No improvement in long-term survival over time for chronic lymphocytic leukemia patients in stereotyped subsets #1 and #2 treated with chemo(immuno)therapy

*Panagiotis Baliakas,[*1] Mattias Mattsson,[*1,2] Anastasia Hadzidimitriou,[3] Eva Minga,[3] Andreas Agathangelidis,[3,4,5] Lesley-Ann Sutton,[1] Lydia Scarfo,[4,5] Zadie Davis,[6] Xiao-Jie Yan,[7] Karla Plevova,[8,9] Yorick Sandberg,[10] Fie J. Vojdeman,[11] Tatiana Tzenou,[12] Charles C. Chu,[7] Silvio Veronese,[13] Larry Mansouri,[1] Karin E Smedby,[14] Véronique Giudicelli,[15] Florence Nguyen-Khac,[16] Panagiotis Panagiotidis,[12] Gunnar Juliusson,[17] Achilles Anagnostopoulos,[18] Marie-Paule Lefranc,[15] Livio Trentin,[19,20] Mark Catherwood,[21] Marco Montillo,[13] Carsten U. Niemann,[11] Anton W. Langerak,[10] Sarka Pospisilova,[8,9] Niki Stavroyianni,[18] Nicholas Chiorazzi,[7] David Oscier,[6] Diane F Jelinek,[22] Tait Shanafelt,[23] Nikos Darzentas,[8] Chrysoula Belessi,[24] Frederic Davi,[16] Paolo Ghia,[4,5] Richard Rosenquist[1,25] and Kostas Stamatopoulos[1,3,18]*

**Equal first authors*

[1]Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, Sweden; [2]Department of Hematology, Uppsala University Hospital, Sweden; [3]Institute of Applied Biosciences, Thessaloniki, Greece; [4]Università Vita-Salute San Raffaele, Milan; [5]Strategic Research Program in CLL, Division of Experimental Oncology, IRCCS San Raffaele Scientific Institute, Milan, Italy; [6]Department of Haematology, Royal Bournemouth Hospital, Bournemouth, UK; [7]The Feinstein Institute for Medical Research, Northwell Health, New York, USA; [8]CEITEC-Central European Institute of Technology, MasarykBrno, Czech Republic; [9]University Hospital Brno, Czech Republic; [10]Department of Immunology, Erasmus MC, University Medical Center Rotterdam, the Netherlands; [11]Department of Hematology, Rigshospitalet, Copenhagen, Denmark; [12]First Department of Propaedeutic Medicine, University of Athens, Greece; [13]Molecular Pathology Unit and Haematology Department, Niguarda Cancer Center, Niguarda Hospital, Milan, Italy; [14]Department of Medicine Solna, Clinical Epidemiology Unit, Karolinska Institutet, and Hematology Center, Karolinska University Hospital, Stockholm, Sweden; [15]IMGT®, the international ImMunoGeneTics information system®, Université de Montpellier, Laboratoire d'ImmunoGénétique Moléculaire LIGM, Institut de Génétique Humaine IGH, UPR CNRS 1142, Montpellier, France; [16]Hematology Department and University Pierre et Marie Curie, Hopital Pitie-Salpetriere, Paris, France; [17]Lund University and Hospital Department of Hematology, Lund Stem Cell Center, Sweden; [18]Hematology Department and HCT Unit, G. Papanicolaou Hospital, Thessaloniki, Greece; [19]Department of Medicine, Hematology and Clinical Immunology Branch, Padova University School of Medicine, Italy; [20]Venetian Institute of Molecular Medicine, Padova, Italy; [21]Department of Hemato-Oncology, Belfast City Hospital, Belfast, UK; [22]Department of Immunology, Mayo Clinic, Rochester, MV, USA; [23]Division of Hematology, Department of Medicine, Mayo Clinic, Rochester, MN, USA; [24]Hematology Department, Nikea General Hospital, Piraeus, Greece and [25]Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden

Correspondence: kostas.stamatopoulos@gmail.com
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Supplementary material includes details regarding the evaluated cohort, the applied methodology as well as Supplemental Tables 1-2 and Supplemental Figures 1-4.

