Opportunities for precision medicine in lymphoma

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October 8, 2018
Objectives

- To understand testing modalities that contribute to diagnosis, prognosis, therapeutic decisions, disease monitoring in patient with lymphoma

- To learn how pathologists contribute to precision medicine in lymphoma patients

- To understand the application of ancillary testing and its impact on precision medicine

- To raise awareness of opportunities to deliver precision medicine for patients with lymphoma
Personalized/Precision Medicine

Definition
Form of medicine that uses information about a person’s genes, proteins, and environment to prevent diagnose and treat disease
National Cancer Institute http://www.cancer.gov/

Not a new concept

“The new language of genomics, as applied to medicine, is less a revolution than an evolution”
Evolution of Molecular Diagnostics in Hematopathology

1980
- Morphologic evaluation

1985
- Introduction of immunopathology, flow cytometry immunohistochemistry

1988
- PCR FFPE tissues

1990
- Rapid growth in molecular genetic application

1994
- Gleevec approved by FDA

2001
- New therapies
  - Combination of small molecules

2008
- Multi-panel detection of structural alterations/mutations with prognostic and therapeutic implications

2018
- WHO classification

...
## Changing Pace of Target Discovery to Therapy

<table>
<thead>
<tr>
<th>Target</th>
<th>Year Target Discovered</th>
<th>Disease(s) and Proportions</th>
<th>Estimated Total # Pts Annually (US)</th>
<th>Drug(s)</th>
<th>Clinical Outcomes</th>
<th>Outcomes from Conventional Chemotherapy</th>
<th>Year Mutation-Targeted Treatment Documented</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR-ABL</td>
<td>1960</td>
<td>CML (100%)</td>
<td>5,000</td>
<td>Imatinib Dasatinib Nilotinib</td>
<td>RR 90% 5y PFS 80% 5y OS 90%</td>
<td>RR 35% 5y OS 70%</td>
<td>2001</td>
</tr>
<tr>
<td>EGFR</td>
<td>1978</td>
<td>EGFR mutated NSCLC (10% of NSCLC)</td>
<td>17,000</td>
<td>Erlotinib Gefitinib</td>
<td>RR 75% Median PFS 11 mos Median OS 31 mos</td>
<td>RR 30% Median PFS 5 mos Median OS 24 mos</td>
<td>2004</td>
</tr>
<tr>
<td>KIT</td>
<td>1998</td>
<td>GIST</td>
<td>6,000</td>
<td>Imatinib</td>
<td>RR 55% Median PFS 27 mos Median OS 58 mos</td>
<td>RR 5% Median OS 20 mos</td>
<td>2002</td>
</tr>
<tr>
<td>BRAF</td>
<td>2002</td>
<td>50% of melanoma 100% HCL</td>
<td>34,000</td>
<td>PLX4032</td>
<td>RR 77% Median PFS 7 mos OS not yet determined</td>
<td>RR 10-20% PFS 1.5 mos OS 8 mos.</td>
<td>2010</td>
</tr>
<tr>
<td>ALK</td>
<td>2007</td>
<td>EML4-ALK NSCLC (5% of NSCLC) ALK+ ALC L</td>
<td>8,500</td>
<td>Crizotinib</td>
<td>RR 55% 6 month PFS 70% OS not yet determined</td>
<td>RR 25% Median PFS 4-6 mos Median OS 12 mos</td>
<td>2010</td>
</tr>
</tbody>
</table>

Gerber DE and Minna JD Cancer Cell 2010
CONCLUSIONS

• Molecular techniques are routinely being employed to provide adjunctive results critical to patient management.

• Molecular techniques also provide opportunities for improved diagnosis, early disease detection and prognosis of lymphomas.

• Molecular techniques now offer opportunities for therapeutic refinement and adaptation to diseases specific to each patient (personalized medicine).

• New genomic tools have led to discovery of novel mutations in lymphoid neoplasms.

• Certain genetic abnormalities previously underappreciated as important lesions in lymphoid malignancies.

Current Status
1) What disease does the patient have? (diagnosis)

2) How much of the disease is there? (residual disease)

3) What drug will the disease respond to? (therapy)

4) Who needs treatment? (prognosis)

5) What dose? (pharmacogenomics, dynamics)
Application of Precision Medicine in Lymphoma

- Diagnosis
- Prognosis
- Therapeutic options/tailored therapies
- Disease monitoring
- Unexpected consequences of targeted therapies
Case 1

A 53 year old man presented with abdominal discomfort.

