I have no personal or financial interests to declare:

I have no financial support from an industry source at the current presentation.
Current Challenges

PTCL

- diagnosis
  - heterogeneity
  - 30-50% of cases are PTCL, not otherwise specified (PTCL-NOS)

- Treatment
  - Most PTCL entities show poor responses to conventional chemotherapy

- Molecular investigation
  - Lack of authentic cell lines and genetically relevant animal models for major PTCL entities
Lymphoma Classification

Morphology/ Clinical
+ Immunohistochemistry
+ Cytogenetics, FISH
+ Molecular Biology

Transcriptomics

Genomics
+ Gene Expression Profiles (GEP)
+ Next Generation Sequencing (NSG)

W.H.Q Classification

PTCL entities
~19
~25
~30
Mature T cell development and activation

Complexity of T-cell immunobiology, numerous subsets and functional plasticity makes meaningful disease classification challenging

HSC: Hematopoietic stem cells
CLP: common lymphoid progenitor

β-Selection phase

Repertoire selection phase

HSC

CLP

NOTCH

IL7

Cortex

CD44+
CD25-

TCRβ Rearrangements

CD44+
CD25+

CD44-
CD25+

Pre-TCRβ Dependent

CD44-
CD25-

Medulla

MHC Class II

DP

CD4

Helper T

CD4+
CD8+

STAT4

T H1

TBX21

IFN-γ

IL4

IL5

IL13

STAT6

GATA3

MHC Class I

CD8

Cytotoxic T

CD8+
CD8-

M mature T cell development and activation, numerous subsets and functional plasticity makes meaningful disease classification challenging.

Iqbal & deLeval Agressive Lymphomas; series: Hem. Malignancies, 2019
W.H.O. classification of mature T/NK-cell neoplasms (2016 revised version)

Nodal
- Peripheral T-cell Lymphoma, Not Otherwise Specified (PTCL-NOS)
- Angioimmunoblastic T-cell lymphoma (AITL)
- Nodal PTCL with T<sub>FH</sub> phenotype
- Follicular PTCL

Extranodal
- Extra-nodal NK/T-cell lymphoma, nasal type (ENK/TCL)
- Enteropathy-associated T-cell lymphoma (EATL)
- Hepatosplenic T-cell lymphoma (HSTL)
- Subcutaneous Panniculitis-like TCL
- Monomorphic epitheliotropic intestinal T-cell lymphoma
- Breast implant-associated anaplastic large cell lymphoma
- Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract

Cutaneous
- Mycosis Fungoides
- Sézary Syndrome
- Primary Cutaneous CD30+ lymphoproliferative disorders
- Primary Cutaneous CD30+ T-cell Disorders
- Primary Cutaneous γδ TCL
- Primary Cutaneous Gamma/Delta TCL
- Chronic lymphoproliferative disorder of NK cells
- Systemic EBV-positive T-cell lymphoma of childhood
- Chronic active EBV infection of T- and NK-cell type, systemic form
- Adult T-cell leukemia/lymphoma
- Adult T-cell leukemia
- Aggressive NK-Cell Leukemia
- T-cell Prolymphocytic Leukemia
- T-cell Large Granular Lymphocytic Leukemia

Adapted from Swerdlow et al. Blood 2016
No major improvement in clinical outcome since last three decades in PTCL

OS of PTCL-NOS/AITL
Overall frequency of PTCL subtypes

- **PTCL-NOS**: 30%
- **AITL**: 22%
- **ALK+ ALCL**: 8%
- **ENKTCL**: 12%
- **ATLL**: 11%
- **HSTL**: 2%
- **EATL**: 5%
- **Others**: 3%
- **ALK-ALCL**: 7%

Additional notes:

- **Park et al.**, *Int J Hematol*, 2014
- **Adams et al.**, *J Clin Oncol.*, 2016
- **Vose et al.**, *J Clin Oncol.*, 2008
- **Iqbal & deLeval**, *Agressive Lymphomas; series: Hem. Malignancies*, 2019

Geographical distribution:

- **USA**: 38%
- **France**: 36%
- **Asia**: 20%
Evolution of molecular analysis techniques

We are beginning to use sophisticated techniques to identify patient- and tumor-related differences that increase response rates and decrease toxicity of lymphoma therapy.

