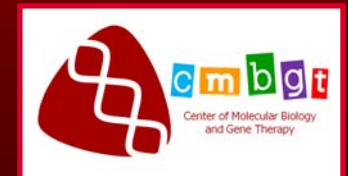


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Immunoglobulin analysis in patients with chronic lymphocytic leukemia

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Šárka Pospíšilová



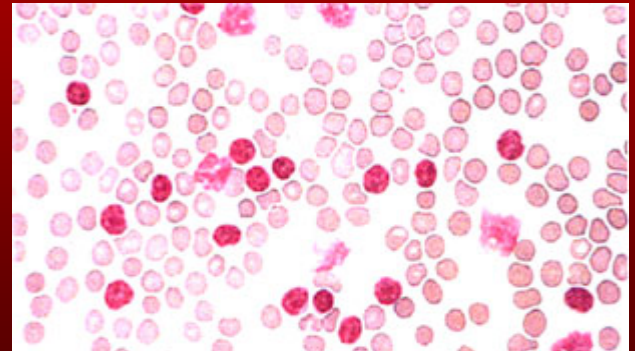
Chronic lymphocytic leukemia

Chronic = slow and protracted course

Lymphocytic = mature B cells affected

Leukemia = malignant disorder

- Most common leukemia in Western world
- Monoclonal expansion of mature B lymphocytes
- Very variable course of disease
- Incidence: 3 patients in population of 100 000 per year
- Median age of diagnosis: 65 years
- Men are at higher risk than women
- Survival: from several years to decades



Diagnosis and prognosis of CLL

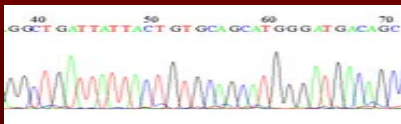
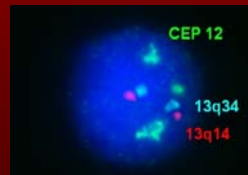
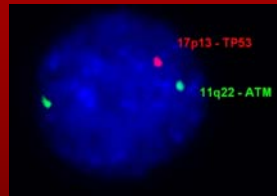
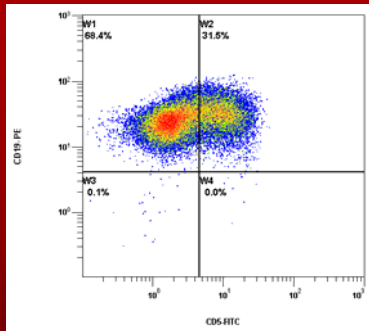
- **Diagnosis**

- Clinical symptoms – fatigue, sweating, fever, lymphadenopathy, hepatosplenomegaly, lymphocytosis
- Haematology – blood smear, more than 5000 B lymphocytes/ μ l for at least 3 months, cytopenia
- Flow cytometry – population CD5+CD19+



- **Prognosis**

- Chromosomal abnormalities: deletions 13q14 (*RB1*, *miR-15a*, *miR-16-1*), 17p13 (*TP53*), 11q22-23 (*ATM*), trisomy 12 +
- **Mutations in *TP53*** – response to therapy
- Mutation status of immunoglobulin heavy chain variable region (IgVH)
- Molecular markers – ZAP-70, CD38
- Experimental molecular markers - expression of microRNA and other genes (*LPL*, *ADAM29*, *LAG3*...)



Dynamism of CLL

- Cells **proliferate but also undergo apoptosis** (not only accumulation but exchange of cells)
- CLL associated **chromosomal aberrations** evolve during the course of the disease
- **Mutation in TP53** can be selected after therapy
- CLL cells can **infiltrate** several organs (e. g. skin)
- Possible **transformation** to more aggressive lymphoma (Richter's syndrome)

→ **monitoring of biological and molecular markers during the course of the disease** (indication of possible complications)

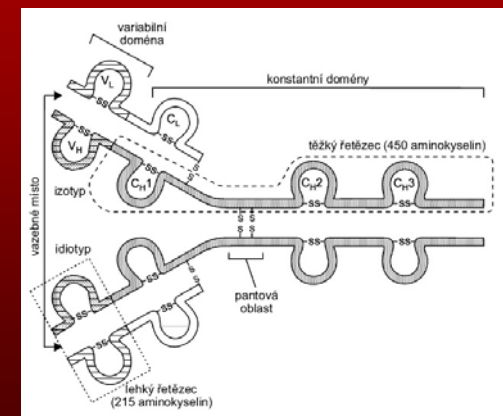
Immunoglobulins – important characteristics of CLL cells