Physical examination and radiologic studies revealed an enlarged spleen which was removed.

The spleen weighed 3.55 kg with dimensions of 25 x 19.5 x 11.7 cm.
Differential diagnosis

- Splenic marginal zone lymphoma
- Splenic B-cell lymphoma, unclassifiable
- Follicular lymphoma
- Mantle cell lymphoma
- Chronic lymphocytic leukemia/SLL
- Hairy cell leukemia

- T cell lymphoma
Immunophenotype

CD5 negative
CD43 negative
Cyclin D1 negative
CD10 negative
Splenic Marginal Zone Lymphoma

- SMZL uncommon indolent lymphoma involving splenic white pulp, blood, and bone marrow
- First-line therapies
  - splenectomy
  - anti-B-cell biologicals
- Median survival 10yr
Molecular genetics of splenic lymphoma

- del7q, +3/+3q [+18, +12]
- No recurrent translocation
- Until recently no known genetic etiology
SMZL Genome: Low Complexity

-19

+8q

del13q

del7q

14
Recurrent NOTCH2 Nonsense Mutations

Reference Sequence

SMZL

Germline

A A A T G C T G C T G C T G A G C T C A C A C C C C A G C T

c.7198C>T

p.R2400X

A A A T G C T G C T G C T G A G C T C A C A C C C C A G C T

Germline

A A A T G C T G C T G C T G A G C T C A C A C C C C A G C T

11 / 28
39%

16 / 44
36%

Read Alignment
Additional 93 SMZL specimens sequenced in validation cohort. 22 additional cases with NOTCH2 mutations identified.

Kiel MJ, .. Elenitoba-Johnson KSJ
Frequency of NOTCH2 Mutations in Various Lymphoma Subtypes and Reactive Lymph Nodes

Kiel MJ, .. Elenitoba-Johnson KSJ
Recurrently targeted pathways in SMZL

Decreased Relapse-free Survival in *NOTCH2*-mutated SMZL

Kiel MJ, .. Elenitoba-Johnson KSJ

Notch signaling can be inhibited by gamma secretase inhibitors

Notch heterodimer

δ-secretase

γ-secretase

DAPT

NICD

CoR

Transcription of target genes: HES, HEY, NF-κB, PPAR

Cell survival and differentiation
Genetics of SMZL

- *NOTCH2* is recurrently mutated in SMZL
- Mutations cluster in C-terminus causing gain-of-function of *NOTCH2*
- *NOTCH2* mutations are specific to MZL
- *NOTCH2* mutations confer an adverse prognosis
- *KLF2* is recurrently mutated in SMZL

Piva R et al., Leukemia 2014 KLF2
Molecular testing in small B cell lymphomas of the spleen

• Splenic marginal zone lymphoma
• Splenic B-cell lymphoma, unclassifiable
• Nonsplenic MALT
• Follicular lymphoma
• Mantle cell lymphoma
• Chronic lymphocytic leukemia/SLL
• Hairy cell leukemia
# Recurrently mutated genes in CLL

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Mutation</th>
<th>Mutated cases/total</th>
<th>Overall frequency (%)</th>
<th>Frequency in IGHV-unmutated (%)</th>
<th>Frequency in IGHV-mutated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOTCH1</td>
<td>Notch 1</td>
<td>P2515Rfs<em>4 Q2503</em> F2482Ffs*2</td>
<td>29/255 1/255 1/255</td>
<td>12.2</td>
<td>20.4</td>
<td>7</td>
</tr>
<tr>
<td>MYD88</td>
<td>Myeloid differentiation primary response gene 88</td>
<td>L265P</td>
<td>9/310</td>
<td>2.9</td>
<td>0.8</td>
<td>5.6</td>
</tr>
<tr>
<td>XPO1</td>
<td>Exportin 1</td>
<td>E571K E571G</td>
<td>3/165 1/165</td>
<td>2.4</td>
<td>4.6</td>
<td>0</td>
</tr>
<tr>
<td>KLHL6</td>
<td>Kelch-like 6</td>
<td>F49L/L65P L90F L58P/T64A/Q81P</td>
<td>3/160</td>
<td>1.8</td>
<td>0</td>
<td>4.5</td>
</tr>
</tbody>
</table>