- Genomic defined personalized medicine
Genomic Signatures in PTCL

- Genetic or molecular abnormalities defining the biology of the major PTCL subtypes

- Molecular diagnosis
  - AITL
  - ALCL
  - PTCLNOS (novel subgroups)

- Pathobiology and target characterization
  - Genetic/epigenetic driver
  - Functional consequences

- Rationalize/Justify the new clinical investigations
Major PTCL entities form tight clusters with cases of PTCL-NOS and other rare entities interspersed.
Gene expression-based molecular predictors of the major subgroups of PTCL

- More than half of the PTCL-NOS cases were not molecularly classified
Molecular characteristics of AITL gene signature

Gene signatures define crucial features in pathobiology & oncogenic pathways

- NF-κB activation pathway
- IL6/STAT3 activation pathway
- TGF-β signaling
- VEGF signaling
- IL12 signaling
- TFH gene signatures

---

Iqbal & deLeval, Aggressive Lymphomas; series: Hem. Malignancies, 2019
Development of prognostic models in AITL

Tumor microenvironment significantly influences AITL prognosis

Refinement of prognostic signatures

Iqbal et al. Blood. 2014
Genomic aberrations in AITL

Genomic copy number analysis using Affymetrix platform

Heavican et al. Blood 2019
Mutation spectrum in AITL

Epigenetic dysregulation

TCR signaling cascade

Major aberration
IDH2R172 mutations defines a unique AITL subgroup

- Histone modifications associated with IDH2 mutation

- IDH2 mutants show significant hypermethylation in proximal promoter region compared to wild type and normal tonsil

Chao et al. Blood 2015
Aberrant CD28 signaling in AITL

CD28 mRNA expression

CD28 mutation spectrum in AITL

Rohr et al. Leukemia 2016
Developing genetically faithful murine models

TET2  70-80%
IDH2^{R172}  20-30%

Human AITL

Murine model

Cre-CD4+;Tet2^{FL/FL}

Overall survival

Long term deficiency of Tet2 in CD4 + T cell leads to T-cell lymphoma with T_{FH} immunophenotype

Heavican et.al, Unpublished
Integrative genomic analysis in AITL

Pathogenetic Evolution

Heavican et al. Blood 2019
— AITL molecular signature reflected $T_{FH}$ cellular origin and suggested role of tumor microenvironment in disease pathobiology

— IDH2 & TET2 mutations co-occur suggesting unique cooperation in T-cell lymphomagenesis

— IDH2 mutations define a unique subset of AITL patients

— Genes involved in TCR signaling and T-cell differentiation are predominantly hyper methylated in IDH2 mutant cases

— Genetically relevant murine models may lead to better understanding of lymphoma biology
III-Delineating molecular subgroups within PTCL-NOS
Refinement of molecular diagnostic signatures

Unique molecular signatures were identified for major PTCL entities

Lymphoma and Leukemia Molecular Profiling Project (LLMPP) initiative
Identification of $\gamma\delta$-PTCL from PTCL-NOS

$\gamma\delta$-PTCL have similar gene expression signature as NKCL but distinct from CT(αβ)-PTCL & HSTCL

<table>
<thead>
<tr>
<th>Markers</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3ε</td>
<td>7/7 (+)</td>
</tr>
<tr>
<td>CD2</td>
<td>2/4 (+)</td>
</tr>
<tr>
<td>CD5</td>
<td>1/7 (+)</td>
</tr>
<tr>
<td>CD7</td>
<td>1/1 (+)</td>
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<tr>
<td>CD8</td>
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<td>CD56</td>
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</tr>
<tr>
<td>TIA1</td>
<td>4/4 (+)</td>
</tr>
<tr>
<td>Granzyme B</td>
<td>2/3 (+)</td>
</tr>
<tr>
<td>TCR-beta</td>
<td>5/5 (-)</td>
</tr>
<tr>
<td>EBER-1</td>
<td>3/5 (-)</td>
</tr>
</tbody>
</table>

Iqbal et al. Leukemia. 2011
STAT3 and STAT5B mutations identified in NK or \( \gamma \delta \)-T cell derived lymphomas

In vitro data analysis showed sensitivity of this mutations to JAK1/2 inhibition

- Stat3 and Stat5B are often mutated at the SH2 domain in NK and \( \gamma \delta \)-T cell lymphomas
- *In vitro* data analysis showed sensitivity of this mutations to JAK1/2 inhibition

Kucuk et.al Nat Commun. 2015
Out of 152 PTCL-NOS cases, a subset of cases were classified as unique PTCL entities.

