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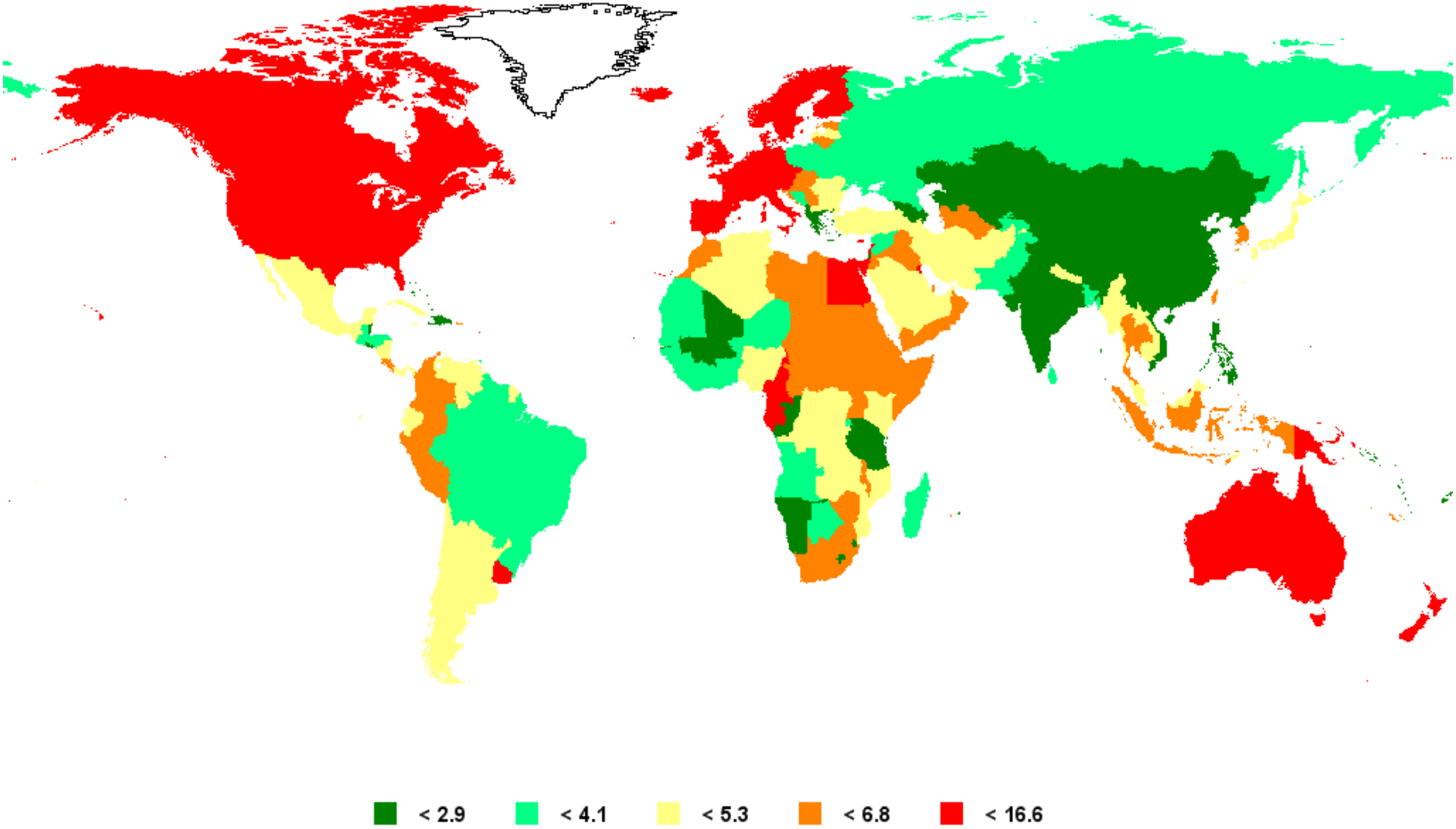
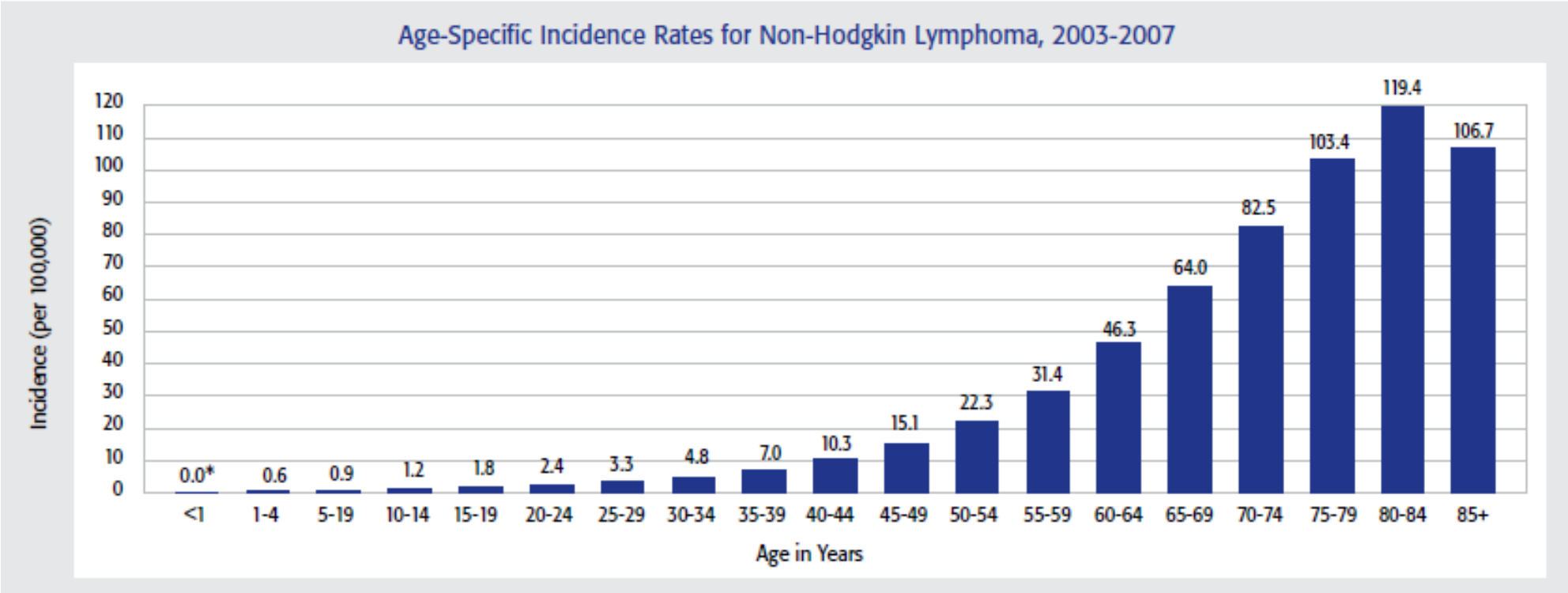
Age Standardized Rate of NHL Worldwide