BRAF IN HAIRY CELL LEUKEMIA

BRAF V600E is strongly associated with classic HCL

BRAF Mutations in Hairy-Cell Leukemia

Enrico Tiacci, M.D., Vladimir Trifonov, Ph.D., Gianluca Schiavoni, Ph.D., Antony Holmes, Ph.D., Wolfgang Kern, M.D., Maria Paola Martelli, M.D., Alessandra Pucciarini, Ph.D., Barbara Bigerna, B.Sc., Roberta Pacini, B.Sc., Victoria A. Wells, B.Sc., Paolo Sportoletti, M.D., Valentina Pettrrossi, Ph.D., Roberta Mannucci, Ph.D., Oliver Elliott, M.Sc., Arcangelo Liso, M.D., Achille Ambrosetti, M.D., Alessandro Pulsoni, M.D., Francesco Forconi, M.D., Livio Trentin, M.D., Gianpiero Semenzato, M.D., Giorgio Inghirami, M.D., Monia Capponi, M.D., Francesco Di Raimondo, M.D., Caterina Patti, M.D., Luca Arcaini, M.D., Pellegrino Musto, M.D., Stefano Pileri, M.D., Claudia Haferlach, M.D., Susanne Schnittger, Ph.D., Giovanni Pizzolo, M.D., Robin Foà, M.D., Laurent Farinelli, Ph.D., Torsten Haferlach, M.D., Laura Pasqualucci, M.D., Raul Rabadian, Ph.D., and Brunangelo Falini, M.D.
**BRAF** exon-15 mutation is sensitive and specific for hairy cell leukemia

<table>
<thead>
<tr>
<th>Tumor Entity</th>
<th>analyzed</th>
<th>mutated</th>
<th>% mutated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hairy Cell Leukemia</td>
<td>48</td>
<td>48</td>
<td>100</td>
</tr>
<tr>
<td>Splenic Marginal Zone Lymphoma</td>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Splenic Lymphoma/Leukemia, Unclassifiable*</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chronic Lymphocytic Leukemia</td>
<td>21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Follicular Lymphoma</td>
<td>35</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diffuse Large B-cell Lymphoma</td>
<td>71</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mantle Cell Lymphoma</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Burkitt Lymphoma</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

High prevalence of MAP2K1 mutations in variant and IGHV4-34–expressing hairy-cell leukemias

Joshua J Waterfall¹, Evgeny Arons², Robert L Walker¹, Marbin Pineda¹, Laura Roth², J Keith Killian¹, Ogan D Abaan¹, Sean R Davis¹, Robert J Kreitman² & Paul S Meltzer¹
RAF kinase is a molecular target
Immunohistochemistry for detection of B-RAF V600E (clone VE1) mutant protein

Andrulis M et al., Am J Surg Pathol 2012
B-RAF V600E (clone VE1) mutant protein IHC for minimal residual disease

Akarca AU et al., Br J Haematol Jul 2013
Take home messages

• Many subtypes of B-cell lymphomas can present in the spleen
• NGS studies identified \textit{NOTCH2} mutations in 25% of SMZL
• Gene mutations in NFkB pathway, chromatin remodeling are also present in SMZL
• Molecular studies may help in subclassification of other B-cell lymphomas that present in the spleen
Aggressive B-cell lymphomas
WHO classification 2017
Aggressive B-cell Lymphoma

• Diffuse large B-cell lymphoma, not otherwise specified
  • Diffuse large B-cell lymphoma, subtypesT-cell/histiocyte-rich large B-cell lymphoma
  • Primary mediastinal (thymic) large B-cell lymphoma
  • Intravascular large B-cell lymphoma
  • DLBCL associated with chronic inflammation (New entity)
  • ALK-positive B-cell lymphoma
  • Plasmablastic lymphoma
  • Large B-cell lymphoma arising in HHV8-associated with Castleman disease
  • Primary effusion lymphoma

• Burkitt lymphoma

• High grade B-cell lymphomas (DHL or NOS)
Prognostic value of distinguishing between HGBL, DLBCL, BL

- Aggressive B-cell lymphomas are clinically, histologically and genetically diverse neoplasia.

