Molecular genetics of peripheral T-cell lymphomas

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## WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

### Entity | Changes in 2017 classification
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**Peripheral T-cell lymphoma (PTCL), NOS** | Subsets based on phenotype and molecular abnormalities being recognized that may have clinical implications but are mostly not a part of routine practice at this time.

**Nodal T-cell lymphomas with T follicular helper (TFH) phenotype** | An umbrella category created to highlight the spectrum of nodal lymphomas with a TFH phenotype including angioimmunoblastic T-cell lymphoma, follicular T-cell lymphoma and other nodal PTCL with a TFH phenotype (specific diagnoses to be used due to clinicopathologic differences).

**ALK-negative anaplastic large cell lymphoma** | Overlapping recurrent molecular/cytogenetic abnormalities recognized that potentially could impact therapy.

**Breast implant-associated anaplastic large cell lymphoma** | Now a definite entity that includes cytogenetic subsets that appear to have prognostic implications (eg, 6p25 rearrangements at IRF4/DUSP22 locus).

**New provisional entity distinguished from other ALK- ALCL; non-invasive disease associated with excellent outcome.**
Peripheral T-cell Lymphoma, Not Otherwise Specified (NOS)
GEP identified specific signatures for PTCLs/NOS, AITL and ALCLs

Molecular profiling improves classification and prognostication of nodal peripheral T-cell lymphomas: results of a phase III diagnostic accuracy study.
Gene expression signatures delineate biological and prognostic subgroups in peripheral T-cell lymphomas

Iqbal J et al., Blood 2014
COO classification impact on prognostication

Iqbal et al., Blood. 2014;123(19):2915-2923
Genomic Complexity: T-bet vs GATA-3 PTCL

Heavican, et. al., 2018
GATA-3: Therapeutic target?

- APC
- T-cell
- TCR
- ITK
- NF-κB
- GATA-3
- Chemotherapy Resistance

Others:
- PI3K/AKT/mTOR
- Inhibition (Ixazomib)
- HDACi
- ITK inhibitor
- Proteasome Inhibition

Quantitative – ↓ GATA-3 Expression

Impaired GATA-3 activity

Wang et al., *Clin Cancer Res*, 2017;23(10):2506-15
Classification of PTCLs/NOS according to their cellular counterparts

Piccaluga PP et al., in preparation
Lennert lymphoma is an independent entity

- Rare morphological variant of PTCL/NOS
- Originally recognized by Karl Lennert as «lymphoepitelioid lymphoma»

- Cell of origin?
- Specific Genetics/GEP?
- Better clinical outcome?

Etebari M et al, 2019
Lennert lymphoma is an independent entity

Consistent cellular derivation from «cytotoxic» CD4+ T-cells

Epithelioid cells are M0-M1 macrophages

Etebari M et al, 2019
Lennert lymphoma is an independent entity

Etebari M et al, 2019
Lennert lymphoma is an independent entity

miRNA profiling indicated further differences

MTOR/PI3K pathway is a suitable therapeutic target in PTCL

Etebari M et al, 2019
Etebari M et al, 2019
Nodal TFH-related Peripheral T-cell Lymphomas
Nodal TFH-related PTCLs

- Angioimmunoblastic T-cell lymphoma (AITL)
- Follicular T-cell lymphoma (FTCL)
- PTCL/NOS with TFH phenotype

TFH-related marker
- CD10
- CD279/PD1
- ICOS
- SAP
- CXCL13
- CCR5
- BCL6

Genetic abnormality
- TET2
- IDH2
- DNMT3A
- RHOA
- CD28
- ITK/SYK
- CTLA4/CD28

Huang 2009; Lemonnier 2012
AITL, FTCL, and PTCL/NOS are distinct based on GEP

Etebary M et al., in preparation
FTCL is distinct from AITL

Etebari M et al, in preparation
The genetic features of AITL

Epigenome
- TET2 47-83%
- DNMT3A 20-30%
- IDH2 20-45%

G17V RHOA
- RHOA 50-70%

TCR signaling
- PLCG1 14%
- CD28 9-11%
- FYN 3-4%

Sakata-Yanagimoto, Nature Genetics 2014
Palomero, Nature Genetics 2014
Vallois, Blood 2016
To examine the clonal evolution in both tumor and tumor-infiltrating B cells

Number of mutated samples (Number of mutations)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Samples</th>
<th>Mutations</th>
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</thead>
<tbody>
<tr>
<td>TET2</td>
<td>16 (26)</td>
<td></td>
</tr>
<tr>
<td>RHOA</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>DNMT3A</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>IDH2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>NOTCH1</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Enrichment of each fraction by LMD

Tumor cells: anti PD1 Ab
B cells: anti CD20 Ab
Pre-LMD, Post-LMD

Leica LMD 7500

Tran, Sakata-Yanagimoto, Blood Cancer J 2017
Specific distribution of mutations in PTCL

Number of mutated samples (Number of mutations)

<table>
<thead>
<tr>
<th></th>
<th>TET2</th>
<th>RHOA</th>
<th>DNMT3A</th>
<th>IDH2</th>
<th>NOTCH1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD1+CD20+</td>
<td>19</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PD1+</td>
<td>7</td>
<td>10</td>
<td>3</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>CD20+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

Tran, Sakata-Yanagimoto, Blood Cancer J 2017
Multi-step and multi-lineage clonal evolution may contribute to AITL development.