GLOBOCAN Data; http://globocan.iarc.fr

Increasing Incidence of NHL with Age in USA



- SEER Data
- Leukemia and Lymphoma Society

Differences Between

Diffuse Large B-cell Lymphoma and Follicular Lymphoma

Diffuse Large B-cell Lymphoma & Follicular Lymphoma

- Approximately 65,000 people were diagnosed with non-Hodgkin lymphoma (NHL) in 2010 in the US (SEER data)
- Diffuse large B cell lymphoma is the most common lymphoma in every country in the world but it is NOT a distinct disease entity; it is highly heterogeneous category consisting of 22 separate variants & subtypes
- Of all lymphomas, that are distinct disease entities, Nodal FL is the most common in the west, and occurs in all ages & at all anatomic sites
- The incidence of FL in the USA is 30%, and it is the highest in the world. And in even most countries in the EAST, ***of the lymphoma disease entities***, it is one of the most common lymphoma.
- PTCL, NOS is not a specific disease entity

Frequency of Follicular Lymphoma Around the World

Country	Frequency	Country	Frequency
Iran	1%	Greece	10%
Mexico	5%	Guatemala	10%
Korea (2)	5-6%	Switzerland	11%
Bulgaria	6%	India (2)	13%
Turkey	6%	France	17%
Taiwan (5)	6-18%	Germany	18%
Japan	7%	Chile	25%
China (3)	7-23%	U.K.	28%
Ecuador	8%	Canada	31%
Thailand	8%	U.S.A.	32%
Hong Kong	8%	S. Africa (whites)	33%

Molecular Pathogenesis of Follicular Lymphoma

Most Exciting & Intriguing Aspects of FL

- In USA, 85% of nodal FL, have the t(14;18) translocation
- In ~40% of normal healthy individuals, rare circulating t(14;18)+ cells are found in the blood
- Paradoxically, the incidence of FL is only 1 per 24,000 individuals
- But, FL is the most common lymphoma disease entity in USA
- Therefore, how does an In Situ FL develop and progress to an overt FL, and how and why further progression and transformation occurs?
- My presentation today deals with these exciting & intriguing aspects of FL, & the most important aspect is:

  -The Biography of the t(14;18)+ cell, and why its journey after survival is unpredictable, long, & tortuous