- Molecular genetics is necessary to convey important prognostic information relevant for therapy.

- Evaluate all DLBCL (with high PI) and other aggressive B cell lymphomas with the panel of FISH probes.
Molecular subgroups of DLBCL are prognostic


<table>
<thead>
<tr>
<th></th>
<th>GCB-DLBCL</th>
<th>ABC-DLBCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>~50%</td>
<td>~30%</td>
</tr>
<tr>
<td>Key genes</td>
<td>CD10, BCL-6, LMO2</td>
<td>PKCβ1, Cyclin D2, IRF/MUM1</td>
</tr>
</tbody>
</table>

High grade B-cell lymphoma

Double Hit Lymphoma
- Aggressive B-cell lymphomas harboring MYC/8q24 gene rearrangement with another recurrent breakpoint
  - BCL2/IGH + MYC/8q24  DHL  60%
  - BCL6/IGH + MYC/8q24  DHL  8%
  - BCL2/IGH + BCL6/IGH  Not DHL  Rare

Aukema SM et al., BLOOD 2010
HGBL

Impact

Diagnosis

Prognosis

Therapy

Classic Burkitt lymphoma
Take home messages

• Molecular genetics is necessary to convey important prognostic information relevant for therapy in aggressive B-cell lymphomas

• Algorithmic approach using immunohistochemistry and FISH analysis will allow subclassification for better risk stratification and treatment

• NGS identifies novel genetic events that may have a role in diagnosis and therapy
Case 3

A 25 year old male presented with a one year history of diffuse lymphadenopathy of cervical, axillary, abdominal regions.

He complained of fevers, weight loss, night sweats.

An excisional biopsy of the cervical lymph node was performed.
### Differential diagnostic considerations

#### Hematopoietic
- Non-Hodgkin lymphoma
  - diffuse large B-cell lymphomas with anaplastic features,
  - anaplastic large cell lymphoma (ALK-positive and ALK-negative)
- Peripheral T cell lymphomas, NOS
- Hodgkin lymphoma

#### Non-hematopoietic
- Melanoma
- Carcinoma (anaplastic variants)
## Immunophenotype

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45</td>
<td>Negative</td>
</tr>
<tr>
<td>CD20</td>
<td>Negative</td>
</tr>
<tr>
<td>CD3</td>
<td>Negative</td>
</tr>
<tr>
<td>CD2</td>
<td>Negative</td>
</tr>
<tr>
<td>CD4</td>
<td>Positive</td>
</tr>
<tr>
<td>CD7</td>
<td>Negative</td>
</tr>
<tr>
<td>CD8</td>
<td>Negative</td>
</tr>
<tr>
<td>CD30</td>
<td>Positive +++</td>
</tr>
<tr>
<td>Perforin</td>
<td>Positive</td>
</tr>
<tr>
<td>ALK-1</td>
<td>Positive +++ N/C</td>
</tr>
</tbody>
</table>

**Diagnosis**

Anaplastic large cell lymphoma, ALK+
Genetics of ALK+ ALCL

t(2;5)(p23;q35)
ALK translocations in human cancer

### ALK translocations in ALCL

- **NPM1**: 2p23, 75%
- **TPM3**: 15%
- **TFG**: ~10%
- **TPM4**: ~10%
- **CLTC**: ~10%
- **ATIC**: ~10%
- **MYH**: ALK
- **MSN**: ALK
- **ALO17**: ALK

### IMT

- **TPM3**: ALK
- **RanBP2**: ALK
- **ATIC**: ALK
- **CLTC**: ALK
- **TPM4**: ALK
- **CARS**: ALK
- **SEC31L**: ALK

### Non-small-cell lung cancer

- **EML4**: ALK
- **KIF5B**: ALK

### Diffuse large B-cell lymphoma

- **SQSTM1**: ALK
- **CLTC**: ALK
- **NPM1**: ALK
ALK is a therapeutic target for ALCL

Phase 1/2 study of PF-2341066, oral small molecule inhibitor of ALK and C-MET in children with relapsed/refractory solid tumors and anaplastic large cell lymphoma