One-third of PTCL-NOS cases were not molecularly classified into WHO recognized PTCL entities.
PTCL-NOS is subdivided into two major subgroups

(A)

(B)

Iqbal et al. Blood 2014
**T\textsubscript{H}1/2 differentiation schematic program**

**T\textsubscript{H}1**
- Cellular immunity
- Inflammation
- T\textsubscript{H}1
- NOTCH3
- TBX21
- EOMES
- STAT4
- JAK2
- IL12
- IL2
- INF\textsubscript{γ}
- Macrophages
- CD8+T-cells
- NK cells

**T\textsubscript{H}2**
- Humoral immunity
- Ab production
- T\textsubscript{H}2
- NOTCH 1/2
- Jagged1/2
- GATA3
- C-MAF
- STAT5
- JAK3
- IL2
- IL4
- IL10
- IL13
- B-cells
- IgE, IgG1, IgG3

Distinct Copy Number Aberrations and potential target genes associated with molecular PTCL subgroups

Heavican et al. Blood 2019

PTCL-GATA3 (CN gain)
PTCL-GATA3 (CN loss)
PTCL-TBX21 CN gain/loss

BCL11B
REL
CD28
TP63, TPRG1
TP53, TPRG1
PRDM1, LAT52
PTPTN
FAS, PTEN
MYC
ATM
PLCG1
STAT3
FoxO1
ITPR3
CDKN2A
FAS, PTEN
FOXO1
TP53
JAK3
IBTKPRDM1
ZC3H12D, LATS1

Relative mRNA Expression
GATA3 vs. TBX21

Median Centered Log2 mRNA Expression
GATA3 vs. TBX21

Overall CDKN2A OS by mRNA Expression
PTCL-NOS
PTCL-GATA3

Proportion

Time (Years)

n=61

Heavican et al. Blood 2019
Unique mutation profiles in molecular PTCL subgroups

Heavican et al. Blood 2019
Generation and validation of the murine models

C57BL/6x129

Trp53<sup>R172H</sup>+/−;Cd4-Cre<sup>+</sup>/−

Pten<sup>fl</sup>+/−;Cd4-Cre<sup>+</sup>/−

Pten<sup>fl/fl</sup>;Cd4-Cre<sup>+</sup>/−

Trp53<sup>R172H</sup>+/−;Pten<sup>fl/fl</sup>;Cd4-Cre<sup>+</sup>/−

Trp53<sup>R172H</sup>+/−;Pten<sup>fl</sup>+/−;Cd4-Cre<sup>+</sup>/−

* p53 mutation

Human PTCL

n= 36

n= 17

n= 11

n= 5

Human PTCL

n= 159
Significant differences in overall survival between genotypes

- Pten loss accelerates tumorigenesis in Trp53 (functional KO) mice & cooperates with Trp53 loss in tumorigenesis
- No tumor observed in Pten heterozygous mice
Genetic analysis within PTCL-TBX21 subgroup
Identification of cytotoxic $\alpha\beta$-PTCL group from PTCL-NOS

(A) Hierarchical clustering

Dendrogram for clustering PTCL-NOS cases using centered correlation and complete linkage

(B) Expression of the CT-PTCL signature in normal CD8+ T-cells stimulated with anti-CD3, anti-CD28 and IL12 for various time intervals (hours)

(C) GSEA analysis

IFN$\gamma$ responsive genes

CD8+ T-cell gene signature

P<0.01

P<0.005

(D) Survival of the CT-PTCL group

OS

EFS

p=0.05

p=0.06

International peripheral T-cell lymphoma Project

Iqbal et al. Blood 2010
Tumor microenvironment influences prognosis in PTCL-TBX21 subgroup