OUTLINE OF PRESENTATION

- Follicular Lymphoma is born in the bone marrow
- It reaches maturity in the Germinal Center- where it can be recognized as an In-situ FL
- Pictures of In-situ, Overt FL, Progression, Transformation
- To understand the molecular pathogenesis of FL, first we must go back to the origins in the bone marrow. For that, we must look at normal B-cell development and differentiation processes
- The biography of the t(14;18) cell
- The function & role of the B-Cell Receptor (BCR) in the development & evolution of FL
- Molecular Pathogenesis of the t(14;18) negative FL

Biography of the t(14;18)+ cell

- Development of an In-Situ FL
- Progression of an In-Situ FL to an overt FL
- Mechanisms that lead to:
  - Progression
  - Transformation to a more aggressive phase

  But, first: histologic & immunophenotypic of the above stages of FL

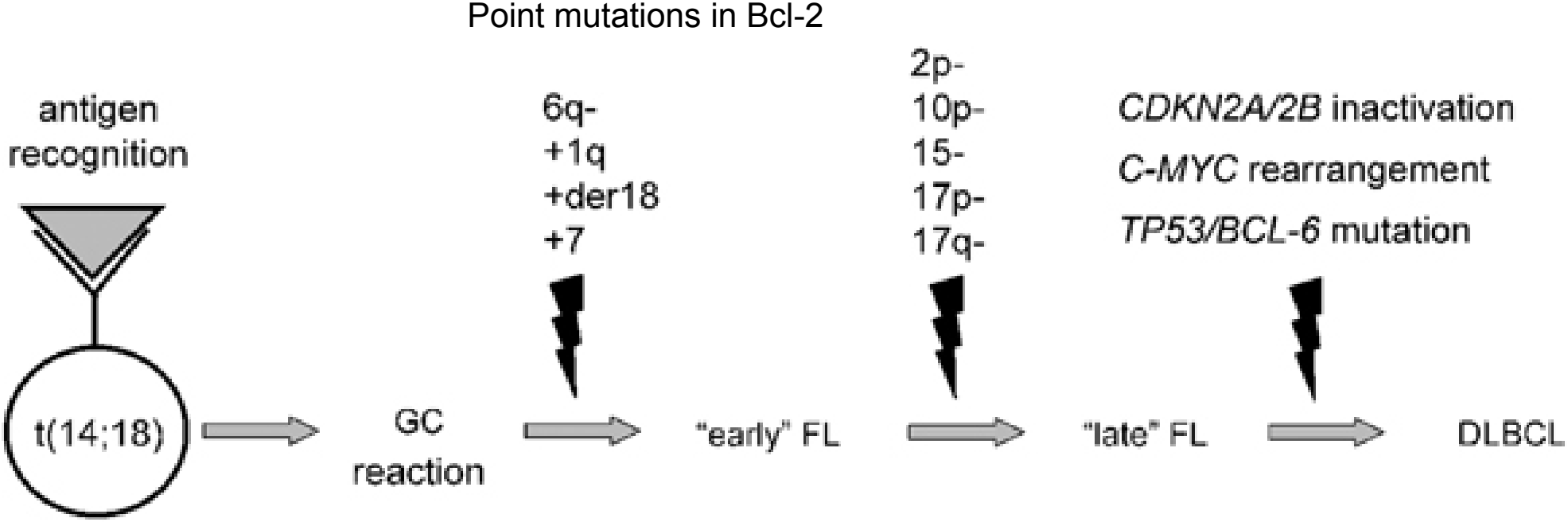
In Situ FL: Development, Progression & Transformation.
Examples of Histology & Immunophenotype

- Development of an In-Situ FL
- Progression of an In-Situ FL to an overt FL
- Mechanisms that lead to:
  - Progression
  - Transformation to a more aggressive phase

MOLECULAR CHANGES IN FL PRECEDE HISTOLOGIC CHANGES

- Multiple “hits” - oncogenic events & further genetic changes occur in the t(14;18)+ cell that lead to autonomous growth of cells, and such changes also produce histologic changes that we recognize under the microscope

Additional Secondary (genetic) Alterations are Necessary for FL Development



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To Understand the Molecular Pathogenesis of How Does an In Situ FL Develop and Progress to an Overt FL, and How & Why Further Progression & Transformation Occurs Requires Knowledge About Normal B-cell Physiology & Development

The Normal B-cell Development Process

Is

Our Savior (> 20 billion antibodies)

&

Our Inadvertent Killer

(Development of B cell lymphoma)

