INTRODUCTION TO THE MOLECULAR BIOLOGY OF LYMPHOMA

January 29, 2016

Urban Novak, MD
Medical Oncology
Inselspital Bern
CANCERS HARBOR GENETIC LESIONS...

Melanoma: ~33’000 changes in the DNA !!!

Acquired through failures during DNA repair

Some lesions may be biologically irrelevant

Oncogenetic if essential biological processes are targeted

“passengers”

“drivers”
GROWTH = A REGULATED PROCESS

(Proto)-ONCOGENE + TUMOR SUPPRESSOR -

Growth
Proliferation
Self renewal

Growth arrest
Cell cycle arrest
Differentiation

DNA damage
Cell cycle arrest
Apoptosis
DNA repair

Fine tuning p53
Grob & Novak, Cell Death Diff 2001
INACTIVATION OF A20 IN MARGINAL-ZONE LYMPHOMAS

Somatic mutations

Normal DNA

Tumor DNA

A20 Allel A

A20 Allel B

Biallelic A20 deletion

A20 CEP6

Biallelic

Monoallelic

Novak, Blood 2009

KNUDSON PNAS 1971!
PRINCIPLES OF LYMPHOMAGENESIS

**Characteristics of**

...**Normal B-cells**

→ Circulating cells
→ Influenced by antigens and cytokines
→ Challenged by the germinal center reaction

...**Lymphomas**

→ Retain features of normal B-cells
→ Influenced by the stroma (CLL, MALT, FL)
→ Blocked differentiation (DLBCL subtypes)
→ Blocked apoptosis (FL, Hodgkin’s disease)
→ Self stimulation (CLL)

→ **Why Ibrutinib Work**
GERMINAL CENTER

MARGINAL-ZONE LYMPHOMA

Novak, Br J Haematol 2011

Memory B cell

High affinity

Expansión

Somatic hypermutation

Class switch

Low affinity

IgM, IgA, IgG, or IgE

Antibodies with different effector functions

DNA damage

Growth

Naïve B cell

IgM, IgD

Plasma cell
GENETIC LESIONS IN LYMPHOMAS: by-products of the AID-dependent DNA remodelling

Germinal Center

physiologic

AID

CSR

DNA BREAKS...

SHM

...ERRORS!!

pathologic

Chromosomal Translocations
(Ig-cMYC, Ig-BCL6)

Aberrant SHM
(multiple oncogenes)

DLBCL/BL

DLBCL
CHROMOSOMAL TRANSLOCATIONS

Gene A

Gene B

Translocation

Fusion protein
(Leukemias)

Deregulated transcription
(Lymphomas)

Burkitt’s & diffuse large B-cell lymphoma
TWO SCENARIOS OF SOMATIC HYPERMUTATION

physiological

B cells in the germinal center

aberrant

DLBCL

Pasqualucci, Nature 2001
Saito & Novak, PNAS 2009
Khodabakhshi, Oncotargets 2012
BCL6 ORCHESTRATES THE GERMINAL CENTER REACTION

Centroblast

BCL6

CD80  ATR  P53  p21  MYC  BCL2  STAT  INFR  IL10R  IL6&7R  PRDM1

Activation  Cell cycle arrest  DNA repair  Apoptosis  Signal transduction  Differentiation  Cytokine response

Naïve B cell

Centrocyte

Memory B cell

GERMINAL CENTER

„proliferate & ignore your DNA damage“

BCL6 ORCHESTRATES THE GERMINAL CENTER REACTION

**Centroblast**

- BCL6
- CD80
- ATnP53
- p21
- MYC
- BCL2
- STAT
- INFR
- IL10R
- IL6&7R
- PRDM1

**Signal transduction**

- Activation
- Cell cycle arrest
- Apoptosis
- Cytokine response
- DNA repair

**Naïve B cell**

**Centrocyte**

- Ag
- CD40L
- CD40
- NF-kB
- MAPK
- IRF4

**Memory B cell**

**Plasma cell**

**GERMINAL CENTER**
**BCL6 ORCHESTRATES THE GERMINAL CENTER REACTION**

**Centroblast**
- **BCL6**: +++
- **MYC**: some
- **BCL2**: none

**Germinial Center**
- BCL6
- MYC
- BCL2
- STAT
- INFγR
- IL6&7R
- PRDM1
- CD80
- ATR
- P53
- p21
- MYC
- DNA repair
- Cell cycle arrest
- Signal transduction
- Differentiation
- Apoptosis
- Cytokine response