Supplemental Table 1. Basic clinicobiological features of the evaluated cohort.

n=3504	n, %
Male	2411/3504, 69%
Age at treatment	63.9 (22-92) years
M-CLL	1286/3504, 37%
del(13q)*	528/953, 55%
Trisomy 12*	239/1201, 20%
del(11q)*	339/1613, 21%
del(17p)*	217/1857, 12%
Subset #2‡	166/3504, 5%
Subset #1¥	110/3504, 3%

M-CLL: CLL carrying mutated IGHV genes, *According to the Döhner hierarchical model, ‡ Assignment to stereotyped subset #2, ¥ Assignment to stereotyped subset #1.

Supplemental Table 2. Distribution of cases included in the study in relation to the time of primary treatment.

	Time of primary treatment			
n=3504	1980-1990	1991-2000	2001-2005	2006-2014
n, %	84, 2%	623, 18%	1386, 39%	1411, 41%

Methods

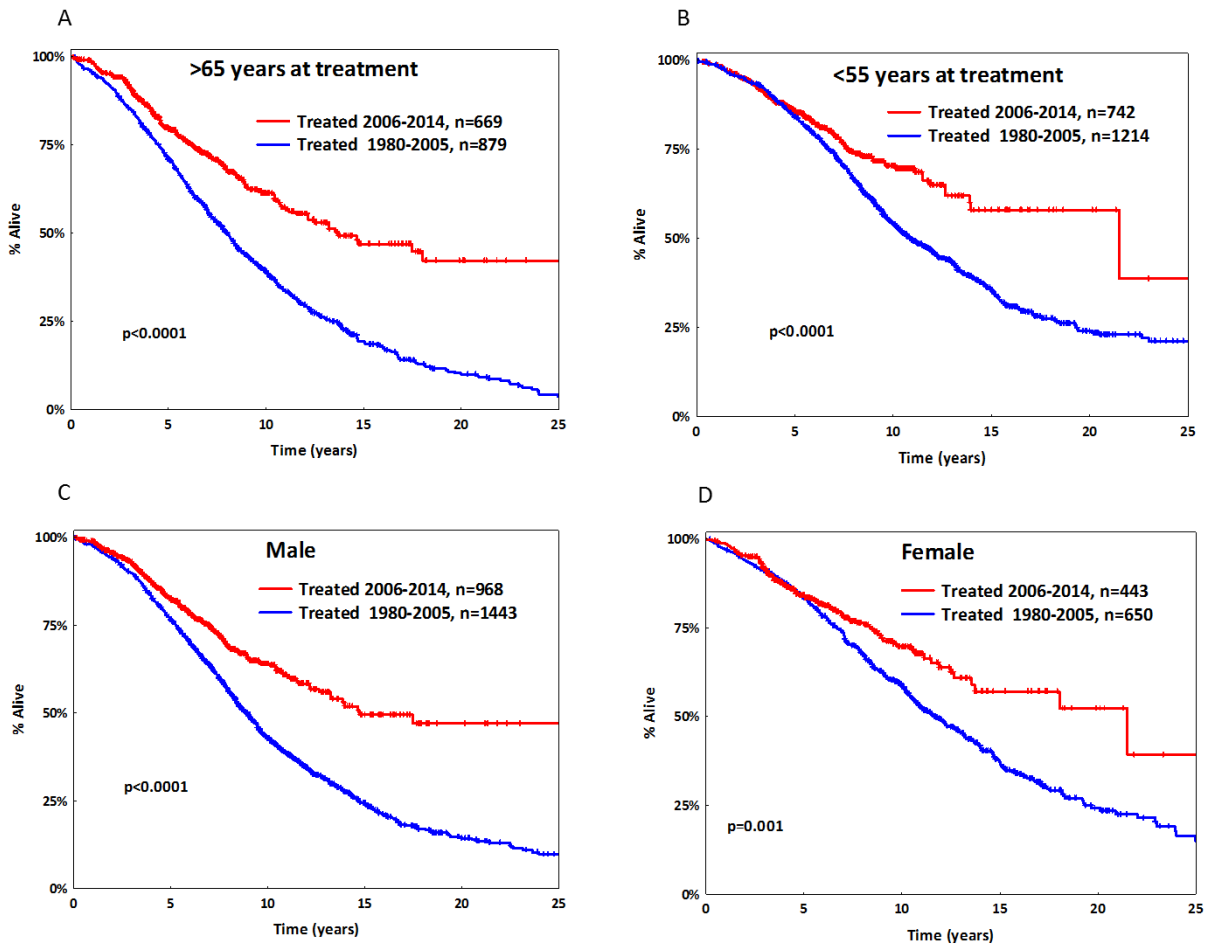
PCR amplification of IGHV-IGHD-IGHJ rearrangements - Sequence analysis