B-cells Are Capable of Producing >20 Billion Highly Specific Antibodies

- To provide effective humoral immunity, B-cells must be extensively diverse to recognize a massive number of antigens/pathogens
- To achieve this, the normal B-cells- randomly, continuously and extensively remodel their DNA,- more than any other cell in the body
- Normal DNA remodeling processes involve double stranded chromosomal breaks (DSB) in normal DNA (6 in the marrow, several in germinal center, & several more thereafter) followed by rejoining of chromosomes
- Mistakes in rejoining process lead to chromosomal translocations: e.g. [t(14;18)], [t(11;14), etc]

DNA Remodeling
&
Generation of the B-Cell Receptor

SEQUENTIAL STEPS IN THE DNA REMODELING PROCESS

- In the bone marrow, in Pro B-cells that have NOT been exposed to antigens, the random VDJ Recombination leads to the generation of the B-Cell Receptor Protein on the surface of the cells
  - Function & structure of the B-cell receptor (BCR) surface protein
- In the Germinal Centers During High Affinity Maturation Process
  - Called Somatic hypermutation & Class switch recombination

THE DETAILS OF THE NORMAL

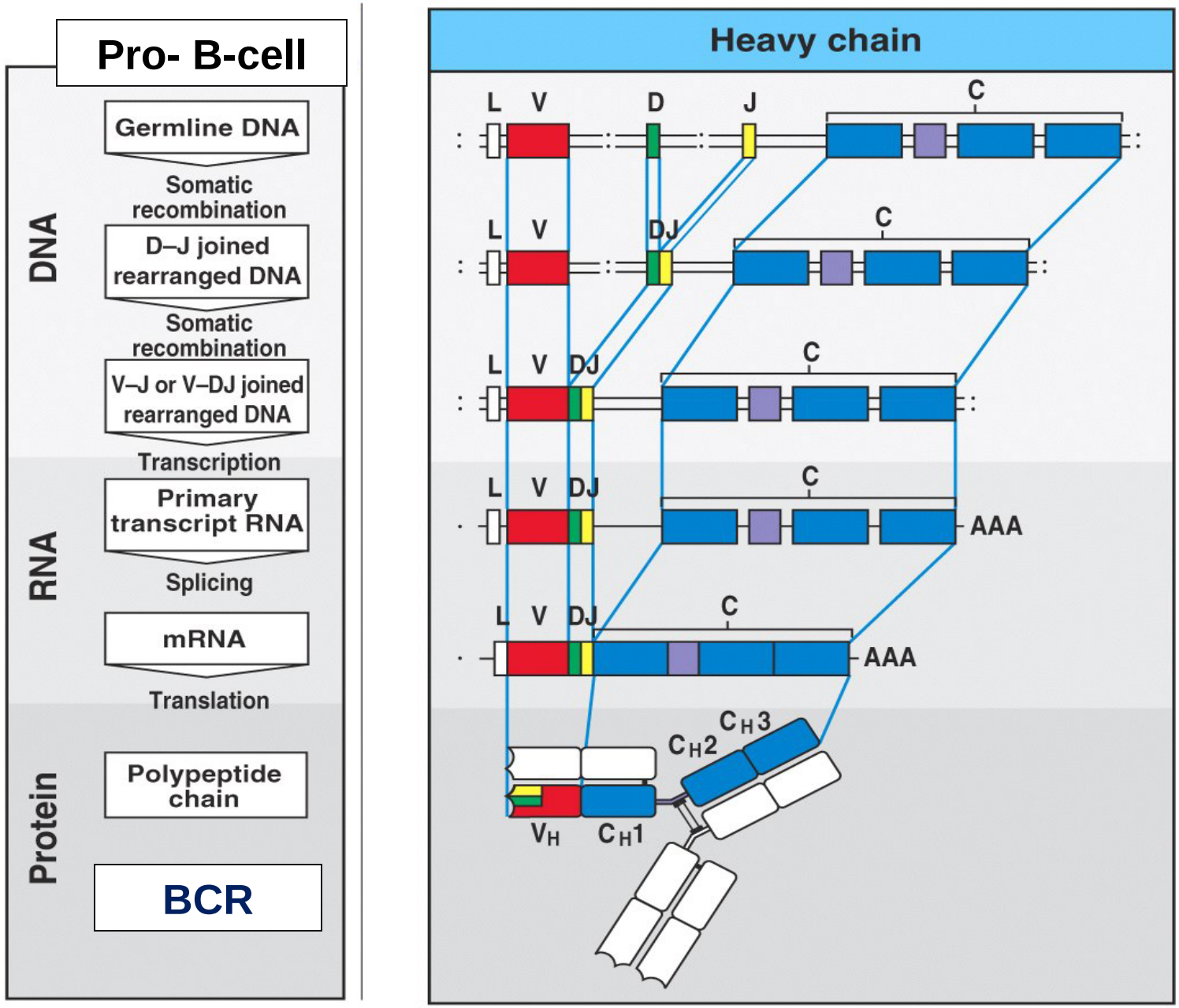
DNA REMODELING

IN THE BONE MARROW

Generation of B-cell Diversity by Random VDJ Recombination in the BM Leads to >10 Billion Highly Specific Antigen Binding Sites