- **Biological point of view**

- Nonrandom distribution of IgVH (role of antigen stimulation in pathogenesis), different from healthy population
- Several Ig rearrangements associated with poorer prognosis (**VH1-69**, **VH3-21**)
- Over 20 % of cases share stereotype receptors (similar structure independent on Ig heavy/light variable segments usage)

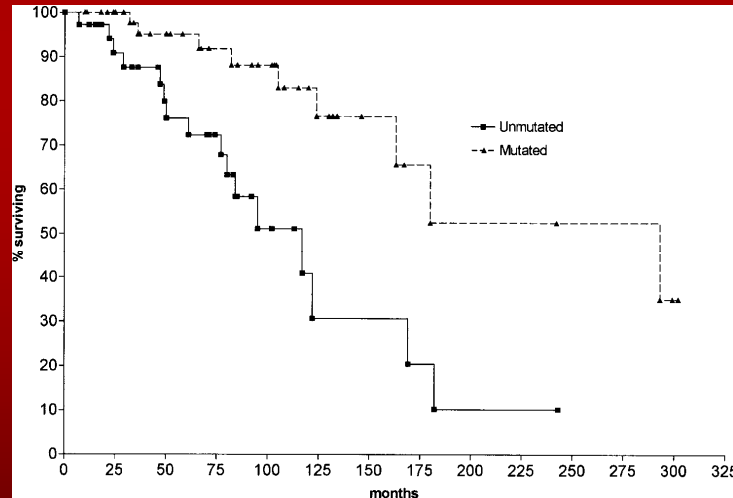
- **Clinical point of view**

- Prediction of disease outcome (prognostication) according to presence of mutations in IgVH
- Unique rearrangement useful for monitoring of minimal residual disease after therapy



IgVH prognostic impact

- Hamblin et al. 1999 and Damle et al. 1999
 - mutated (indolent) vs. unmutated (aggressive) ~ survival 10 vs. 25 years
 - Cut off - 98 % homology with germline of closest IgVH subgene

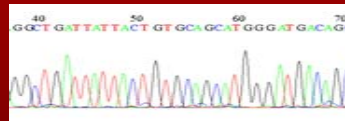
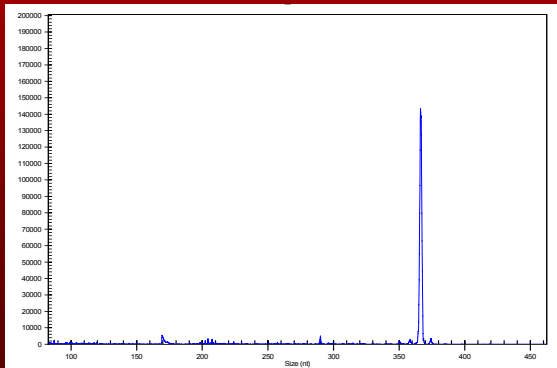
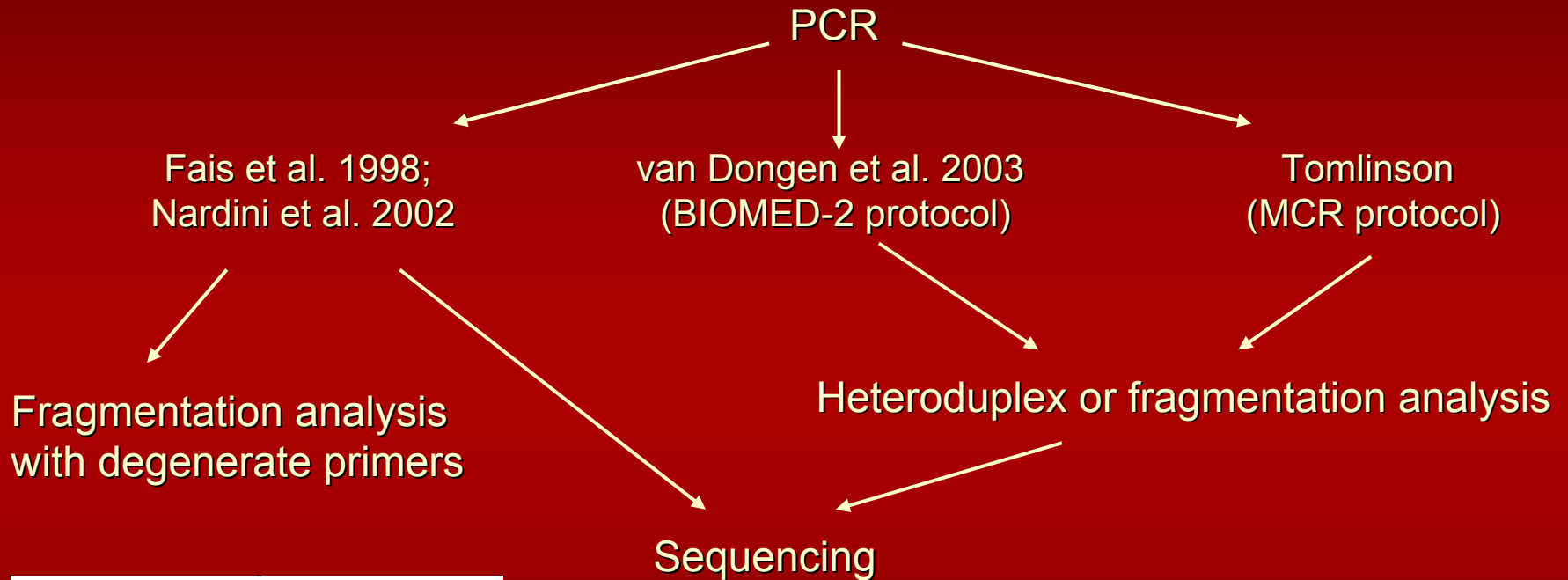


