european research initiative on CLL

IgCLL group activities in 2009 and beyond

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George Papanicolaou Hospital,
Thessaloniki, Greece

Standardization

Troubleshooting

Standardization and Validation

Standardization and Validation

Immunoglobulin gene mutational status

Leukemia (2007) 21, 1–3
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www.nature.com/leu

EDITORIAL

ERIC recommendations on IGHV gene mutational status analysis in chronic lymphocytic leukemia

P Ghia[1], K Stamatopoulos[2], C Belessi[3], C Moreno[4], S Stilgenbauer[5], F Stevenson[6], F Davi[7], R Rosenquist[8], on behalf of the European Research Initiative on CLL (ERIC)

- **Online support and troubleshooting for interpretation of IGHV sequences**
  A review board of experts is available online to discuss general queries on IGHV gene interpretation or analyze actual IGHV sequences that can be difficult to interpret in everyone's daily activity; to submit queries and/or sequences please go to the » submission form.

Review Board
Chrysoula Belessi, Athens/Greece
Frederic Davi, Paris/France
Paolo Ghia, Milan/Italy
Anton Langerak, Rotterdam/The Netherlands
Kathy Potter, Southampton/UK
Richard Rosenquist, Uppsala/Sweden
Kostas Stamatopoulos, Thessaloniki/Greece

TROUBLE IG SEQUENCES

IGHV-(IGHD)-IGHJ recombination

- **Rearrangements carrying non functional genes**
- **Problematic IGHV-IGHD-IGHJ junctions**

Somatic Hypermutation

- **Stop codons**
- **Absence of CDR3 anchors**
- **Insertion / Deletions**

Database of ‘ trouble sequences’ collected since February 2007

Over 80 trouble sequences have been submitted to the review board for analysis in 2.5 years (an average of 30 per year).

1. Borderline mutated case
2. Missing HCDR3 landmarks
3. Truncated IGHV sequences
4. Insertions and deletions
5. No IGHV sequence
6. Double productive
7. Double productive with discordant mutational status
8. Single unproductive
9. Intraclonal diversity

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Significance of ‘trouble sequences’

Analysis of trouble sequences provides:

- An opportunity to investigate clinical correlations.
- An opportunity to decipher the molecular mechanisms that were used to generate the IG sequences.
- This information can be applied to normal B cells as well as the CLL B cells.

New analytical tools

REFINEMENT OF ANALYTICAL TOOLS

IMGT, Montpellier

Marie-Paule Lefranc
Veronique Giudicelli
Xavier Brochet

BAT, CERTH, Thessaloniki

Nikos Darzentas
Anastasia Hadzidimitriou
Andreas Agathagelidis

Insertions/Duplications – Deletions

- **Frequency in CLL: ~3%**
- **An analytical nightmare (until recently…)**

Belessi et al. Eur J Immunol. 2006;36:1963-74.

Im Muno Gene Tics Information system®
http://www.imgt.org

the IGCL group

Result summary:

Nucleotide insertions have been detected and automatically removed for this analysis: they are displayed as capital letters in the user submitted sequence above.

localization in V-REGION	nb of inserted nt	inserted nt	causing frameshift	from V-REGION codon	from nt position in user submitted sequence
FR3-IMGT	3	CAC	no	67	108

IMGT/V-QUEST results after removal of the insertion(s):
Potentially productive IGH rearranged sequence: no stop codon and in-frame junction
(Check also your sequence with BLAST against IMGT/GENE-DB reference sequences to eventually identify out-of-frame pseudogenes)

V-GENE and allele	IGHV3-21*01, or IGHV3-21*02	score = 1040	identity = 97,71% (213/218 nt)
J-GENE and allele	IGHJ6*01, or IGHJ6*02 or IGHJ6*04 (a)	score = 56	identity = 72,73% (16/22 nt)
D-GENE and allele by IMGT/JunctionAnalysis	IGHD3-16*02	D-REGION is in reading frame 2	
[CDR1-IMGT.CDR2-IMGT.CDR3-IMGT] lengths and AA JUNCTION	[8.8.9]	CATDRNGMDVW	

