Lymphoma Pathogenesis as the Ultimate Diagnostic and Therapeutic Guidance

Mariusz A. Wasik
University of Pennsylvania

12:15 PM-1:15 PM session

SH/EAH: 9-7-17
Approach to diagnosis and therapy of lymphomas and other malignancies: current status

- Diagnosis: mostly descriptive, based predominantly on the morphological appearance and phenotype of malignant cells

- Therapy: mostly empirical, predominantly based on the results of large cohort clinical trials with anti-proliferative agents with limited cell/tumor-type specificity
Paradigm shift in approach to diagnosis and therapy of cancer

- Diagnosis and, consequently, therapy based on the biology of malignant cells and pathogenesis of the disease

- Both will gradually become highly specific for individual patients
Immunohistochemical stain panel for DLBCL

Cell lineage-specific
CD20, CD79a, PAX-5

DLBCL subtype-specific
CD10, BCL-6, MUM1(IRF-4)

Prognostic markers
Ki-67, c-MYC, BCL-2, BCL-6 (+ FISH)

Therapeutic targets
CD30, PD-1 (PD-L1), CD19, CD20, BCL-2
Elements of an antibody-drug conjugate (ADC)

**Antibody**
- Specific for a tumor-associated antigen that has restricted expression on normal cells

**Cytotoxic agent (payload)**
- Kills target cells when internalized and released

**Linker**
- Attaches the cytotoxic agent to the antibody; newer linker systems are designed to be systemically stable and release the cytotoxic agent in targeted cells

References:
Reinvention of medicine at the molecular level

Molecular Anatomic Pathology

Molecular Pathophysiology

Targeted Pharmacology
Chronic Myeloid Leukemia (CML)

1. “Philadelphia chromosome” found in 100% of cases - 1960-ties

2. Ph chromosome: result of translocation involving chromosomes 9 and 22 t(9;22) - 1970-ties

3. t(9;22) carries novel fusion kinase BCR-ABL - 1980-ties

4. Inhibitor of BCR-ABL identified - 1990-ties

5. Clinical trials performed with inhibitor approval based on high rate of durable responses - 2000-nds

6. Normal life span in the inhibitor responders - 2017
## Disease-specific mutations in lymphomas as therapeutic targets

<table>
<thead>
<tr>
<th>Disease category</th>
<th>Gene</th>
<th>Hotspot amino acids</th>
<th>Hotspot regions</th>
<th>Mutation frequency</th>
<th>Other lymphoid malignancies</th>
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<tr>
<td>Hairy cell leukemia</td>
<td>BRAF</td>
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<td>~100%</td>
<td>MM (3%), CLL (2%)</td>
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<td>WM</td>
<td>MYD88</td>
<td>L265P</td>
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<td>79–100%</td>
<td>IgM-MGUS (10–87%), ABC-DLBCL (19%), CLL (4%)</td>
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<td>AITL and AL-PTCL</td>
<td>RHOA</td>
<td>G17V</td>
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<td>53–68% in AITL, 62% in AL-PTCL</td>
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<td>T-LGL</td>
<td>STAT3</td>
<td>Y640F, D661Y/VH1, N647I</td>
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<td>28–40%</td>
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<td>NK/T-cell lymphoma</td>
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<td>NOTCH2</td>
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<td>Frequency CLL IGHV-UNMUT</td>
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Tier: 1 = Activating, 2 = Truncating
SLL/CLL study: conclusions and lessons

- Differential mutational profile in mutated vs. non-mutated SLL/CLL supports the notion that these are two distinct subtypes of the same general disease.

- The disease is genetically highly heterogeneous with clusters of aberrantly expressed genes seemingly contributing to its pathogenesis.

- **SLL/CLL emerges as a group of closely related malignancies with two subgroups rather than a single disease.**

- Pathogenesis of the disease for a large subset of patients remains unclear.

- The identified mutations point to attractive therapeutic targets, possibly to be targeted simultaneously.
Mutational genomic profiling of T- and B-cell lymphomas

McKinney et al. 2017
CRS occurs primarily in solid organs (lymph nodes, spleen, liver) not in bone marrow, at least early on, regardless of the organ tumor burden.