PCR amplification and sequence analysis of IGHV-IGHD-IGHJ rearrangements were performed on either genomic DNA (gDNA) or complementary DNA (cDNA), as previously described. PCR amplicons were subjected to direct sequencing on both strands. Sequence data were analyzed using the IMGT® databases and the IMGT/V-QUEST tool (http://www.imgt.org). Only productive rearrangements were evaluated. Output data from IMGT/V-QUEST for all productive IGHV-IGHD-IGHJ rearrangements were parsed, reorganized, and exported to a spreadsheet through the use of computer programming. Information was extracted regarding IG gene repertoires, VH CDR3-IMGT length and amino acid sequence and SHM; to identify and cluster stereotyped rearrangements, we used a purpose-built bioinformatics method, as previously described (bat.infspire.org/arrest/)(1).

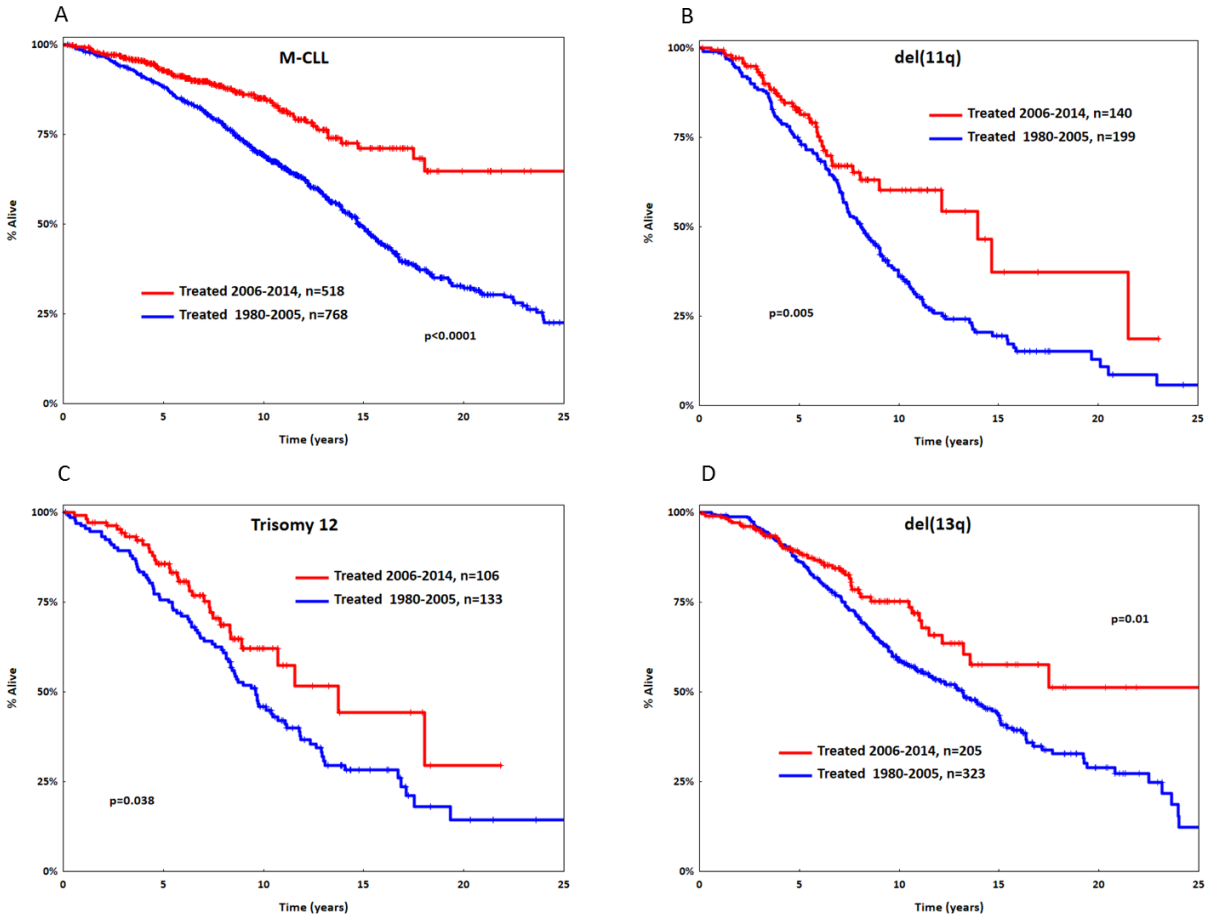
FISH analysis

Preparations for FISH analysis were counterstained with 4,6-diamidino-phenyl-indole (DAPI) using probes for the 13q14, 13q34, 11q22, 17p13 regions and trisomy 12; a minimum of 200 interphase nuclei were examined.

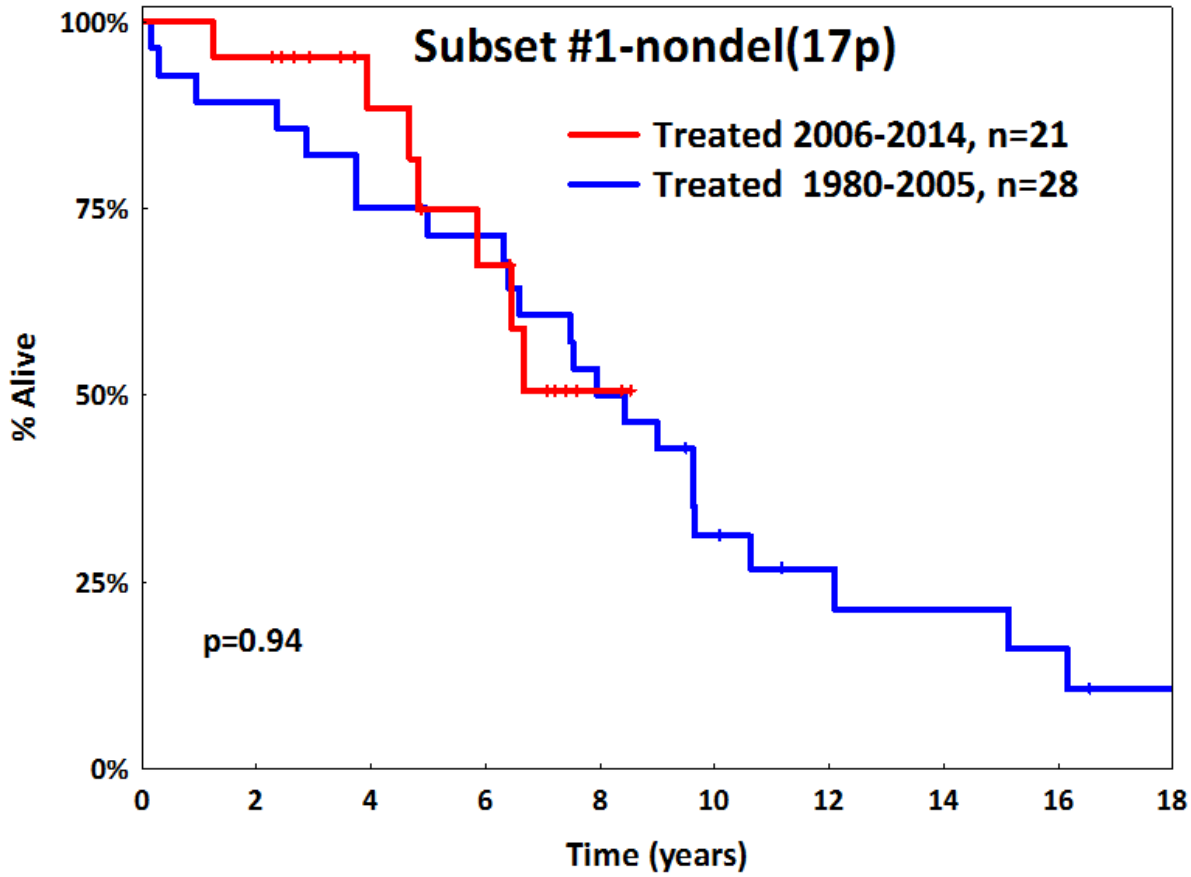
Supplemental Figure 1. Inferior overall survival for cases treated between 1980-2005 (blue line), compared to cases treated between 2006-2014 (red line) independently of age at the time of treatment (A, B) or gender (C, D).