Assumed that no changes of IgVH status occur during course of disease.

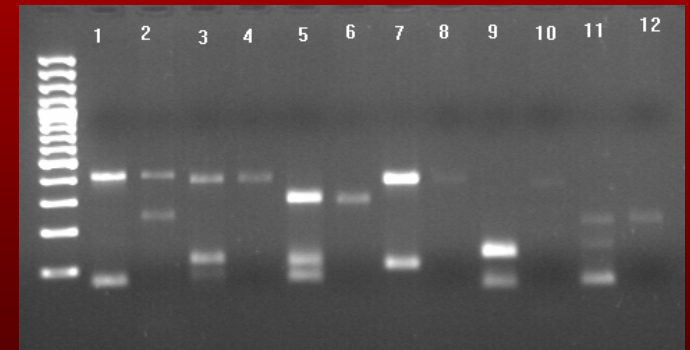
Monoclonal disorder...?

- Most CLL cases are **monoclonal ones**
- In one CLL IgVH clone, several hypermutation patterns can be detected using high throughput sequencing → **intraclonal evolution** (Cambell et al. 2008)
- **Biclonal CLL** cases are rarely observed (Hsi et al. 2000; Ferhnout et al. 1997; Gonzáles – Campos et al. 1997; Chang and Cerny 2006)

Methods for immunoglobulins analysis



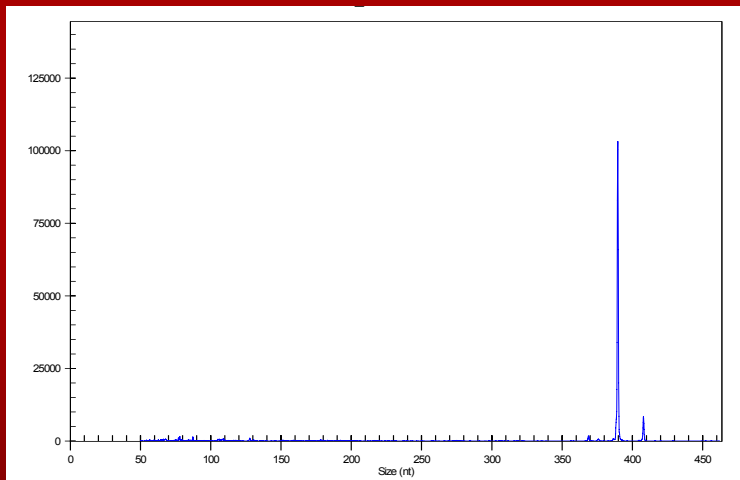
Database search
(IMGT/V-QUEST)