- **Combinatorial Diversity in Bone Marrow:**
  - **IGH VDJ (6,000) & Light chain VJ (320) recombination**
- **Joining of heavy and light chains to form Fab**
  - **1.92 million combinations (6,000 x 320)**
- **Junctional Diversity (RAG & TdT mediated in BM)**
  - **Involves addition and subtraction of one to multiple nucleotides to the 1.92 million combinations that leads to over 10 billion specificities (nuclear DNA to B-Cell Receptor[BCR] protein)**

Cellular and Molecular Immunology, Abbas et al.


Pro- B-cell
DNA
Germline DNA
Somatic recombination
D–J joined rearranged DNA
Somatic recombination
V–J or V–DJ joined rearranged DNA
Transcription
RNA
Primary transcript RNA
Splicing
mRNA
Translation
Protein
Polypeptide chain
BCR
Heavy chain
L
V
D
J
C
DJ
AAA
V_H
C_H1
C_H2
C_H3

Further Generation of B-cell Diversity Occurs in the Germinal Center that Leads to Additional >10 Billion Highly Specific Antigen Binding Sites

- **Further B-cell diversification occurs in the germinal center during the Process of High Affinity Maturation via the BCR**

Cellular and Molecular Immunology, Abbas et al.

Role of the Normal B-Cell Receptor (BCR)

The Function of the BCR

- The BCR, throughout the life of a B-cell, transmits signals for survival, proliferation or initiate program cell death (apoptosis)
- Altered BCR signaling may support lymphoma development
- In follicular lymphoma, BCR-mediated signaling via phosphorylation of Btk, Syk, Erk1/2, and p38 occur:
  - more rapidly in FL cells than in infiltrating benign B cells
  - achieve greater levels of per-cell signaling in FL than in benign B-cells
  - sustain this level of signaling for hours longer than benign B cells

Irish JM et al. (2006) Blood. 108:3135-42.

The Function & Role of the BCR (cont)

- High affinity maturation occurs mainly in the germinal center by the process of somatic hypermutation (SHM), wherein the DNA of the VDJ genes accumulate point mutations, which involves chromosomal DSB with rejoining, and it also involves T-cell interactions

Role of The Normal High Affinity Maturation Process

- The aim of the high affinity maturation process is to produce B-cells that have high affinity BCR (antigen binding site that has the best fit for the antigen)

AND

Eliminate cells that have low affinity BCR for antigen

Comparison of the Affinity Maturation Processes in a Normal B cell with that of the B-cell that has acquired the t(14;18) Translocation

Affinity Maturation in the Germinal Center: Comparison of Normal with the t(14;18)+ Cell

- Normal affinity maturation involves random mutations of the normal DNA (VDJ genes), wherein, in most cases, a low affinity BCR with poor fit to antigen is produced ("crippled"), followed by clonal selection, which involves elimination of the crippled cell(s)
- Similarly, the t(14;18)+ B-cell, enters a follicle and undergoes "Affinity Maturation", and in most cases a low affinity BCR (crippled cell) results, & instead of being eliminated, it is illegitimately rescued due to constitutive expression of BCL2 that leads to inhibition of apoptosis (long living)

Properties of the t(14;18)+ Cell, and its further Biography

Properties of the t(14;18)+ cell

- The t(14;18)+ B-cell has:

  - one functional BCR (from the non-translocated allele) that responds to antigen like a normal B-cell and

  - a non-functional BCR (translocated) allele that cannot respond to antigen

- Circulating t(14;18) + cell(s) is found in ~ 40% of the blood samples of normal healthy individuals and they can survive up to 10 years & have memory B-cell phenotype (CD27+, IgM+ and mutated)
- Paradoxically, incidence of FL is only 1 case per 24, 000 individuals
- Thus, most t(14;18)+ cells die (due to neglect)

Biography of the t(14;18)+ Cell

- Survival of the t(14;18)+ cells that are crippled
- Additional oncogenic events & further genetic changes that lead to autonomous growth of crippled cells

Struggle for Survival of t(14;18)+ Cell that Has a Low Affinity (Crippled) BCR in the GC

- Most t(14;18) cells do not survive the hostile environment of the germinal center because:

  - these crippled cells have to compete with normal GC cells to survive & propagate. The normal GC cells have high affinity BCRs & thus rapidly proliferate, & crowd out the crippled cell(s)