Supplemental Figure 2. Inferior overall survival for cases treated between 1980-2005 (blue line) compared to cases treated between 2006-2014 (red line) for all M-CLL (A), and independently of the presence of del(11q) (B), trisomy 12 (C) or del(13q) (D).

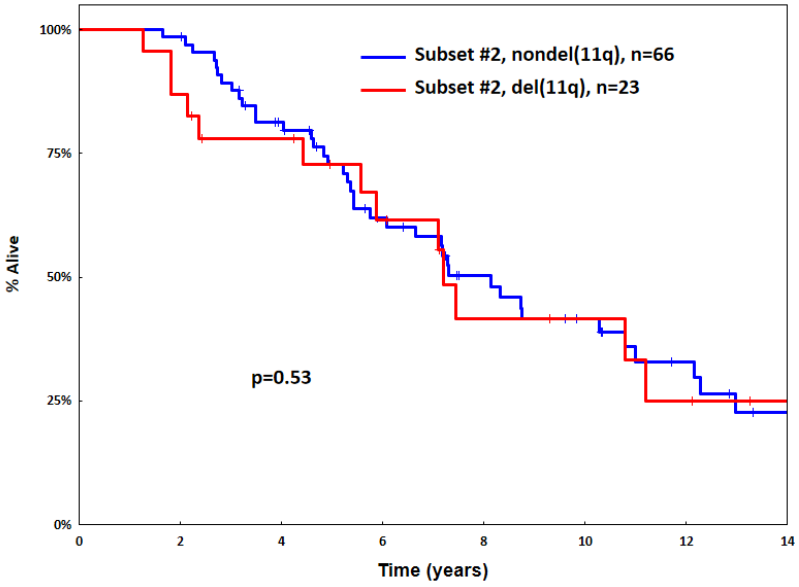


Supplemental Figure 3. No improvement in overall survival over time for cases belonging to subset #1 even after excluding del(17p) cases.

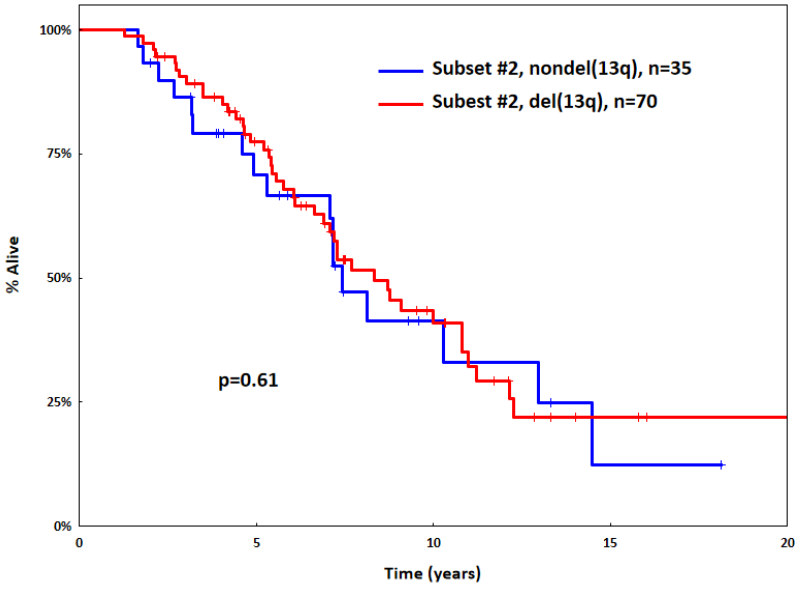


Supplemental Figure 4. No impact of del(11q) (A) or del(13q) (B) on the overall survival of subset #2 cases.

A



B



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