Clonal evolution – case report I

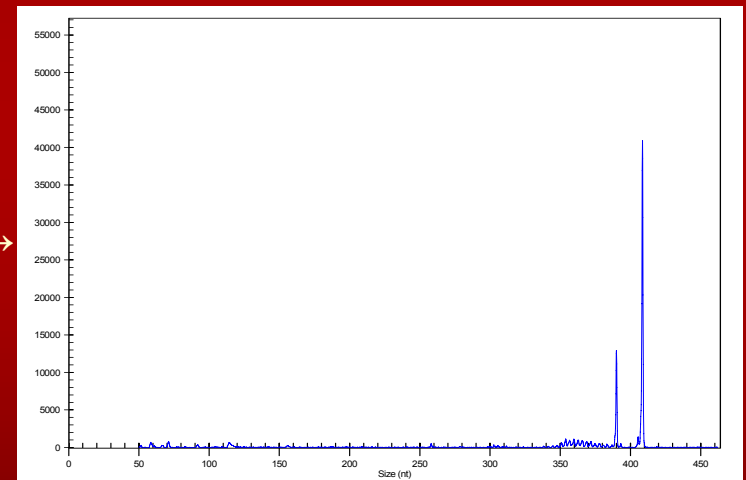
Patient WJ – clone alteration mut VH3-30 → unmut VH1-69

September 2005



Therapy FC →

May 2007



Shift from prognostically favorable group to group with poor prognosis.

Clonal evolution – case report II

Patient AB – clone alteration unmut VH3-21/VLλ1-51 → unmut VH1-69/VLκ1-39

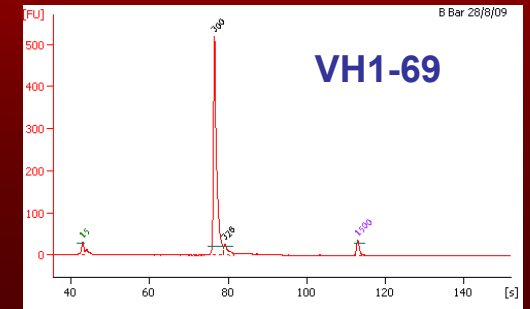
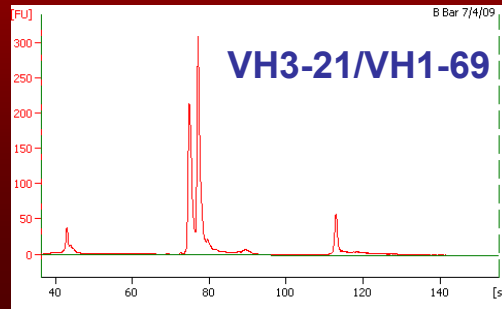
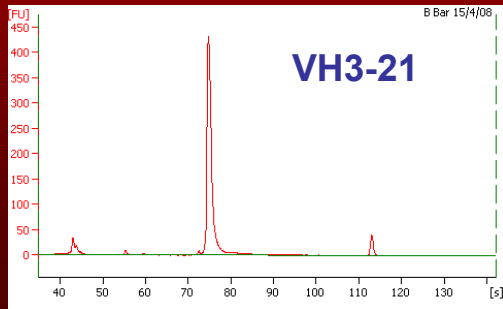
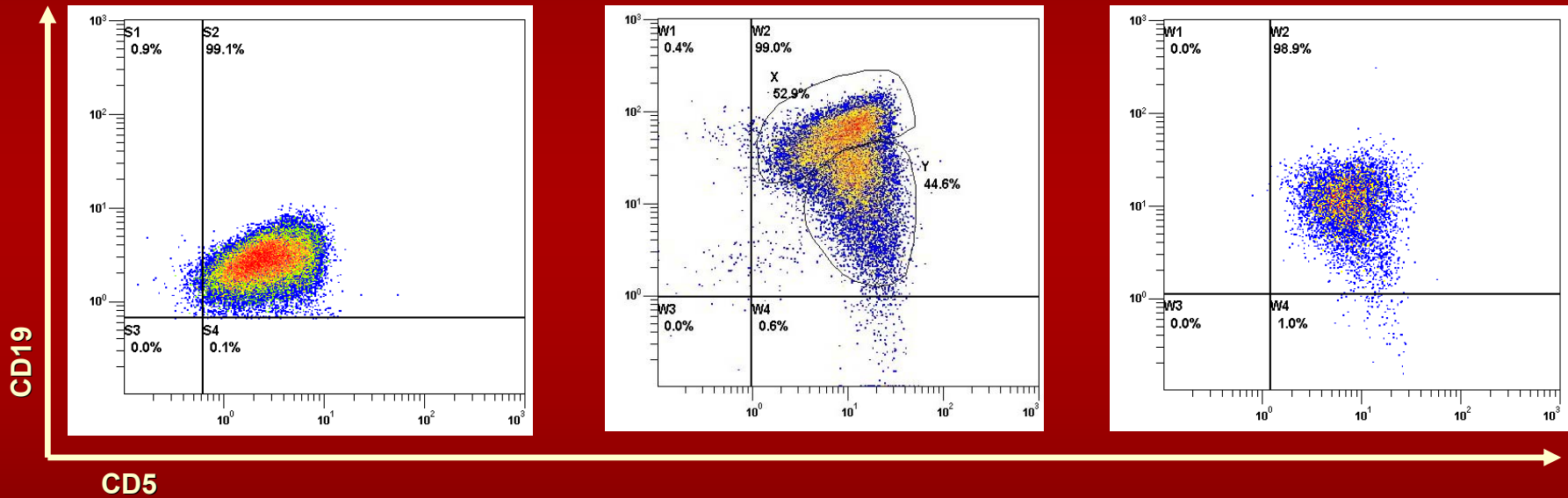
April 2008