- the same T-cell (CART-cell) clones expand in various organs to vastly different degree

- CART cells co-activate also non-CART cells ("epitope-spreading phenomenon")

- CART cells are not the source of IL-6; endothelial and stromal cells, most likely APC, are
Targeting kinases in the BCR signaling pathway
Combination of CART19 cells with BTK inhibitor

Double targeting of MCL by two very different mechanisms

Enhanced anti-lymphoma activity

Reduced tumor escape
CART19 cell-Ibrutinib combination as novel therapy for MCL and other types of lymphoma

- Both CART19 and Ibrutinib display anti-MCL activity with the former agent being more potent
- CART-19/Ibrutinib combination is more effective against MCL than either agent alone, both *in vitro* and *in vivo*
- Ibrutinib partially suppresses expression of PD-1 and other “checkpoint” receptors and, hence, augments CART19 effect
- Ibrutinib alleviates CART19-induced CRS

CR in 8/9 CLL pts with advanced disease treated with the CART19/Ibrutinib combination

*S. Gill et al. ASCO 2017*
In September of 2003, a 58-year old male underwent a routine colonoscopy which revealed several polyp-like structures containing diffuse infiltrate of atypical small/medium lymphocytes. The lymphocytes were of B-cell origin, as demonstrated by staining for CD20, and co-expressed CD5, Cyclin D1, and BCL2 leading to the diagnosis of mantle cell lymphoma (MCL).

Oncology consultation revealed extensive, generalized lymphadenopathy; histology of an inguinal lymph node in November 2003 also showed involvement by MCL.
- monthly injections of rituximab (anti-CD20 antibody) lasted until April of 2005; the disease remained stable
- in January 2007, he experienced cecal obstruction leading to surgical resection and R-CHOP therapy (6 cycles)
- in March 2008, an isolated paratracheal mass was noted; it contained MCL and was treated with radiation (3600 cGy)
- in July 2009, a tonsillar biopsy showed MCL; R-bendamustine therapy was applied
- in March 2012 he displayed duodenal MCL involvement; R-bendamustine therapy was repeated
- in October 2012: mesenteric and retroperitoneal lymphadenopathy has developed; MCL was confirmed and treated with bortezomib and dexameth.
- in March 2013 large retroperitoneal mass was found and treated with radiation but bilateral pleural effusions containing MCL have developed and were treated experimentally with BTK inhibitor ibrutinib
- in July 2013 bilateral pleural effusions were treated with R-hCEV followed by Revlimid and Cytoxan. Meningitis-like symptoms with CSF involvement by the lymphoma occurred with the patient passing away in October of 2013
Glass half full:
the patient received state-of-the art clinical care and lived for 10 years having a lymphoma type associated with median survival time of 4 years

Glass half empty:
the patient never achieved complete remission, received numerous toxic therapies, lived through many life-threatening lymphoma rebounds, experienced disease progression and, finally, died of the highly aggressive lymphoma
Studies undertaken with MCL cells from this patient

- establishment of two cell lines fully recapitulating morphology, phenotype, and genotype (IgH-Cyclin D1 FISH) of the primary MCL cells

- DNA sequence analysis: whole exome sequencing (WES) of the normal cells, primary MCL cells, and MCL cell lines

- in-depth study of selected aberrant genes

- whole genome DNA methylation (WGM) analysis of the MCL primary cells and cell lines

- establishment of the in vivo ("mouse avatar") MCL model using the patient’s primary cells and cell lines

- novel combination therapies in the in vitro and in vivo models
Novel therapeutic targets identified in patient’s MCL cells

**Genomic studies:**

- Cyclin D1: CDK4/6 inhibitor (confirmed in cellular studies)
- ATM: PARP inhibitor
- KMT2D/MLL-2: HDAC inhibitor, novel MLL-Menin inhibitor

**Cell culture studies:**

- CD19: CART19 immunotherapy
- CART19-BTK inhibitor combination
- BTK-CDK4/6, mTOR-BTK, mTOR-CDK4/6 inhibitor combination
Translocation t(2;5)(p23;q35) in anaplastic large T-cell lymphoma

- 80 kD NPM (nucleophosmin)/ALK hybrid protein

- oligomerization motif of NPM fused to cytoplasmic portion of ALK (includes the entire kinase catalytic domain)
NPM-ALK hybrid tyrosine kinase is:

• **aberrantly expressed**
  (due to persistent activation of the NPM promoter)