ADVL0912  Children’s Oncology Group

WHO 2017 Classification

Mature T-cell neoplasms

**Cutaneous**
- Mycosis fungoides (MF)
- Sézary syndrome
- Primary cutaneous CD30+ T-cell Disorders
- Primary cutaneous ALCL
- Primary cutaneous γδ TCL
  - Primary cutaneous CD8+ aggressive epidermotropic cytotoxic TCL
  - Primary cutaneous acral CD8+ TCL
  - Primary cutaneous CD4+ small/medium T cell lymphoproliferative disorder*
  - Hydroa vacciniforme-like
- Primary cutaneous CD8+ aggressive epidermotropic cytotoxic TCL
- Primary cutaneous CD4+ small/medium T cell lymphoproliferative disorder*
- Sézary syndrome
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- Primary cutaneous CD30+ T-cell Disorders
- Primary cutaneous ALCL
- Primary cutaneous γδ TCL
  - Primary cutaneous CD8+ aggressive epidermotropic cytotoxic TCL
  - Primary cutaneous acral CD8+ TCL
  - Primary cutaneous CD4+ small/medium T cell lymphoproliferative disorder*
  - Hydroa vacciniforme-like

**Extranodal**
- NK/TCL nasal type
  - Enteropathy-associated TCL
  - Monomorphic epitheliotropic intestinal T-cell lymphoma*
  - Hepatosplenic TCL
- Subcutaneous Panniculitis-Like TCL
- Systemic EBV+ T-cell lymphoproliferative disorder of childhood

**Nodal**
- Peripheral TCL-NOS
  - Anaplastic large Cell lymphoma (ALK +)
  - Anaplastic Large Cell lymphoma (ALK -)
  - Breast implant-associated ALCL*
  - Angioimmunoblastic TCL
  - Follicular T cell lymphoma*
  - Nodal peripheral T cell lymphoma with TFH phenotype*

**Leukemic**
- Adult T-cell leukemia/lymphoma
- T-cell prolymphocytic leukemia
- T-cell large granular lymphocytic leukemia
- Chronic lymphoproliferative disorder of NK cells*
- Aggressive NK-cell leukemia

*Provisional entity.

Swerdlow SH et al, eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: IARC; 2017
## Recurrent structural abnormalities in T-cell neoplasms

<table>
<thead>
<tr>
<th>Disease entity</th>
<th>Structural Abnormality</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK+ ALCL</td>
<td>NPM1-ALK</td>
<td>84%</td>
</tr>
<tr>
<td></td>
<td>Various-ALK</td>
<td>16%</td>
</tr>
<tr>
<td>T-PLL</td>
<td>TRA-TCL1A</td>
<td>70-80%</td>
</tr>
<tr>
<td></td>
<td>TRA-MTCP1</td>
<td>10%</td>
</tr>
<tr>
<td>HSTL</td>
<td>i(7q)(q10)</td>
<td>&gt;80%</td>
</tr>
<tr>
<td>EATL</td>
<td>9q gains</td>
<td>~70%</td>
</tr>
<tr>
<td></td>
<td>16q12.1 loss</td>
<td>~30%</td>
</tr>
<tr>
<td>ALK- ALCL, c-ALCL</td>
<td>IRF4/DUSP22 translocations</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td>TYK2/ NPM1-TYK2</td>
<td>17%/4%</td>
</tr>
<tr>
<td></td>
<td>VAV rearrangements</td>
<td>4%</td>
</tr>
<tr>
<td></td>
<td>PCM-JAK2</td>
<td>rare</td>
</tr>
<tr>
<td>PTCL</td>
<td>P53-related genes</td>
<td>6%</td>
</tr>
<tr>
<td>F-PTCL</td>
<td>ITK-SYK</td>
<td>18-38%</td>
</tr>
<tr>
<td>AITL</td>
<td>ITK-SYK</td>
<td>rare</td>
</tr>
</tbody>
</table>
## Recurrent somatic gene mutations in T-cell neoplasms

