Gene expression profiling in peripheral T-cell lymphoma

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Rationale for GEP studies on PTCL

— The diagnosis of PTCL is challenging even among expert hematopathologists, compounded by the rarity and diversity of PTCLs
— May have a prominent reactive cellular infiltrate masking the neoplastic cell population
— No unique cytogenetic abnormality in the major PTCL subtypes
— Approximately 50% of the cases are categorized as PTCL, not otherwise specified (PTCL-NOS)
— Gain better understanding of the biology of PTCL including clinically significant pathways
Frequency of common subtypes of PTCL

- PTCL-NOS: 25.9%
- AITL: 18.5%
- ENKTCL: 10.4%
- ATLL: 9.6%
- ALCL: 6.6%
- 5.5%
- 4.7%
- 2.5%
- 1.7%
- 1.4%
- 0.9%

Other disorders:
- Peripheral T-cell Lymphoma, not other specified
- Angioimmunoblastic
- Natural killer/T-cell lymphoma
- Adult T-cell leukemia/lymphoma
- Anaplastic large cell lymphoma, ALK+
- Anaplastic large cell lymphoma, ALK-
- Enteropathy-type T-cell
- Primary cutaneous ALCL
- Hepatosplenic T-cell
- Subcutaneous panniculitis-like
- Unclassifiable PTCL

International T-Cell Lymphoma Project, J Clin Oncol; 26:4124-4130 2008
PTCL subtype and ENKTL cases with acceptable GEP

- Gene expression profiling data was generated on HG U133 plus 2 arrays (Affymetrix Inc)
Refinement of molecular diagnostic signatures for PTCL subgroups

- Unique molecular signatures were identified for major PTCL entities

**Lymphoma and Leukemia Molecular Profiling Project (LLMPP) initiative**
- Of 152 PTCL-NOS cases, a subset of cases were classified into
  
  i. AITL [14%]
  ii. ALK(-)ALCL [11%]
  iii. ATLL [03%]
  iv. γδ-PTCL [09%]

- Of 117 AITL cases, 26 cases (22%) changed to PTCL-NOS.
Robust molecular signature for ALK(-)ALCL

Gene signature/pathway enrichment summary in ALK(-)ALCL

- Proliferation signature
- MYC induced gene signature
- IRF4 induced gene signature
- Absence of TCR signaling molecules

ALK(-)ALCL is molecularly distinct from PTCL-NOS and ALK(+)ALCL
DUSP22 and TP63 rearrangements in ALK- ALCL

A. Overall Survival by ALK Status

B. Overall Survival by Genetic Subtype

C. Overall Survival by Genetic Subtype, Non-transplanted Patients Only

Parilla Castellar et al. Blood 2014, August 28, 124(9) 1473-1480
JAK/STAT3 pathway activation through mutations and fusion transcripts

Novel fusions combining a transcription factor (NFkB2 or NCOR2) with a tyrosine kinase (ROS1 or TYK2).

Crescenzo and Abate et.al, Cancer Cell 2015
One-third of PTCL-NOS cases were not molecularly classified into WHO recognized PTCL entities.

WHO recognized T/NK tumor subtypes

- n=251
  - AITL
  - ATLL
  - ALK(-)ALCL
  - ALK(+)ALCL
  - NKCL
  - γδ-PTCL
- n=121
  - 32% PTCL-NOS

One-third of PTCL-NOS cases were not molecularly classified into WHO recognized PTCL entities.
PTCL-NOS can be further divided into two major subgroups.
GATA3 expression identifies a subset of PTCL, NOS with inferior survival.

\[
\text{A} \quad p = .0001 \\
\begin{array}{cccccc}
\text{GATA3}^- & \text{1.0} & \text{0.8} & \text{0.6} & \text{0.4} & \text{0.2} & \text{0.0} \\
\text{GATA3}^+ & \text{1.0} & \text{0.8} & \text{0.6} & \text{0.4} & \text{0.2} & \text{0.0} \\
\end{array}
\]

\text{B} \quad p = .01

\begin{array}{cccccc}
\text{# Patients at risk} & 32 & 17 & 11 & 8 & 7 & 5 \\
\text{GATA3}^- & 32 & 19 & 16 & 14 & 11 & 10 \\
\text{GATA3}^+ & 34 & 6 & 1 & 0 & 0 & 0 \\
\end{array}

Tianjiao Wang et al. Blood 2014;123:3007-3015
TBX21 expression by Immunohistochemistry is Predictive of survival in PTCL

T-bet expression in Chinese PTCL cohort (n=142)

Tumor microenvironment significantly influences the prognosis in TBX21 subgroup of PTCL-NOS.

Cytotoxic-plasma cell signature

Quartile (D)

OS in TBX21 subgroup

Quartile

Proportion

Time (years)

p=0.2

p=0.05

Tumor microenvironment significantly influences the prognosis in TBX21 subgroup of PTCL-NOS.