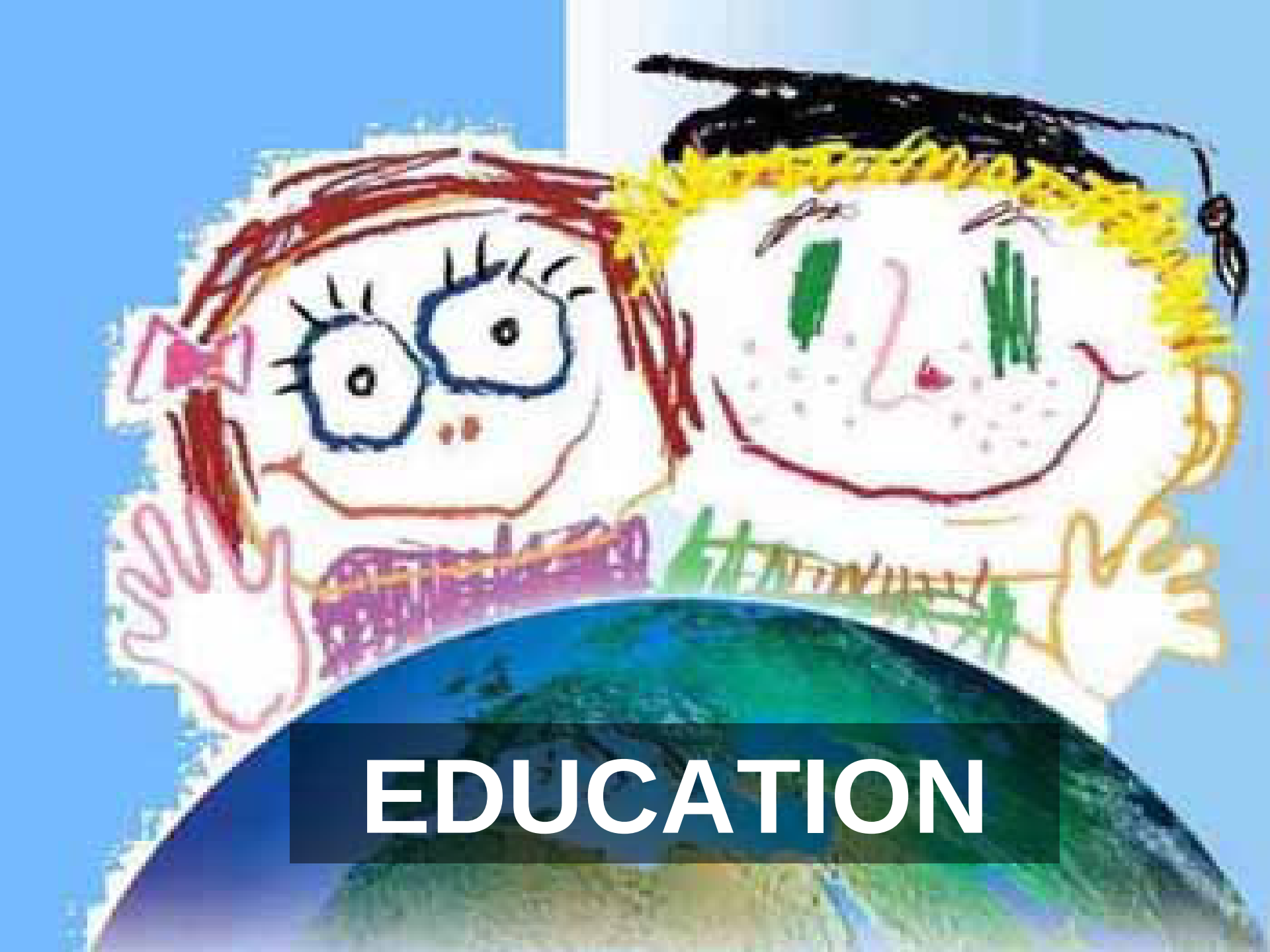
A paradigmatic example of bioinformatics advances meeting clinical needs!