**Centrozyte**
- **BCL6**: +

**Memory B cell**

**Naïve B cell**

**Burkitt’s Lymphoma**

**Follicular Lymphoma**

**Plasma cell**

„you graduated, go ahead“
PATHOLOGIC CO-EXPRESSION OF BCL2 & BCL6 IN DIFFUSE LARGE B-CELL LYMPHOMAS

Saito & Novak, PNAS 2009

BCL2
BCL6 & BCL2
BCL6

Physiology

Pathology

- BCL6 & BCL2
- Other combinations

Cases (%)
0 20 40 60 80 100

- No MIZ1 (no binding)
- Lots of MIZ1 (activation)
- MIZ1 normal

BCL2 mutations → MIZ1 hyperactive
BCL2 MUTATIONS DESCRIBE THE CLINICAL EVOLUTION OF FOLLICULAR LYMPHOMAS

- Follicular lymphoma (FL)
- Transformed follicular lymphoma (tFL)

11% over 5 years!

Link, J Clin Oncol 2013

Biopsies

Years after diagnosis
**Clinical application:**

**Clonal relationship:**

Relapse or new lymphoma?

**Physiology** *(Diversity = normal !)*

**Pathology** *(e.g. in lymphomas)*
“As is our pathology so is our practice; what the pathologist thinks today the physician does tomorrow.” Sir William Osler, 1909
“TRUE” DIAGNOSTIC MUTATIONS

MYD88 (L265P) mutations
Waldenstrom’s: ~90 %
Predictive for…
→ Ibrutinib (Treon, NEJM 2015)

BRAF V600E mutations
Hairy cell leukemia: 100 %
Predictive for…
→ Vemurafenib (Tiacci, NEJM 2015)

Diagnostic dissection of nodal & splenic MZL & Waldenstrom’s
→ mutational analysis of NOTCH2, MYD88 & PTPRD?
Rossi, JEM 2012; Kiel, JEM 2012; Spina, ASH 2014#705
1. **Prognostic biomarkers**

→ *Information on disease outcome, irrespective of therapy*

→ PET+ DLBCL: no ! better treatment than R-CHOP (ASH 2014 # 391 & 392)

→ **Biomarkers in CLL:**

1. NOTCH1 M **prognosticator of its transformation** (Rossi Blood 2011 & BJH 2012)

2. **Predictive biomarkers (≠ necessarily therapeutic targets)**

→ *predicting the response to a given treatment*

Some biomarkers are **prognostic & predictive** (deletion 17p in CLL)

→ first prognostic, then predictive (HER2 in breast cancer)

→ **examples in lymphomas ?**
THE MOLECULAR SUBTYPES OF DLBCL ARE DIFFERENT

...but their assessment is not standardized!

Hans, Blood 2004

Reber, Swiss Medical Weekly 2013
DLBCL SUBTYPES: NO LONGER PROGNOSTIC (?) AND NOT YET PREDICTIVE

**PROGNOSTIC?**

PFS (2y)  ABC 40% *(Lenz, NEJM 2008)*
non-GC 62% *(Fu, JCO 2008)*

PYRAMID trial (R-CHOP +/- Bortezomib)

R-CHOP performed better (2y PFS 78%)

*Leonard ASH 2015#811*

Selected population in randomized trials?