Survival model creation in AITL

Samples divided into Training and validation set

Cases divided to minimize OS bias

Survival model Creation

PS3 upregulated Cytotoxic T

Monocytic

B-cell

Other gene signature

University of Nebraska Medical Center
**Survival prediction on AITL: role of tumor microenvironment**

<table>
<thead>
<tr>
<th>Signature Cluster</th>
<th>Effect of high expression</th>
<th>Training p-value</th>
<th>Validation p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53 upregulated signature</td>
<td>Poor prognosis</td>
<td>0.001</td>
<td>0.014</td>
</tr>
<tr>
<td>Cytotoxic signature</td>
<td>Poor prognosis</td>
<td>0.005</td>
<td>0.046</td>
</tr>
<tr>
<td>Monocytic/dendritic signature</td>
<td>Poor prognosis</td>
<td>0.011</td>
<td>0.010</td>
</tr>
<tr>
<td>B-cell signature</td>
<td>Good prognosis</td>
<td>0.002</td>
<td>0.017</td>
</tr>
</tbody>
</table>

**Training set**

- **Tumor microenvironment significantly influences the prognosis in AITL**
- **Role of macrophages (M1) vs (M2) and dendritic cells are being investigated**
IDH2 mutation is specifically associated with molecularly defined AITL cases

![Graph A](image)

**Graph A:**
- **AITL**
  - IDH2R172
  - TET2
  - DNMT3A

- **PTCL, NOS**
  - GATA3 subgroup
  - TBX21 subgroup
  - Unclassifiable subgroup
  - TET2
  - DNMT3A

- **ALCL, ALK (-)**
  - TET2
  - DNMT3A

Legend:
- Wild-type
- Mutant, VF > 10
- Mutant, 3 < VF < 10

![Graph B](image)

**Graph B:**
- **DNMT3A**
  - (912 aa)
  - PWWP
  - ZNF
  - MTase

- **TET2**
  - (2002 aa)
  - CD
  - DBSH

Legend:
- AITL
- Insertion/Deletion
- ALCL, ALK (-)
- Missense mutation
- PTCL, NOS
- Nonsense mutation
RhoA mutation map in PTCL

**G17**
- AITL (n=39)
  - 28 (71.8%)
  - 0
  - 1 (2.6%)
- PTCL, NOS (n=41)
  - Tbx21 (n=19)
    - 5 (26.3)
    - 1 (5.3)
    - 0
  - Gata3 (n=12)
    - 2 (18.2)
    - 0
    - 0
  - Unclassified (n=10)
    - 3 (33.3)
    - 0
    - 0
- ALCL, ALK- (n=12)
  - 0
  - 0
  - 1 (8.3)

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**G17**
- AITL
  - *G17E* case includes an S26R mutation in same clone
  - ^K18M major clone has G17V minor clone
- PTCL, NOS
- ALCL, ALK-

**GTP/Mg**

**++**

**-**

**binding "boxes"**

**PKN/PRK1 binding site**

**Effector region “Switch I”**

**number (% of screened)**
STAT3 and 5B mutations identified in NK or γδ-T cell derived lymphomas

IL2 is secreted into the Extracellular Space and binds to its receptor IL2R in the Cell Membrane. The IL2R complex consists of IL2Rα, IL2Rβ, and IL2Rγ. In the Cytoplasm, the bound IL2 activates JAK1/2, leading to the phosphorylation of STAT5B. STAT5B then forms a dimer and translocates to the Nuclear Membrane. Upon nuclear translocation, STAT5B promotes transcription of target genes, including IL2Rα, mir155hg, BCL-XL, etc.

AZD148 is a small molecule that inhibits STAT3 activity, thereby blocking the downstream effects of IL2 signaling. This leads to a decrease in the expression of target genes and promotes cell growth and survival.

Key points:
- IL2 binding to IL2R
- JAK1/2 activation
- STAT5B dimerization and nuclear translocation
- Target gene transcription
- AZD148 inhibition of STAT3
- Promotes cell growth and survival
Cell-of-origin for PTCL subgroups Can that be assigned?

Adapted from O’Shea and Paul. 2010. Science

AITL

PTCL-GATA3/Th2 subgroup

PTCL-NOS/Th1 subgroup

ALCL (ALK+)

ALCL (ALK-)?

ATLL

Laurence de Leval, and Philippe Gaulard Blood 2014;123:2909-2910
Tim McKeithan
Javeed Iqbal
Alyssa Bouska
Can Kucuk
Xiaozhou Hu
Bei Jiang
Weiwei Zhang
Chao Wang
Joe Rohr
Cindy Lachel
Yuping Li
Xinhua Wang

Andrew Canonn
Zhongfeng Liu
Shaungping Guo
Karen Deffenbacher
Cuiling Liu

Kai Fu
Tim Greiner
Dennis Weisenburger
Julie Vose

I-PTCL
LLMPP
Nordic and ACT
Sichuan University
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