Upcoming developments in 2010

- **IMGT V-QUEST "Alerts"**
  - Sequence ambiguities or troubles
  - Short sequences leading to gene assignment problems

- **Identification of IGHC genes**

- **Analysis of incomplete IGHD-J rearrangements**
  - Biological significance (Tsakou et al. ASH 2009)
  - Practical significance: MRD testing


EDUCATION

ERIC
european research initiative on CLL

3rd Educational Workshop

Immunoglobulin Gene Analysis in Chronic Lymphocytic Leukemia

24-25 September 2009

Thessaloniki - Greece

Target audience:

Workshop for scientists interested to learn more about immunoglobulin gene anal CLL

Structure:

This two-day educational workshop will comprise a mixture of lectures and applie sessions held by experts in the field on immunoglobulin gene interpretation in CLL

Main topics covered during the workshop:

- Latest recommendations on IGHV gene mutational status
- IG gene interpretation and trouble shooting
- New bioinformatic tools
- Trouble cases in clinical routine
- Stereotyped B-cell receptors in CLL

Chair:

Kostas Stamatopoulos

Scientific committee:

Chrysoula Belessi (Greece)
Frederic Davi (France)
Paolo Ghia (Italy)
Marie-Paule Lefranc (France)
Richard Rosenquist (Sweden)
Kostas Stamatopoulos (Greece)

Sponsorship/support:

ERIC european research initiative on CLL

IMGT Information system®

ELN European LeukemiaNet

G. PAPANIKOLAOU GENERAL HOSPITAL THESSALONIKI, GREECE

60 participants, 17 countries

Austria	2
Belgium	2
Czech Republic	2
Denmark	4
Germany	1
Greece	17
Iran	1
Ireland	1
Italy	8
Netherlands	3
Poland	2
Romania	1
Serbia	1
Spain	4
Sweden	8
UK	2
US	1

Workshop activities and innovations

- Questionnaire: current everyday practice throughout Europe

  Material, PCR methodology, Interpretation of PCR results, Sequencing, Reporting to the clinic

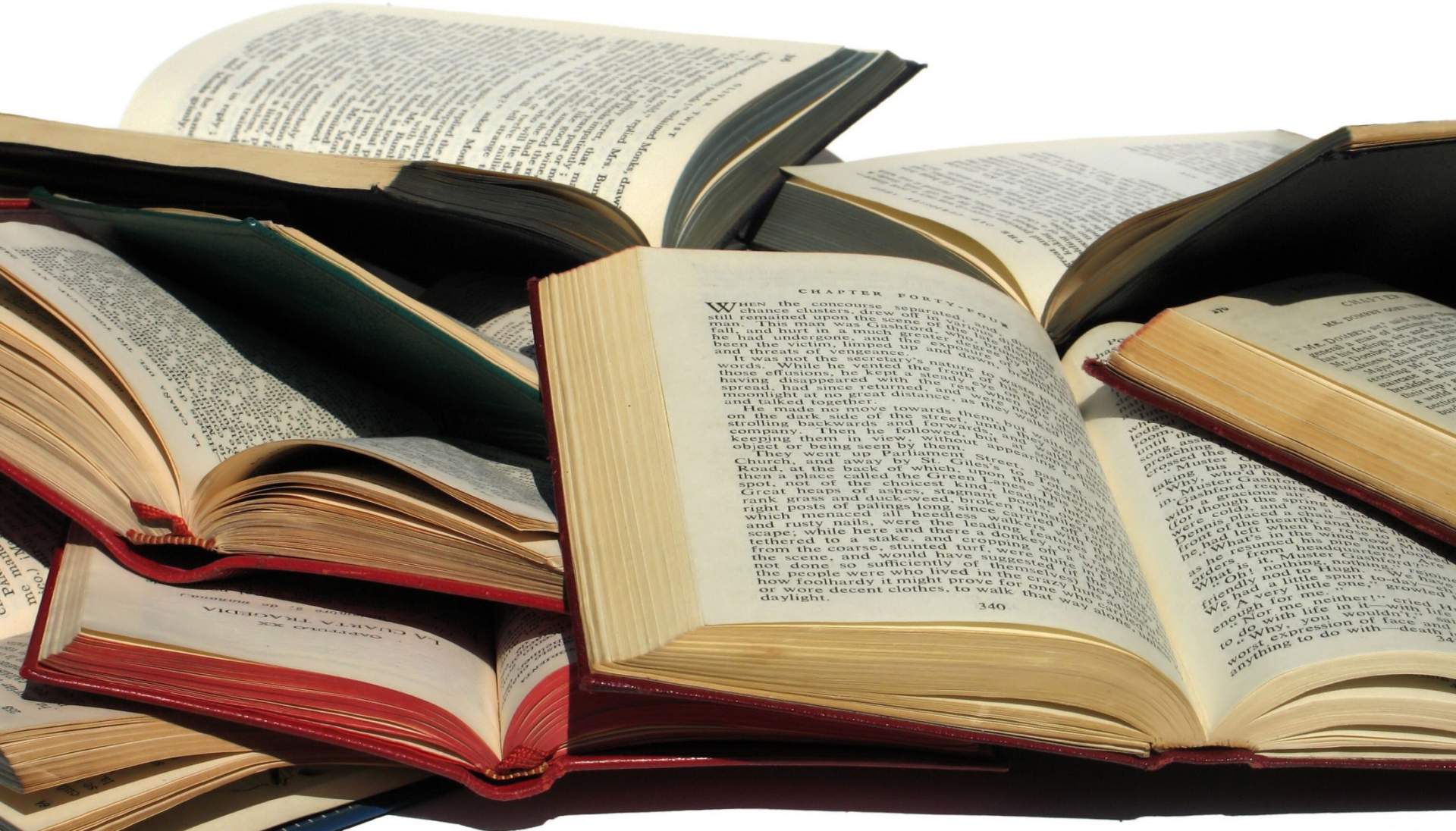
- Exercises and web-based assessment and self-assessment