<table>
<thead>
<tr>
<th>Gene</th>
<th>Disease entity</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>JAK1/JAK3</strong></td>
<td>T-PLL, NKTCL/EATL</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-35%</td>
</tr>
<tr>
<td><strong>STAT3</strong></td>
<td>T-LGLL, GD HSTCL/EATL, CLPD-NK</td>
<td>27-40%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30%</td>
</tr>
<tr>
<td><strong>STAT5B</strong></td>
<td>T-PLL, GD HSTCL/EATL, T-LGLL</td>
<td>36%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rare</td>
</tr>
<tr>
<td><strong>CD28</strong></td>
<td>AITL, MF/SS</td>
<td>11%, rare</td>
</tr>
<tr>
<td><strong>FYN</strong></td>
<td>AITL, PTCL, NOS</td>
<td>3%, rare</td>
</tr>
<tr>
<td><strong>PKCG1</strong></td>
<td>AITL, MF/SS, PTCL, NOS</td>
<td>12%, 20%, 15%</td>
</tr>
<tr>
<td><strong>PRKCB</strong></td>
<td>NKTCL</td>
<td>33%</td>
</tr>
<tr>
<td><strong>CARD11</strong></td>
<td>MF/SS, NKTCL</td>
<td>15%, 24%</td>
</tr>
<tr>
<td><strong>TNFRSF1B</strong></td>
<td>MF/SS, NKTCL</td>
<td>6%, rare</td>
</tr>
<tr>
<td><strong>TET2</strong></td>
<td>AITL, F-PTCL</td>
<td>33-47%, 58%</td>
</tr>
<tr>
<td><strong>IDH2</strong></td>
<td>AITL</td>
<td>~25%</td>
</tr>
<tr>
<td><strong>DNMT3A</strong></td>
<td>AITL, PTCL, NOS</td>
<td>11% overall</td>
</tr>
<tr>
<td><strong>SETD2</strong></td>
<td>EATL I/II</td>
<td>32%</td>
</tr>
<tr>
<td><strong>RHOA</strong></td>
<td>AITL, PTCL, NOS</td>
<td>67%; 18%</td>
</tr>
<tr>
<td><strong>CCR4</strong></td>
<td>MF/SS</td>
<td>7%</td>
</tr>
</tbody>
</table>

### Key Pathways

- **JAK/STAT**
- **Co-stimulatory TCR signaling**
- **NF-κB**
- **Epigenetic**
- **Other**
Case 4: Clinical History

- 66 year old man
- enlarged inguinal lymph node
- multiple lesions on arm
- skin biopsy performed

- What is the diagnosis?
- Is there prognostic significance?
- What therapies are relevant?
Case 4: Differential Diagnosis

Primary cutaneous CD30+ lymphoproliferative disorders
  Lymphomatoid papulosis

Primary cutaneous anaplastic large cell lymphoma

ALK+ anaplastic large cell lymphoma
Histiocytic sarcoma
Extramedullary myeloid tumor
Transformation of mycosis fungoides
Sézary syndrome
Primary cutaneous gd T-cell lymphoma
ALK-negative ALCL has a better prognosis than PTCL-NOS

5-year overall survival of ALK-negative ALCL and PTCL-NOS
(CD30+ ≥80% of cells)

Rearrangements in ALK- ALCL

- 73 ALK- ALCL
  - 22/73 (30%) *DUSP22* translocated
  - 6/73 (8%) *TP63* translocated
  - Mutually exclusive
  - 45 were ALK/DUSP22/TP63 triple negative

Parillia Castellar ER et al *Blood* 2014
TYK2 rearrangements in ALK- ALCL

Brief Report

LYMPHOID NEOPLASIA

A novel recurrent NPM1-TYK2 gene fusion in cutaneous CD30-positive lymphoproliferative disorders


(Blood. 2014;124(25):3768-3771)
NPM1
- Nucleophosmin 1
- Multiple functions particularly in nucleolus associating with ribonucleolar proteins

TYK2
- Non-receptor tyrosine protein kinase
- First member of JAK family
- Signal transduction by interferons and interleukins
Diagnostic and therapeutic implications

*TYK2* translocation

Signaling of immunoregulatory cytokine

- IFNAR1/2
- IL-13Ra1
- IL-12Rβ1
- IL-10R2
- IL4Ra
- OSMR gp130

TYK2

JAK2

JAK1

NPM1-TYK2 Fusion

- NPM1-TYK2+ MyLa
- NPM1-TYK2+ CD30+ LPD
- NPM1-TYK2- CD30+ LPD
Knockdown of NPM-TYK2 decreases cell proliferation.