- Cytotoxic-plasma cell signature
- Cytotoxic
- Pan-B plasma-cell
- Immunoglobulin

(B) H&E, CD3, TIA1

(C) H&E, CD3, TIA1

(D) OS in TBX21 subgroup

Iqbal et al. Blood 2014
Distinct **DNMT3A** mutational spectrum in PTCL-TBX21

- **AITL** n = 39% (69/176)
- **PTCL-NOS**
  - GATA3 n = 27% (6/22)
  - TBX21 n = 40% (12/30)
  - \(T_{FH}\)like n = 10% (1/10)

Unpublished
Prognostic significance of DNMT3A mutations

PTCLs (excluding ALCLs)

PTCL-NOS

PTCL-TBX21

PTCL-GATA3

Unpublished
**DNMT3A\(^\Delta\)** mutations define CD8\(^+\) T-cell subgroup in PTCL-TBX21

**PTCL-TBX21**

<table>
<thead>
<tr>
<th>WT</th>
<th>DNMT3A(^\Delta)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="img1.png" alt="Image 1" /></td>
<td><img src="img2.png" alt="Image 2" /></td>
</tr>
</tbody>
</table>

*n=14*  *n=9*

- Activated CD8\(^+\) T cells
- N.E.S. = 1.62
- \(p < 0.01\)
- \(q = 0.12\)

**Bioinformatics programs**

**GSEA**

- Cibersort analysis

**DNMT3A** mutation
- Cytotoxic Signature
- Activated CD8\(^+\)T cell signature
- CD4/CD8 by IHC

**Unpublished**
PTCL-NOS can be subdivided into two molecular subgroups with distinct molecular pathobiology and cellular-origin.

Molecular subgroups of PTCL-NOS evolve using different oncogenic pathways.

Tp53 role in T-cell differentiation in PTCL-GATA3 subgroup is further warranted.

DNMT3A mutation likely defines a CD8+ T cell cytotoxic subgroup in PTCL-TBX21.
Speculations of cell-of-origin for PTCL subsets

- AITL
- PTCL-NOS/GATA3 subgroup
- PTCL-NOS/TBX21 subgroup
- ALCL (ALK+)
- ALCL (ALK-)?
- ATLL

GEP and immunophenotypic findings corroborate these speculations
PTCL entities are genetically distinct
The complexity of PTCL can finally be addressed with the integration of global genomic analyses, which demonstrated that molecularly defined PTCL subgroups have diverse genetic features and arise by distinct genetic pathways.
PTCL subtyping assay development for clinical setting

<table>
<thead>
<tr>
<th>Fresh frozen RNA using Affymetrix platform (Blood, 2014)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HG-U133 (Affymetrix platform)</strong></td>
</tr>
</tbody>
</table>

- Total RNA isolation
- Refined gene signature
- Normalization and model correction
- Molecular diagnosis

<table>
<thead>
<tr>
<th>FFPE RNA using NanoString platform 2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refined PTCL signature on nCounter™</td>
</tr>
</tbody>
</table>

Validation of diagnostic model for PTCL classification

- Total RNA isolation
- Refined gene signature
- Normalization and model correction
- Molecular diagnosis
Integrating new genomic information for targeted therapy in PCTL

PTCL-NOS GATA3: constitutively active PI3K and mTOR pathways

ALK-ALCL: enriched mTOR pathway signatures

ALK-ALCL: constitutively active JAK/STAT3 pathway

PTCL-NOS TBX21: constitutively active NFκB and STAT3 pathways

Molecularly defined AITL: oncogenic pathways NFκB, TGFβ and IL-6 signaling identified

HSTL: high frequency STAT3 mutation

NCI Rare Tumor Initiative (Refractory TCGA-like approach)

Comprehensive genomic characterization of PTCL entities

- PTCL-NOS
- AITL
- ALCL
- ATLL
- NCKL
- rare one

Projected numbers ~500 PTCLs

- Fresh Frozen PTCL cases with available germline
- Other sites (anticipated) in Asia for rare PTCLs
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