- t(14;18)+ crippled cells also search for antigenic stimulation by migrating from follicle to follicle looking for growth signals (Oeschger, Hansmann et al*)
- Also these cells seek safe sanctuaries, cognate T-cell help, & further antigenic stimulation (HCV, etc)
- Also, the t(14;18)+ cell reactivate recombinase-activating genes (RAG1 & RAG2) that would allow the crippled cell to revise its low affinity antigen BCR to a high affinity, thereby giving it a second chance to be selected for growth

*(Blood 2002; 99:2192-2198)

Additional Oncogenic Events that Promote Survival and Propagation of the t(14;18)+ cell that has a Crippled BCR

- **Factors that cause genomic instability**
  - Environmental factors: exposure to pesticides, chemicals, excessive smoking, etc
  - Constitutive AID expression upregulates DNA remodeling by excessive somatic hypermutation (SHM) and class-switch recombination (CSR) which cause excessive chromosomal breaks and aberrant translocations in Ig and Non-Ig genes
- **Eventual antigen independence**
- **Inactivation of tumor suppressor genes (p53, p16), & activation of oncogenes & other pathways (c-myc)**

Biography of the t(14;18)+ Cell: Further Evolution

- Additional oncogenic events and further genetic changes lead to autonomous growth of crippled cells

- Information gathered from Gene Expression Profiling Consortia Studies

Gene Set Enrichment Analysis

- Rosenwald A et al. N Engl J Med. 2002;346:1937–1947
- Dave SS et al. N Engl J Med. 2006;354:2431–2442
- Dave SS et al. N Engl J Med. 2004;351:2159–2169.
- Wright G et al. Proc Natl Acad Sci U S A. 2003;100:9991–9996
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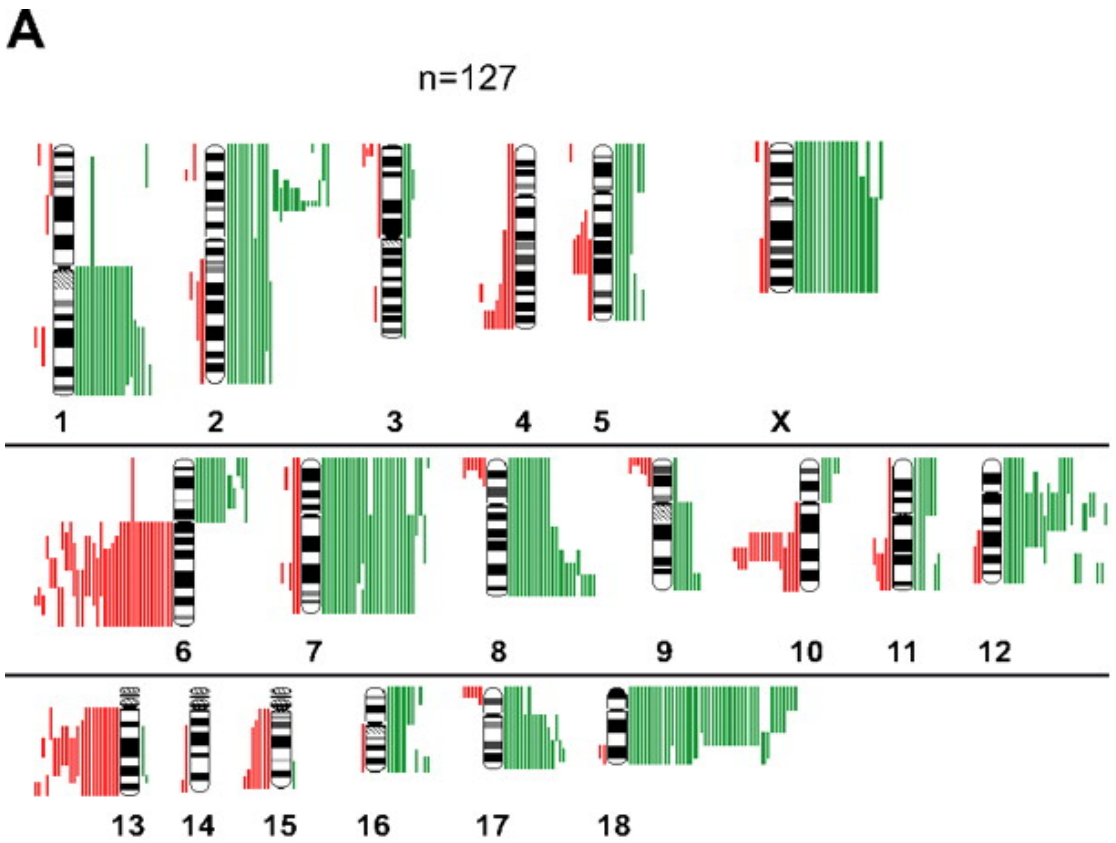
- Kovanen PE, et al. J Biol Chem. 2003;278:5205–5213.
- Shaffer AL, et al Immunity. 2001;15:375–385.
- Su AI, et al. Proc Natl Acad Sci U S A. 2004;101:6062–6067
- Rosenwald A et al. Cancer Cell. 2003;3:185–197
- Baechler EC et al. Proc Natl Acad Sci U S A. 2003;100:2610