• **constitutively activated**
  (due to NPM domain-mediated oligomerization and ALK kinase domain-triggered autophosphorylation)

• **highly oncogenic**
  (documented *in vitro* and *in vivo* in mouse models)

MA Wasik et al. Sem Oncol 2009
NPM-ALK utilizes IL-2R-type signaling to transform target CD4+ T cells

Table 1. Signaling pathways activated in common by IL-2 and NPM/ALK

<table>
<thead>
<tr>
<th>Pathway</th>
<th>p-value</th>
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<td>JAK/Stat Signaling</td>
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<td>IL-2 Signaling</td>
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<td>Circadian Rhythm Signaling</td>
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<tr>
<td>p53 Signaling</td>
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</tbody>
</table>

M Marzec et al. J Immunol 2013
NPM-ALK/STAT3-promoted immune evasion of lymphoma cells

ALK+ TCL cells are protected from immune response by both secreting tolerogenic cytokines (IL-10&TGFβ) and expressing immunosuppressive cell-membrane protein CD274 (PD-L1)

Kasprzycka et al. PNAS 2006, Marzec et al. PNAS 2008
NPM-ALK promotes DNMT1 expression and induces epigenetic silencing of IL-2Rγ

Zhang et al. PNAS 2011
NPM-ALK-mediated reprogramming of CD4+ T cells

TCR signaling

IL-2-type cytokine signaling

Cell proliferation, survival, growth, metabolism, etc.
NPM-ALK induces expression of HIF1α mRNA: protection of malignant cells from effects of hypoxia

Marzec et al. 2011
NPM-ALK induces expression of ICOS gene: “parasitic” utilization of stimulatory signals generated by immune cells

Zhang et al. 2011
Hallmarks of cancer: NPM-ALK as omnipotent oncogene

M. Werner et al. Blood 2017
NPM-ALK-transfected CD4+ T cells form tumors

Q. Zhang et al. 2013
Growth of NPM-ALK-transformed CD4+ T cells is dependent on ALK
Outcome of ALK inhibition in ALK+ ALCL patients

Adults: 11 pts with advanced/resistant disease: 7 sustained CR; 3 of them underwent allotransplant (Gambacorti-Passerini et al. NEJM 2011, JNCI 2014)

Discontinuation of ALK inhibitor resulted in disease recurrence (Gambacorti-Passerini et al. NEJM 2016)

Children: 7 out of 9 pts achieved CR in C1-C5; 3 underwent auto-BMT. Follow up study of 26 pts achieved >80% CR rate (Mosse et al. LO 2013, JCO 2017)

CR in chemotherapy- & ALK inhibitor-resistant ALK+ ALCL patient in response to anti-PD1 antibody (Hebart et al. AIM 2016)
Paradigm shift in approach to diagnosis and therapy of lymphoma

- Diagnosis and, consequently, therapy should be based on well understood and documented pathogenesis of the lymphoma in individual patients.

- The emergence of targeted therapies calls for the expansion of the diagnostic aims from classifying lymphoma according to the WHO schemes to identification of therapeutic targets.
Increased understanding of biology is already affecting patient management and will continue to do so.

Understanding the biology

Accurate classification/Identification of therapeutic targets

Rational treatment approaches

Improved patient management

Ultimate goal: disease pathogenesis-driven, personalized diagnosis & therapy of cancer
Interpretation of Sequence Variants in Cancer

**Tier I: Variants of Strong Clinical Significance**
Therapeutic, prognostic & diagnostic

- **Level A Evidence**
  - FDA-approved therapy
  - Included in professional guidelines

- **Level B Evidence**
  - Well-powered studies with consensus from experts in the field

**Tier II: Variants of Potential Clinical Significance**
Therapeutic, prognostic & diagnostic

- **Level C Evidence**
  - FDA-approved therapies for different tumor types or investigational therapies
  - Multiple small published studies with some consensus

- **Level D Evidence**
  - Preclinical trials or a few case reports without consensus

**Tier III: Variants of Unknown Clinical Significance**

- Not observed at a significant allele frequency in the general or specific subpopulation databases, or pan-cancer or tumor-specific variant databases
- No convincing published evidence of cancer association

**Tier IV: Benign or Likely Benign Variants**

- Observed at significant allele frequency in the general or specific subpopulation databases
- No existing published evidence of cancer association

Li et al. 2017