Therapy FC →

April 2009

Progression,
therapy R →

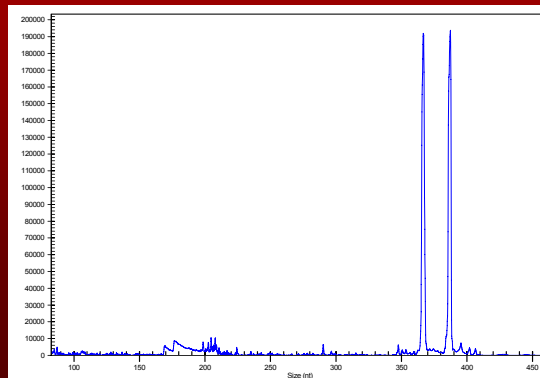
August 2009



Biclonal CLL – unique subgroup

- Biclinality ~ two independent CLL arising in one patient
- Can bring insight into the CLL pathogenesis (selection of more B cell clones in a process of an antigen stimulation)

→ **Searching for another biclonal/oligoclonal patients**



Oligoclonality and its evolution in our patients

- Detection of more than one functional IgVH rearrangements per a patient in **27 cases**
 - 24 cases with two functional IgVH rearrangements
 - 3 cases with three functional IgVH rearrangements
- Necessary to exclude cases with lack of allelic exclusion (expression of Ig from both alleles)
 - 12 cases with lack of allelic exclusion
 - **15 cases with expansion of more than one clone**
- 10 patients with follow-up
 - **Lost of mutated clone in 5 cases** (persisting clone VH1-69 in 4 cases)
 - Arising of unmutated clone in 3 cases (VH1-69 in 2 cases)
 - 2 cases – lack of allelic exclusion

Follow up in our patients

Patient	Year of sampling	VH1	homology	VH2	homology	VH3	homology
6	2009	1-69	100	4-59	96,84		
	2010			x	x		
14	2008	1-69	100	1-69	99,65	2-05	97,6
	2010					x	x
15	2009	1-69	100	2-05	92,78		
	2010			x	x		
18	2009	4-34	100	3-7	95,14		
	2010			x	x		
26	2005	3-30	91	1-69	100		
	2007	1-69	100	3-30	91		
21	2006	4-39	100	3-33	100	x	x
	2010					3-30	100
25	2009	3-23	92,7	x	x		
	2010			1-69	100		
27	2008	3-21	100	x	x		
	2009	x	x	1-69	100		
11	2009	1-69	100	2-70	100		
	2010						
20	2009	1-03	100	4-34	100		
	2010						

Many thanks to all coworkers

Šárka Pospíšilová
Jana Kotašková
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Hana Jelínková
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Martin Trbušek
Šárka Pavlová
Jana Lochmanová
Ludmila Ročňová
Jana Šupíková
Petr Dobeš
Marek Mráz
Jitka Kabáthová
Jan Verner



Thank you for your attention!