Array Comparitive Genomic Hybridization(CGH)

- Microarrays are created by the deposit and immobilization of small amounts of DNA (known as probes) on a solid support, such as a glass slide, in an ordered fashion.
- Probes vary in size from oligonucleotides manufactured to represent areas of interest (25–85 base pairs) to genomic clones such as bacterial artificial chromosomes (80,000–200,000 base pairs).
- The primary advantage of a CGH is the ability to simultaneously detect aneuploidies, deletions, duplications, and/or amplifications of any locus represented on an array; in fact, one assay using this technique is equivalent to thousands of FISH experiments.

CGH Analysis: Gains & Losses in t(14;18)+ 127 FL Cases



- **Gains**
  - 1q
  - 2p
  - 7
  - 8q
  - 12q
  - 18q
  - X
- **Losses**
  - 6q
  - 10q
  - 13q
- These abnormalities establish the presence of secondary alterations that suport autonomous and unregulated proliferation

Leich E et al. 2009. Blood 114: 826

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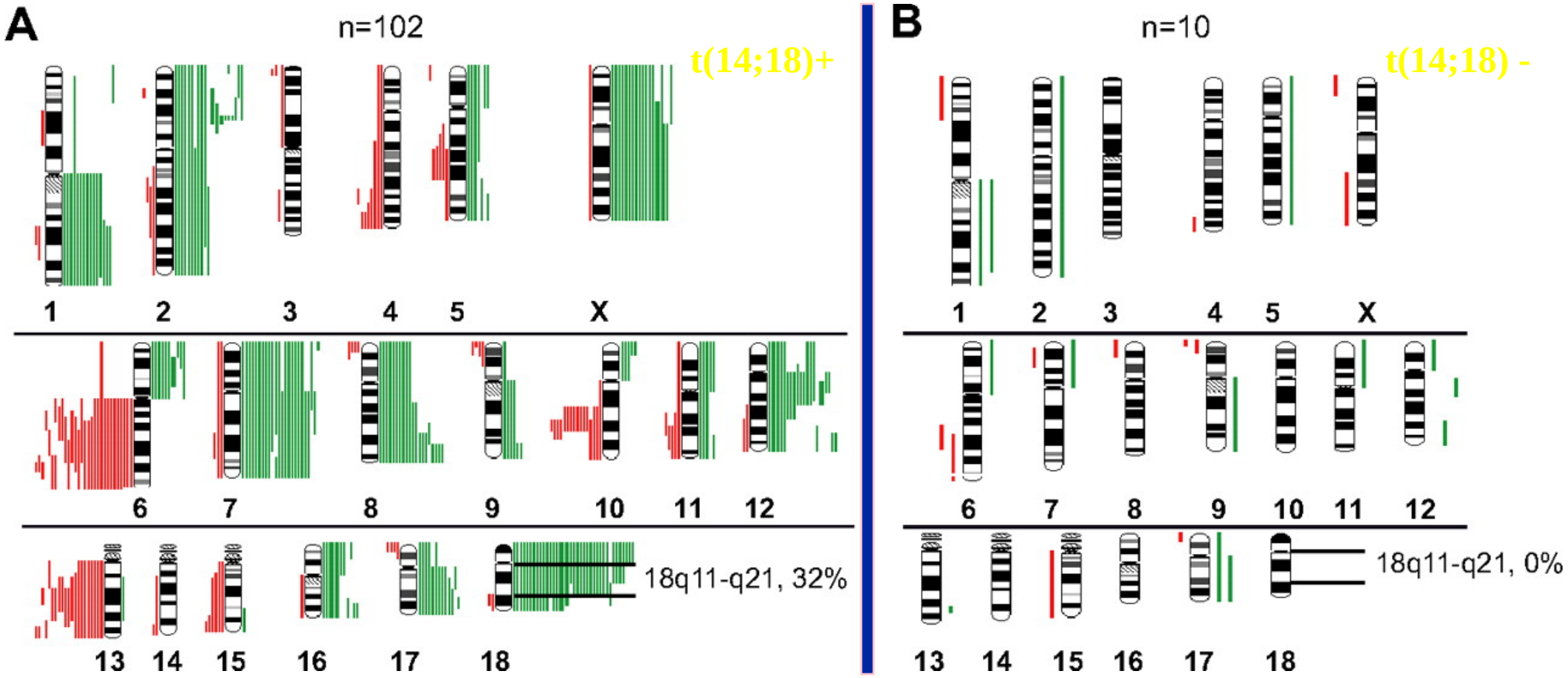
Molecular Pathogenesis of t(14;18) Negative Follicular Lymphomas