- Premalignant cells
- Hematopoietic Progenitors
- Aging
- Premalignant cells
- BONE MARROW
- RHOA mut
- IDH2 mut
- TET2 mut
- DNTM3A mut
- NOTCH1 mut
- EBV infection
- AITL tumor cells
- Infiltrating B cells
- BONE MARROW
- LYMPH NODES
VAV1 alterations in AITL/PTCL-NOS

VAV1 alterations

VAV1alties in AITL/PTCL-NOS

VAV1 alterations

VAV1

VAV1-STAT2

VAV1 alterations

RHOA mut (+) 0/41 (0%)

RHOA mut (-) 7/85 (8.5%)
G17V RHOA mutant enhanced phosphorylation of VAV1 and PLCγ1 in Jurkat cells

Bustelo Xose R, Small GTPase 2014

Autoinhibition via formation of high order structure
RHOA-VAV1 activation in angioimmunoblastic T-cell lymphoma

- **RHOA** mut (+) (70%)
- **RHOA** mut (-) (30%)
- **VAV1** mut (+)

**G17V RHOA**

**Src kinase**

**Dasatinib?**

**TCR**

**VAV1 mutants**
Anaplastic Large Cell Lymphomas
Systemic Anaplastic Large Cell Lymphoma

**Key Points**

- ALK-negative anaplastic large cell lymphoma is a genetically heterogeneous disease with widely disparate clinical outcomes.
- ALK-negative ALCs have chromosomal rearrangements of DUSP22 or TP63 in 30% and 9% of cases, respectively.
- DUSP22-rearranged cases have favorable outcomes similar to ALK-positive ALCs, whereas other genetic subtypes have inferior outcomes.

**References**

- Parrilla Castellar 2014
- Boi 2013
- Abate 2015
- Crescenzo 2015
CD30 is synthesized in the Golgi apparatus in a proteic form [90 kDa]

Then, it undergoes glycosylation [120 kDa] and moves to the cytoplasmic membrane

Gene located at 1p36
CD30/CD30L signaling
IRF4-CD30-NFκB Feedback Loop in ALCL

Proteasome/NFκB inhibitors, BET inhibitors, IMiDs; brentuximab

Bandini et al. Cancers (Basel) 2018
Boddicker et al. Blood 2015
Weilemann et al. Blood 2015
# CD30 expression in T-cell lymphomas

<table>
<thead>
<tr>
<th>CD30 IHC Score</th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>4</th>
<th>Score ≥2+</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTCL, NOS</td>
<td>31</td>
<td>11</td>
<td>18</td>
<td>11</td>
<td>16</td>
<td>45/87</td>
</tr>
<tr>
<td>(87 cases)</td>
<td>(35.63%)</td>
<td>(12.64%)</td>
<td>(20.69%)</td>
<td>(12.64%)</td>
<td>(18.39%)</td>
<td>(51.72%)</td>
</tr>
<tr>
<td>AITL</td>
<td>24</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td></td>
<td>3/42</td>
</tr>
<tr>
<td>(42 cases)</td>
<td>(51.14%)</td>
<td>(21.42%)</td>
<td>(11.90%)</td>
<td>(9.52%)</td>
<td></td>
<td>(21.42%)</td>
</tr>
<tr>
<td>ENTL</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>7/10</td>
</tr>
<tr>
<td>(10 cases)</td>
<td>(20.00%)</td>
<td>(10.00%)</td>
<td>(30.00%)</td>
<td>(10.00%)</td>
<td>(10.00%)</td>
<td>(70.00%)</td>
</tr>
<tr>
<td>MF</td>
<td>13*</td>
<td>15**</td>
<td>2†</td>
<td></td>
<td>2‡</td>
<td>4/32</td>
</tr>
<tr>
<td>(32 cases)</td>
<td>(40.63%)</td>
<td>(46.88%)</td>
<td>(6.25%)</td>
<td></td>
<td>(6.25%)</td>
<td>(12.50%)</td>
</tr>
<tr>
<td>Transformed MF</td>
<td>–</td>
<td>–</td>
<td>3</td>
<td>6</td>
<td>–</td>
<td>9/9</td>
</tr>
<tr>
<td>(9 cases)</td>
<td></td>
<td></td>
<td>(33.33%)</td>
<td>(66.67%)</td>
<td></td>
<td>(100%)</td>
</tr>
<tr>
<td>EATL type 1</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td></td>
<td>7</td>
<td>9/9</td>
</tr>
<tr>
<td>(9 cases)</td>
<td></td>
<td></td>
<td>(22.22%)</td>
<td></td>
<td>(77.78%)</td>
<td>(100.00%)</td>
</tr>
<tr>
<td>EATL type 2</td>
<td>3</td>
<td>–</td>
<td>–</td>
<td></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(3 cases)</td>
<td>(100%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All types</td>
<td>73</td>
<td>36</td>
<td>33</td>
<td>17</td>
<td>28</td>
<td>83/192</td>
</tr>
<tr>
<td>(192 cases)</td>
<td>(38.02%)</td>
<td>(18.75%)</td>
<td>(17.18%)</td>
<td>(8.85%)</td>
<td>(14.58%)</td>
<td>(43.22%)</td>
</tr>
</tbody>
</table>

