<td>T-PLL</td>
<td>ATM deletion/mutation</td>
<td>70%</td>
<td>Aberrant ATM1043S expression and activation of ATM pathway</td>
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<tr>
<td>T-PLL</td>
<td>PDCD1LG2 mutations</td>
<td>30-40%</td>
<td>Aberrant PDCD1LG2 expression and activation</td>
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<tr>
<td>CLPD-NK</td>
<td>STAT3 mutations</td>
<td>75%</td>
<td>Aberrant STAT3 expression and activation</td>
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<tr>
<td>ATLL</td>
<td>AK3 mutations</td>
<td>70%</td>
<td>Aberrant AK3 expression and activation</td>
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<tr>
<td>SS</td>
<td>TET2 deletion</td>
<td>70%</td>
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Recurrent genetic alterations in mature T/NK-cell leukemias

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<td>ALK+ACLL</td>
<td>t(2;5)(p23;q35) (NPM-ALK)</td>
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<td>ALK-ACLL</td>
<td>DUSP22-HIP4 focus on 6p25.3 (30%)</td>
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<tr>
<td>AITL</td>
<td>(ind T/NK-like PDL1/NOG)</td>
</tr>
<tr>
<td>PKCI-NOS</td>
<td>&quot;GATA3&quot; (FABSA, CCA, CD10, CCB, ETV6, and AKT)</td>
</tr>
<tr>
<td>HTCL</td>
<td>11q22 (CTC-ALK)</td>
</tr>
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The table above lists the recurrent genetic alterations in mature T/NK-cell leukemias. The table includes several genes and their respective alterations such as TRADD-TGFBBI, ATM, T-PLL, CLPD-NK, ATLL, SS, etc. The effect of these alterations is also mentioned, which includes aberrant expression or activation of various pathways.
Detection and classification of T-cell neoplasms is a challenge...

- Lower incidence (than B-cell disorders)
- Diverse and heterogenic group of disorders
- Classification driven by pathology/clinical
- Even within one entity, immunophenotypic heterogeneity
- Immunophenotypic overlaps with normal, reactive
- Clonality assessment cumbersome
- Few entities with recurrent genetic aberrations for confirmation

as a consequence:

- Knowledge of normal immunophenotypic pattern is essential
- Knowledge of typical phenotypes of T/NK-NHLs is important
- Diagnosis cannot be established solely based on immunophenotype

Surrogate information

- Clinical indication for sending material?
- Lymphocytosis
- Morphologically atypical lymphocytes
- Organomegaly (spleen/liver)
- Lymphnode enlargement
- Effusions
- Skin lesions, erythrodermia
- Eosinophilia*
- Fever*, wasting* (B-symptoms), rashes*
- Autoimmune phenomena*: cytopenia, hemolytic anemia
- B-cell deregulation*: hyper/hypogammaglobulinemia
- Age, gender, ethnicity
- EBV, HTLV status

* Paraneoplastic manifestations (cytokine production, cytotoxic activity)

How to recognize and classify T NHLs with immunophenotyping?

- Recognizing abnormal and the differentiation from activated phenotypes
  - Altered expression (up/downregulation)
  - Abnormal patterns
  - In combination with scatter characteristics

- Maturation stage?

- Expression of classification antigens?
  - CD30, cyTCL1, CD279

Progressive differentiation

- Effector function
- Antigen dependence

Mahnke et al. Eur J Immunol 2013
### Cell Origin of T-cell Lymphoma

**PLL**

De Leval & Gaulard, ASH Educational Book, 2008

### Antigens investigated in T-cell malignancies

- **pan T-cell**: CD3, CD4, CD8, CD7, CD2, CD5, TCR\(\alpha\)b, TCR\(\gamma\)d, CD45
- **co-stimulatory antigens**: CD28, CD27, CD26
- **maturiation-related**: CD45RA, CD45RO
- **homing receptors**: CCR7 (CD197)
- **activation-related**: CD38, HLA-DR, CD25, CD11b, CD11c
- **NK-associated**: CD16, CD94
- **cytotoxicity-related**: CD56, CD57, Granzyme, Perforin
- **classification markers**: CD30 (ALCL), CD10 (AITL), cyTCL1 (PLL), CD279/PD-1 (AITL)

### T-cell lymphoma cases: Panel used

#### Screening PacB PacO FITC PE PerCP Cy5.5 PECy7 APC APC-7H APM

<table>
<thead>
<tr>
<th>Case</th>
<th>CD4</th>
<th>CD45</th>
<th>CD7</th>
<th>CD69/CD16</th>
<th>CD19</th>
<th>smCD3</th>
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1) Exclusion of doublets and debris:
2) Select lymphocytes:
   - double check in individual plots (e.g. SSC/CD3)
3) Lymphocytes: select B, T
4) check B-cells
   - Plasma cells/T- progenitors?
   - Kappa/lambda should overlap for CD19 and CD20
5) check NK-cells
   - Include: CD56+/CD8-/CD16-
   - Only CD4-
6) check T-cells

TCRγδ pos cells

All other T-cells:

Normal T-cell subpopulations:
CD4+CD8+ (dim) CD4+ (dim)CD8+ CD4-CD8-

Normal Patterns of activation: CD5 and CD38

naive recently activated late activated/ terminally differentiated

Reactive T cells in a 10y old with EBV infection

Screening: what to look for

Green: normal T-cells
Grey: B- and NK-cell
Red: abnormal T-cells

Example:

large granular lymphocyte leukemia (LGL)

Prolymphocytic leukemia (PLL)

T-cell lymphoma cases: Panel used

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T-cell lymphoma cases: Panel used

Maturation stages
Different possible combinations: shown CCR7/CD27/CD45RA/CD45RO

Normal Patterns of activation

naive
recently activated
late activated/
terminally differentiated

Pictograms from M. Lima, Porto, Portugal
T-cell lymphoma cases: Panel used

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Cy: cytoplasmic, Perf: perforin, Grz: granzyme

TCL1: T-cell lymphoma breakpoint 1

Over-expression of this oncogene is due to translocation in proximity of TCR-regulatory elements. -> classification marker for PLL

www.control-T.de

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**Normal Patterns of activation**

- **naive**
- **recently activated**
- **late activated/terminally differentiated**

### CD30 expression in T-NHL

**Algorithm for differential diagnosis of NHL**

- **Anaplastic Lymphoma (ALCL)**
- **Large Granular Lymphocytic Leukemia (T-LGL)**
- **Prolymphocytic Leukemia (T-PLL)**
- **Angioimmunoblastic T-cell Lymphoma (AITL)**

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**Pictograms by M. Lima, Porto, Portugal**

- **naive**
- **recently activated**
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Strategy for immunophenotypic classification of T-cell neoplasms

based on «normal counterparts»

- identify, quantify, and characterize abnormal T- or NK-cell population
- establish clonality criteria: aberrant immunophenotypes, TCRβ usage
- maturation stage [naive, memory, effector]
- functional properties (expression of NK/cytotoxicity-related antigens)
- activation status [resting/activated]
- sample type/cell type -> localization (leukemic, nodal, extranodal)

What are the most relevant aberrant immunophenotypic features and are they compatible with any disease entity?

**your case match 0/7?**

**WHO entities**

- based on clinical, histopathology, morphology, genetics and immunophenotype
Thank you for your attention

thanks to:
Margarida Lima, Porto, Portugal
Julia Almeida, Salamanca, Spain