10 to 15% of Follicular Lymphoma are t(14;18) Negative

Leich E et al. 2009. Blood 114: 826

CGH Analysis: Gains and Losses in FL With and Without t(14;18) Translocation



- Chromosomal gains and losses in 102 t(14;18)+ FL cases compared to 10 t(14;18)- FL cases. Gains are displayed in green bars and losses are displayed in red bars.
- No gains in 18q (Bcl-2 gene) in t(14;18) negative cases

t(14;18) Negative Follicular Lymphoma

- DNA level: t(14;18) positive & negative groups had chromosomal gains and losses in similar regions
- NO gains in 18q (Bcl-2 gene) in t(14;18) negative cases
- At the RNA level, the following gene expression signatures are enriched in t(14;18) negative FL:
  - activated B-cell (post germinal center)
  - NF-kB , Immune response-1, T-cells , Cell cycle & proliferation
- At the protein level, CD10- in 68%, MUM-1+ in 11%,

  Ki-67 high in 91%, Granzyme B+ in 54%
- These results demonstrate that the t(14;18) negative cases belong to the spectrum of the classic FL , but have distinct genetic features, gene expression & immunologic profiles that differ from t(14;18)+ cases
- Clinically, no differences in overall survival was observed between the t(14;18) negative & positive groups

Leich E et al. 2009. Blood 114: 826

Frequency of BCL6 Rearrangements in FL

- 3q27 translocation or rearrangement of BCL6 gene locus is observed in some cases of FL, especially Grade 3B FL
- BCL6 rearrangement was found in 26% of t(14;18)- and 19% of t(14;18)+ FL (Gu, K et. al)
- In another study, BCL6 rearrangement was found in 18% of t(14;18)- and 27% of t(14;18)+ FL (p=0.475, Leich E et. al)
- These results suggest an important causative or additive role for BCL6 in t(14;18)- FL

Gu, K et al. 2009 Mod Pathol. 22: 1251
Leich E et al. 2009. Blood 114: 826

Frequency of the t(14;18) translocation in: different countries, histologic grades, primary sites, and other diseases

Differences In Frequency of the t(14;18) Translocation In Different Regions of the World

- In Nodal FL, the t(14;18) is seen in:

  85% in USA, 75% in Europe , 5-50% in Asia

- Grade 1, 98%; Grade 2, 15%; Grade 3A, 80%; Grade 3B, < 50%
- At extranodal sites this translocation is present in only 10 to 50% of cases (except duodenum)
- In Diffuse Large B-cell Lymphoma t(14;18) is seen in: 35%
- Rarely in CLL

Rabkin CS et al. J Natl Cancer Inst Monogr (2008) 39: 48

Diagnostic Utility of Bcl-2 Protein Expression (IHC stain) in Tissues

- **Frequency in nodal FL: Overall 80%**

Grade 1: 98%, Grade 2: 85%, Grade 3A: 75%, Grade 3B: 60%

- **In extranodal: 20-50%**

- **BCL-2 negative cases:**
  - **Absence of Bcl-2 may be due to mutations in the Bcl-2 gene that eliminates the epitopes recognized by the most commonly used antibodies (use antibodies to other epitopes)**
  - **In Bcl-2 negative cases, use other members of the BCL-2 family: $BCLX_L$, BAD and BAK**

Frequency of Follicular Lymphoma Around the World

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Japan	7%	Chile	25%
China (3)	7-23%	U.K.	28%
Ecuador	8%	Canada	31%
Thailand	8%	U.S.A.	32%
Hong Kong	8%	S. Africa (whites)	33%

Molecular Pathogenesis & Implications For Practice

- Molecular pathogenesis (MP) of FL at nodal & extranodal sites is complex, different, & has multiple pathways of evolution, and the underlying differences in the MP are reflected in the differences in natural history & prognosis of these FL
- 85% of nodal FL have the bcl-2 gene rearrangement/ t(14;18) translocation
- 10% of nodal FL patients do not have the t(14;18), but have Bcl-6 gene rearrangement; & 10 % have both the bcl-2 & bcl-6 gene rearrangements
- <5% of nodal FL are negative for bcl-2 & bcl-6, but have other genetic abnormalities
- All patients with overt FL have many genetic abnormalities
- Histologic criteria for diagnosing nodal & extranodal FL are identical, & probably most important for diagnosis, especially because bcl-2 protein expression and t(14;18) positivity by FISH is found in < 50 % of extranodal cases