**PREDICTIVE ?**

Randomized evidence by adding bortezomib, a purported NF-κB inhibitor, to R-CHOP

→ outcome of the NF-κB dependent subtype not improved

*Offner Blood 2015, Leonard ASH 2015 # 811, Davies ASH 2015 # 812*
MOLECULAR BIOLOGY OF THE DLBCL SUBTYPES: LOST IN COMPLEXITY & SPACE?!?

Prognosis (Ennishi, ASH 2014 #703):
Good: STAT3 M; bad: MYD88 M

THE NF-κB SIGNALING PATHWAY
A DIFFICULT THERAPEUTIC TARGET !?!

canonical

alternative

Immunhistochemistry?

Compagno, Nature 2009

NF-κB pathways
THE ACHILLES’ HEEL OF ABC-DLBCL OFFERS MULTIPLE THERAPEUTIC TARGETS

- PKC
- ENZASTAURIN
- SYK
- BORTEZOMIB (VELCADE®)
- FOSTAMATINIB
- IBRUTINIB (IMBRUVICA®)
- CAL-101 (IDELALISIB®)
- PI3K
- BTK
- AKT
- mTOR
- BCL2
- DNA damage response
- Differentiation
- Cell cycle arrest
- Apoptosis
- IRF4
- NF-kB
- CD79A,B
- CD40 Ligand
- CD40 Receptor

REM trial

IBRUTINIB REMoDL trial
**BCR-Mutations Are Not Predictive for the Response to Ibrutinib**

- **M. Waldenström (~90% MYD88 mutations):**
  - ~80% response (Treon, NEJM 2015)

- **CLL:** BTKM in 5/13 pts. refract. to ibrutinib
  - (Woyach, NEJM 2014)

- **HL:** active with few M (BCR 20%, BTK 10%)
  - (Hamadani NEJM 2015; Mata ASH 2015 # 178)

> Most responses in BCR WT ABC

> oncogenic BCR signalling by non-genetic mechanisms

*Wilson, ASH 2012 & Nat Med 2015*

*Cheung, ASH 2015 # 2642*
Within the phase II SPARK (MCL2001) study…

- single-agent ibrutinib in MCL progressing after bortezomib
- Response: ORR 66.4 %, CR 18.2 %

...Balasubramanian (ASH 2014 # 78) explored the patients with:

*primary resistant disease (23 %) or a moderate benefit (20 %)*

Mutations in PIM1 kinase, mTOR, the oncogenes BCL2 & ERBB4, the epigenetic modifiers WHSC1, MLL2 & CREBBP &

NF-κB pathway (TAB2, TRAF3 & NIK) in pts. with a moderate clinical benefit

Combinations of ibrutinib and NF-κB inhibitors worth exploring

→ ongoing phase I/II SAKK trial is combining ibrutinib & bortezomib
ANALYSIS OF CIRCULATING LYMPHOMA DNA: A LIQUID BIOPSY

Clonotypes detected in the blood of 13/17 (81%) DLBCLs

*Armand, BJH 2013*

Retrosp. analysis on 126 DLBCL pts.
- Circulating DNA correlated with progression (not OS) at 5y
- Relapse predicted 3.5 month before clinical manifestation
- Specificity better than PET

*Roschewski, Lancet Oncol 2015; Kurtz, Blood 2015*

ASH 2015 abstracts 114, 127 & 130 on the possible use of circulating DNA:
- Assessment of tumor burden, minimal residual disease & relapse
- Accurate detection of tumor heterogeneity and clonal evolution
- Detection of additional mutations from clones distant from site of biopsy

...but consider which treatment will improve the outcome?