- Light chain analysis

Next Workshop

Italy 2011

Publications



ERIC
european research initiative on CLL

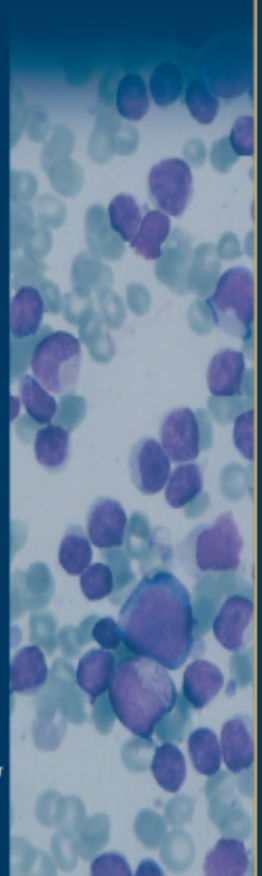
The first book!

ISBN 978-88-7556-377-6

P. Ghia • R. Rosenquist • F. Davì

Immunoglobulin Gene Analysis in Chronic lymphocytic Leukemia

LWW



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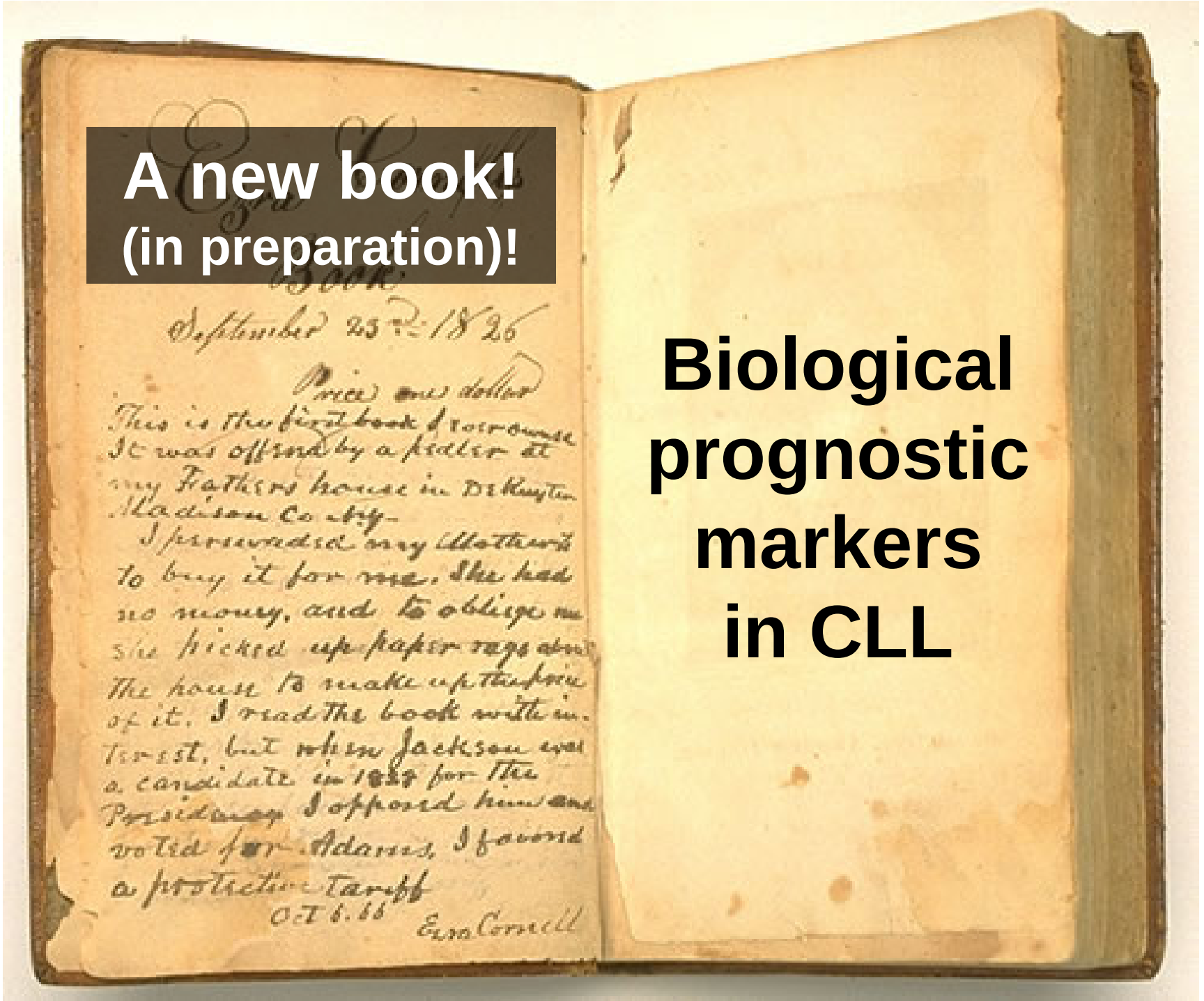
Immunoglobulin Gene Analysis in Chronic Lymphocytic Leukemia

Wolters Kluwer | Lippincott Williams & Wilkins



Biological prognostic markers in CLL

Biological prognostic markers in CLL

Stamatopoulos, Ghia, Rosenquist, eds.

Foreword	Emili Montserrat, Michael Keating
Section I - Clinico-biological background of CLL	
CLL biology	Nicholas Chiorazzi, Federico Caligaris-Cappio
Clinical prognostic markers (Rai/Binet staging, serum markers)	Kanti Rai, Eva Kimby
Section II - Cytogenetics/molecular cytogenetics	
Genomic aberrations	Stephan Stilgenbauer, Hartmut Doehner
p53 dysfunction	Andrew Pettit, Sarka Pospisilova
Section III - Immunoglobulin genes	
IGHV gene mutation status as prognostic marker	Paolo Ghia, Richard Rosenquist
Stereotyped B-cell receptors in CLL	Chrysoula Belessi, Kostas Stamatopoulos
Section IV - Immunophenotyping	
CD38 onwards: the evolution of flow cytometric markers in CLL	Rajendra Damle, Silvia Deaglio
ZAP-70	Florence Cymbalista, Francesc Bosch
From MBL to MRD: to the limits of flow cytometry	Andy Rawstron, Paolo Ghia
Section V – RNA-based methods/micro-RNA	
RNA-based molecular markers	Frederic Davi, Anne-Mette Buhl
Micro-RNA in CLL	George Adrian Calin, Carlo Croce
Section IV – Clinical application of new prognostic markers	
Clinical application of new prognostic markers	David Oscier, Michael Hallek
Conclusions	Daniel Catovsky, Tom Kipps

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