Minimal residual disease detection in mantle cell lymphoma: methods and significance of four-color flow cytometry compared to consensus *IGH*-polymerase chain reaction at initial staging and for follow-up examinations

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Treatment protocols

Untreated patients with Ann Arbor stage II to IV MCL were eligible for inclusion in the trials. Computed tomography (CT) examinations of neck, chest, abdomen, and pelvis as well as bone marrow biopsies for cytology and histology (in general assessed by local pathologists) were mandatory for initial staging.

Patients younger than 65 years ("MCL younger study") were randomized to receive either six cycles of rituximab plus CHOP (R-CHOP), myeloablative radiochemotherapy and autologous stem cell transplantation (as detailed by Lenz *et al.*).[1] or three cycles of R-CHOP alternating with three cycles of R-DHAP followed by myeloablative radiochemotherapy and autologous stem cell transplantation (as detailed by Lefrere *et al.*).[2] Staging examinations were performed before treatment, after four cycles of induction chemotherapy, after induction and at 3-monthly intervals thereafter. Patients older than 65 years ("MCL elderly study") were randomized to receive either eight cycles of R-CHOP or six cycles of R-FC (rituximab, fludarabine, cyclophosphamide) followed by interferon-α or rituximab maintenance therapy. Staging examinations were scheduled before therapy, after half of the induction cycles and at 2-monthly intervals during maintenance therapy (for detailed study protocols refer to: www.european-mcl.net). The institutional review boards of all participating centers approved the protocols.

Consensus IGH-PCR and GeneScanning

For consensus *IGH*-PCR, 500 ng genomic DNA was amplified using FR1 (frame-work region 1)-*IGH* primer sets as published by the BIOMED-2 Concerted Action.[3] FAM-labeled PCR products were size separated on a high-resolution polyacrylamide gel and laser-induced fluorescence analyzed using an ABI 310 genetic analyzer (Applied Biosystems, ABI, Darmstadt, Germany) as described previously (GeneScanning).[4] In case of a polyclonal signal after FR1-*IGH* PCR, FR2- and FR3-*IGH* primer sets were used in additional PCR reactions.[3] Follow-up samples were defined as monoclonal if peaks of identical sizes as those in pre-treatment samples were identified.

Detection of IGH-CCND1 fusion indicating t(11;14)(q13;q32) translocation by fluorescence in situ hybridization (FISH) and PCR

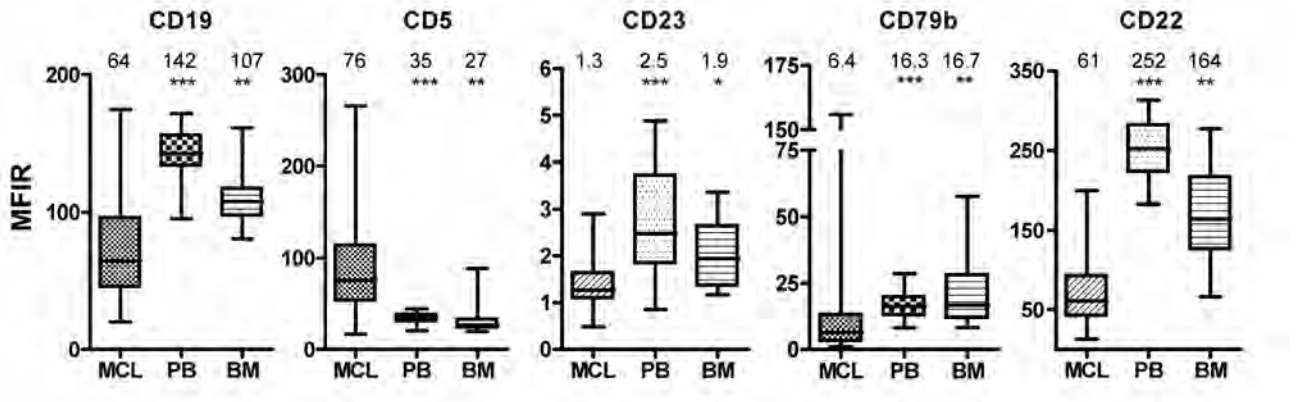
Interphase-FISH for the detection of the t(11;14)(q13;q32) translocation was performed with the LSI IGH/CCND1 XT dual color fusion probe (Vysis/Abbott Molecular Inc., Des Plaines, IL, USA) on peripheral blood or bone marrow cells left over from cytogenetic analysis or on peripheral blood or bone marrow smears. In selected cases, the LSI CCND1 Dual Color Break Apart Rearrangement Probe (Vysis/Abbott) was additionally applied. Hybridization and evaluation followed recently described procedures.[5,6] All initial samples were assessed for the t(11;14) translocation by PCR using a published method.[7] Patients were designated as t(11;14)-positive if at least PCR or FISH in one sample was positive. Since not diagnostic specimens, but only bone marrow and/or peripheral blood were centrally assessed for the presence of the t(11;14) translocation by FISH and PCR, a negative result does not exclude the presence of this translocation in the tumor tissue.

Supplementary Table S1. Patients' characteristics and details on samples included in this study.

Patients, n.	98
EU MCL younger trial, n.	55 (56.1 %)
EU MCL elderly trial, n.	43 (43.9 %)
Age, years, median (range)	62 (38 – 83)
Sex (female: male)	22:76
Clinical Stage	
Stage II, n.	4 (4.1 %)
Stage III, n.	15 (15.3 %)
Stage IV, n.	79 (80.6 %)
BM histology positive	73 (74.5 %)
Gastrointestinal involvement	27 (27.6 %)
Elevated lactate dehydrogenase	30 (30.6 %)
t(11;14) detectable by FISH and/or PCR in bone marrow/peripheral blood samples	71 (72.4 %)
Samples, n.	281
bone marrow, n.	83 (29.5 %)
peripheral blood, n.	198 (70.5 %)

Supplementary Table S2. Applicability of standardized gates for the detection of MCL. Eighty $CD19^{+}CD5^{+}$ LCR^{+} MCL samples were assessed. The inclusion rate gives the percentage of all MCL cells that satisfied the gating strategy in a given sample. The lower part of the table lists the proportion of all 80 MCL samples that were considered positive by immunophenotype provided that different percentages of the whole MCL population were required to satisfy the gates.

	Criteria of MCL dection				
	$CD22^{low}$	$CD23^{low}$	$CD79b^{low}$	$CD22^{low}$+ $CD79b^{low}$	$CD22^{low}$+ $CD23^{low}$
Inclusion rate (%)					
median	93	97	97	86	88
range	31 -100	71 -100	1 -100	0.5 -100	29 -100
% of positive samples according to required inclusion					
95 %	41	69	64	25	28
90 %	66	83	73	40	45
85 %	70	89	75	51	58
80 %	79	98	76	60	70
75 %	88	98	77	65	85
70 %	91	100	79	69	93
65 %	93	100	80	74	93
60 %	93	100	85	80	93



Supplementary Figure S1. Immunophenotype of MCL cells from 80 samples in comparison to benign $CD19^{+}CD5^{+}$ lymphocytes of 8 bone marrow and 25 peripheral blood samples. Median MFIR, interquartile range (boxes) and range (whiskers) are given. Numbers at the top of the graphs denote the median MFIR. Statistical significance in comparison to MCL samples: ***p<0.0001; **p<0.01; *p<0.05.

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