PTCL, NOS; peripheral T-cell lymphoma, not otherwise specified; AITL: angioimmunoblastic T-cell lymphoma; ENTL: extranodal NK/T-cell lymphoma, nasal type; MF: mycosis fungoides; EATL: enteropathy-associated T-cell lymphoma; *2 cases in tumoral phase; **1 case in tumoral phase; †folliculotrophic variant; ‡pagetoid reticulosis subtype.

0 no staining; 1+ <25% positive cells; 2+ 25–50% positive cells; 3+ >50–75% positive cells; 4+ >75% positive cells
Aberrant ERBB4 Transcripts in ALK-ALCL

By courtesy of A. Feldman
**DUSP22 Rearrangements in ALK- ALCL**

risk-adjusted management; 
\( ? \)kinase inhibitors (unpublished)

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By courtesy of A. Feldman


Melard et al, Oncotarget 2016
TP63 Rearrangements in ALK- ALCL

risk-adjusted management;
other

By courtesy of A. Feldman
ALK Activates STAT3 to Drive Lymphomagenesis

risk-adjusted management; ALK inhibitors; JAK/STAT inhibitors

By courtesy of A. Feldman

JAK-STAT3 Activated Across Many ALCLs

JAK/STAT inhibitors; other kinase inhibitors

Crescenzo et al. Cancer Cell 2015
Hu et al. Leukemia 2017

CD30, Granzyme, IL2RA
T cell lymphoma phenotype
Anaplastic large cell lymphoma

• Anaplastic large cell lymphoma, ALK+
• Anaplastic large cell lymphoma, ALK-
• Cutaneous anaplastic large cell lymphoma

• Breast implant associated Anaplastic large cell lymphoma
Breast Implant associated Anaplastic Large Cell Lymphoma (BI-ALCL)

- Malignancy that arises around mammary implants in patients undergoing prosthetic surgery for breast augmentation or reconstruction
- Provisional entity in the 2017 WHO classification
- BI-ALCL has an ALK-negative phenotype
- Cases confined to the peri-implant breast seroma fluid without invasion of the fibrous capsule have shown an excellent prognosis
- Etio-pathogenesis still elusive
BI-ALCL correspond to activated CD4+ cells (Treg?)
No TH17 signature in BI-ALCL
BI ALCL presents with STAT3 activation and TRG shut off

Di Napoli A et al, Mod Pathol 2018
BI-ALCL is distinct from syALCL based on GEP

Di Napoli A et al, Mod Pathol 2018
RPS10 is over-expressed in BI-ALCL but not in other PTCLs

**Table A**

<table>
<thead>
<tr>
<th></th>
<th>mRNA</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIALCL1</td>
<td>5.773</td>
<td>2+</td>
</tr>
<tr>
<td>BIALCL2</td>
<td>1.139</td>
<td>3+</td>
</tr>
<tr>
<td>BIALCL3</td>
<td>1.140</td>
<td>2+</td>
</tr>
<tr>
<td>BIALCL4</td>
<td>0.773</td>
<td>1+</td>
</tr>
<tr>
<td>BIALCL5</td>
<td>0.356</td>
<td>1+</td>
</tr>
<tr>
<td>BIALCL6</td>
<td>0.067</td>
<td>1+</td>
</tr>
<tr>
<td>BIALCL7</td>
<td>0.041</td>
<td>1+</td>
</tr>
<tr>
<td>BIALCL8</td>
<td>0.028</td>
<td>1+</td>
</tr>
<tr>
<td>BIALCL9</td>
<td>0.005</td>
<td>1+</td>
</tr>
</tbody>
</table>

**Figure B**

![Graph showing expression levels of RPS10 in different cell types.]

**Figure C**

![Images showing histological slides.]

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Di Napoli A et al., Mod Pathol 2018
Cancer-associated programs are associated with RPS10 expression

Di Napoli A et al, Mod Pathol 2018
Summary

• PTCL/NOS
  • GEP-based COO classification
    • TBX21 vs. GATA3
      → TKI, proteasome inhibitors, HDACi,

• AITL – TFH related PTCLs
  • TET2, IDH2, DNMT3A
    → Demethilating agents?
  • RHOA, VAV1
    → SRC inhibitors (Dasatinib)

• ALCL
  • STAT3
    → JAK/STAT inhibitors?
  • ALK-TP63
    • DUSP22
  • CD30 Axis
    → BV
THANK YOU

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