Graph showing the effect of knockdown on cell proliferation over time. The graph compares the proliferation rates of Vector, shTYK2-1, and shTYK2-2 treatments. The graph indicates a significant decrease in proliferation for the shTYK2-2 treatment compared to the Vector control.
Prognostic and Therapeutic Importance of Subclassification of ALK- ALCL

- DUSP22 Rearrangement
- TP63 Rearrangement
- NPM1-TYK2
- VAV Rearrangement
Integrated genomic analysis of T-PLL identifies novel highly recurrent activating mutations

- **JAK-STAT** mutations in 76% of T-PLL
- **IL2RG** implicated in human cancer for the first time
- Inhibition of JAK or pSTAT5 may represent a therapeutic strategy for T-PLL patients

*Kiel M et al., Blood. 2014 Aug 28;124(9):1460-72*
Molecular studies will impact therapeutic decisions in T-cell lymphoma

<table>
<thead>
<tr>
<th>Lymphoma</th>
<th>Genetic features</th>
<th>Therapeutic relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALCL</td>
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<tr>
<td>cALCL</td>
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<tr>
<td>PTCL</td>
<td>ITK/SYK</td>
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<tr>
<td>T-PLL/ENKTCL</td>
<td>JAK3</td>
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<tr>
<td>T-PLL</td>
<td>STAT5B</td>
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<td>T-LGLL, NK-LPD</td>
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<tr>
<td>AITL</td>
<td>TET2, IDH2, DNMT3A</td>
<td>Epigenetic modulator</td>
</tr>
<tr>
<td>PTCL, NOS</td>
<td>DNMT3A</td>
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</tr>
<tr>
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Molecular studies will impact therapeutic decisions in B-cell lymphoma

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<tr>
<td>Burkitt</td>
<td>MYC/IGH</td>
<td>CVAD vs RCHOP</td>
</tr>
<tr>
<td>Double Hit DLBCL</td>
<td>MYC/IGH; BCL2/IGH</td>
<td>Not responsive</td>
</tr>
<tr>
<td></td>
<td>BCL6/IGH</td>
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</tr>
<tr>
<td>Gastric MALT</td>
<td>API2/MALT1</td>
<td>MALT1 inhibitors</td>
</tr>
<tr>
<td>Hairy cell leukemia</td>
<td>B-RAF mutation</td>
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<tr>
<td>CLL/SLL</td>
<td>IgH SHM</td>
<td>No need for aggressive Tx</td>
</tr>
<tr>
<td>CLL/SLL; SMZL</td>
<td>NOTCH mutation</td>
<td>Gamma secretase inhibitor</td>
</tr>
<tr>
<td>FCL/DLBCL</td>
<td>EZH2, MLL, p300</td>
<td>Demethylating agents</td>
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</table>
Emerging applications

- Molecular diagnostic signatures of DLBCL and PTCL subgroups based on gene expression (NanoStringTM platform)

- Targeted NGS panel for subclassification and identification of targeted therapies

- High throughput sequencing of IGH and TCR for diagnosis and disease monitoring

- Circulating tumor DNA for early disease detection and disease monitoring
High throughput sequencing of IGH or TCR genes

Capillary electrophoretogram

Monoclonal

Monoclonal

Polyclonal background

Polyclonal

Gerline

Rearranged TCR

HTS-TCR

Clonal

Relative frequency

Number of clones

Polyclonal

Relative frequency

Number of clones
Circulating tumor DNA for lymphoma

- cfDNA for MRD for DLBCL
- Retrospective and prospective studies show accurate genotyping to detect somatic mutations of allelic abundance >20%
- Non-invasive tool to track treatment-resistant clones in DLBCL

Rossi D et al., Blood 2017
Jian Y et al, Genome Biology 2014
Roschewski M et al., Blood 2016
Roschewski M., Lancet Oncol 2015
Assessment of ctDNA during and after lymphoma treatment facilitates the detection of both emerging resistance mutations and minimal residual disease (MRD) before progression, with potential for noninvasive prediction of relapse and histological transformation.

Kaplan-Meier analysis of PFS in patients with at least one ctDNA-positive plasma sample after the end of curative therapy compared to patients without detectable ctDNA after the end of curative therapy.

Florian Scherer et al., Sci Transl Med 2016;8:364ra155
Take home points

• Diagnosis
• Prognosis
• Therapeutic options/tailored therapies
• Disease monitoring
• Unexpected consequences of targeted therapies
QUESTIONS?