*costs effectiveness
less stress for patients (than CT or PET?)*
**GENETIC LESIONS PROVIDE AN IMMUNE ESCAPE**

---

**No surface HLA-expression**

- in >60 % of DLBCL (Challa-Malladi, Cancer Cell 2011)
- Rare in other B-NHLs (Fangazio, ASH 2014#1692)
- frequent in FL transformation (Pasqualucci Cell Reports 2014)

---

**CIITA fusions**

- surface HLA class II expression ↓
- overexpression the ligands of PD-1/2  
  Steidl, Nature 2011

---

„Immune privilege lymphomas“:

- PMBL, Hodgkin‘s, testicular, & primary CNS lymphomas

  Twa, Blood 2014

---

**B**

- 9p24.1 Break-apart
- 9p24.1 Amplified
IDENTIFYING PATIENTS THAT MIGHT BENEFIT FROM PD-1 BLOCKAGE

PD-1 engagement by its ligand transient ↓ T-cell function (exhaustion)


PD-L1: expressed in 10% (EBV+) Non-GC DLBCL, poor outcome (Kiyasu, Blood 2015)

In Hodgkin’s disease:

Armand, NEJM 2014
Roemer, ASH 2015 # 176

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Polysomy 9p</th>
<th>PDL1/2 Gain</th>
<th>PDL1/2 Amplification</th>
<th>IHC-positive HRS cells</th>
<th>Nuclear pSTAT3</th>
<th>EBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

Table: Efficacy Results

<table>
<thead>
<tr>
<th>Tumor</th>
<th>N</th>
<th>Complete Response (%)</th>
<th>Partial Response (%)</th>
<th>Stable Disease (%)</th>
<th>Progression Free Survival Rate at 24 Weeks (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse Large B Cell Lymphoma (DLBCL)</td>
<td>11</td>
<td>1 (27)</td>
<td>3 (27)</td>
<td>3 (27)</td>
<td>(24)</td>
</tr>
<tr>
<td>Follicular Lymphoma (FL)</td>
<td>1</td>
<td>1 (10)</td>
<td>0 (20)</td>
<td>6 (80)</td>
<td>(68)</td>
</tr>
<tr>
<td>Other B Cell Lymphoma</td>
<td>8</td>
<td>0 (63)</td>
<td>5 (63)</td>
<td>0 (38)</td>
<td>(38)</td>
</tr>
<tr>
<td>Primary Mediastinal B Cell Lymphoma</td>
<td>2</td>
<td>0 (60)</td>
<td>0 (20)</td>
<td>2 (100)</td>
<td>(30)</td>
</tr>
<tr>
<td>Mycosis Fungoides (MF)</td>
<td>13</td>
<td>1 (7)</td>
<td>2 (15)</td>
<td>9 (69)</td>
<td>(59)</td>
</tr>
<tr>
<td>Peripheral T Cell Lymphoma (PTCL)</td>
<td>5</td>
<td>1 (40)</td>
<td>0 (20)</td>
<td>0 (80)</td>
<td>(15)</td>
</tr>
<tr>
<td>Other T Cell Lymphoma</td>
<td>5</td>
<td>0 (20)</td>
<td>1 (20)</td>
<td>0 (20)</td>
<td>(20)</td>
</tr>
<tr>
<td>Multiple Myeloma (MM)</td>
<td>27</td>
<td>0 (67)</td>
<td>0 (67)</td>
<td>18 (67)</td>
<td>(15)</td>
</tr>
<tr>
<td>Chronic Myelogenous Leukemia</td>
<td>1</td>
<td>0 (100)</td>
<td>0 (100)</td>
<td>0 (100)</td>
<td>(100)</td>
</tr>
</tbody>
</table>

Lesokhin, ASH 2014 # 291
SUMMARY & TAKE HOME MESSAGES

1. Errors during B-cell development (e.g. GC) involved in lymphomagenesis

2. Variety of genetic lesions reflects the clinical heterogeneity of lymphomas

3. Whole-genome approaches have identified...
   - ...prognostic & diagnostic markers (e.g. BRAFV600E & MYD88)
   - ...druggable targets (e.g. EZH2 mutations)

4. Postgenomic era: new tools used to address specific questions, including:
   - clonal evolution (e.g. FL / tFL or DLBCL, CLL / Richter’s)
   - mechanisms of resistance (e.g. to ibrutinib: CLL ≠ MCL)

Clinicians need: **predictive markers & surrogates for pathways**!

(e.g. are COO subtypes predictive; what determines the response to Ibrutinib?)