

Minimal residual disease monitoring by 8-color flow cytometry in mantle cell lymphoma: an EU-MCL and LYSA study

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MRD by flow cytometry in Mantle Cell Lymphoma

Supplementary Data

Treatment of patients in the EU-MCL trials.

The trials investigated the role of different induction protocols followed by either 2 different high-dose regimens with ASCT (MCL Younger) or 2 different maintenance therapies (MCL Elderly).

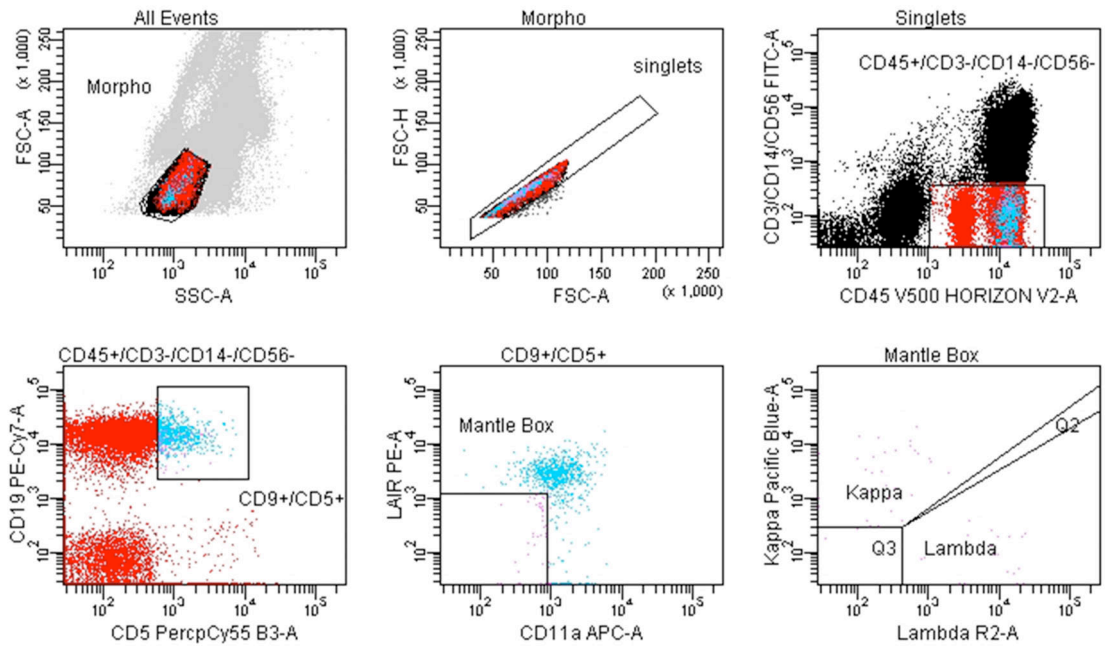
Younger patients received either 6 cycles rituximab with cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) followed by myeloablative radiochemotherapy with autologous blood stem cell support or 6 cycles of alternating R-CHOP/R-DHAP (rituximab with high-dose cytarabine and cisplatin) regimens followed by high-dose cytarabine containing myeloablative radio-chemotherapy and ASCT. Older patients were randomized to induction with either 8 cycles of R-CHOP or 6 cycles of rituximab, fludarabine, cyclophosphamide (R-FC). After a second randomization, all patients in clinical remission (CR) received maintenance treatment with either interferon- α or rituximab at 2-monthly intervals, until clinical relapse. Peripheral blood (PB) and/or bone marrow (BM) samples were collected at diagnosis and simultaneously to clinical assessment at midterm staging (after 3 or 4 cycles of induction therapy), end-of-induction (4 weeks after the last induction cycle and before ASCT or maintenance) and post-induction at 3-monthly intervals after ASCT for MCL Younger and 2-3-monthly intervals for MCL Elderly patients, until clinical relapse in both trials^{2,9,11}.

Statistical analyses

For continuous and categorical data the Mann-Whitney test or the χ^2 -test/Fisher's exact test was used for group comparisons. Agreement of MRD positivity as defined by both methods was assessed using Cohen's kappa for different scenarios: positive RQ-PCR including all BQR, positive RQ-PCR including BQR with 2-3/3 positive triplicates and positive RQ-PCR \geq 0.01%. A Bland Altman analysis was performed to assess the level of agreement between two measurement methods in samples where both were positive.²⁸ Measurements were log-transformed prior to analysis. The limits of agreement were calculated using a linear mixed effects model taking into account the correlation among the repeated measurements in each subject. Correlations between MRD values generated by RQ-PCR and MFC were measured with the Pearson's correlation coefficient and their representation plotted using GraphPad

Software (GraphPad Software Inc, San Diego, CA, USA). RD according to MRD status in PB and/or BM at end-of-induction was analyzed by Kaplan-Meier estimates and compared using the log-rank test. A *P*-value of 0.05 was considered the limit of significance in all analyses. Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, North Carolina, USA).

Figure S1. Example of assessment of specificity and sensitivity of the CD11a/LAIR-1 combination on a normal blood sample.



Population	#Events	%Parent	%Total
All Events	1,000,000	###	100.0
Morpho	258,024	25.8	25.8
singlets	257,679	99.9	25.8
CD45+/CD3-/CD14-/CD56-	21,321	8.3	2.1
CD19+/5+	825	3.9	0.1
Mantle Box	55	6.7	0.0
Kappa	31	56.4	0.0
Q2	0	0.0	0.0
Q3	1	1.8	0.0
Lambda	23	41.8	0.0

Table S1. Number of samples analyzed by MFC and RQ-PCR at different time points.

	Patients, n	MRD samples	Midterm evaluation	End of induction	Follow-up or maintenance
MFC, n (PB/BM)	105	294 (210/84)	49 (24/25)	48 (30/18)	197 (156/41)
RQ-PCR, n (PB/BM)	97	894 (639/255)	126 (71/55)	98 (57/41)	670 (511/159)
Comparison of MFC and RQ-PCR MRD, n (PB/BM)	61	284 (207/77)	48 (24/24)	47 (30/17)	189 (153/36)

Table S2. LAIR-1 and CD11a sub-populations and their respective Kappa/Lambda light chain ratio in physiological B CD19+CD5+ cells from 10 normal blood samples. Results are expressed in median and standard deviation (SD). * The high SD is due to the very low number of events in the CD19+CD5+LAIR-1-CD11a- sub-population.

	All events	CD19+	CD19+ CD5+	CD19+CD5+ LAIR-1 pos CD11a neg	CD19+CD5+ LAIR-1 pos CD11a neg	CD19+CD5+ LAIR-1 neg CD11a neg	CD19+CD5+ LAIR-1 neg CD11a pos
Total events Median (SD)	1000000 (0)	24722 (12111)	1178 (800)	141 (112)	1551 (670)	7 (2)	81 (60)
K/L ratio Median (SD)		1.60 (0.20)	1.61 (0.19)	1.29 (0.31)	1.71 (0.21)	2.00 (2.10*)	2.00 (0.14)

Figure S2. Minimal residual disease quantification by MFC in 211 PB samples and 83 BM samples from patients included in the EU-MCL trial. The number of negative/total samples in each category is indicated on the x-axis. 10 PB and 10 BM samples from 11 patients without detectable tumor cells at diagnosis were excluded.

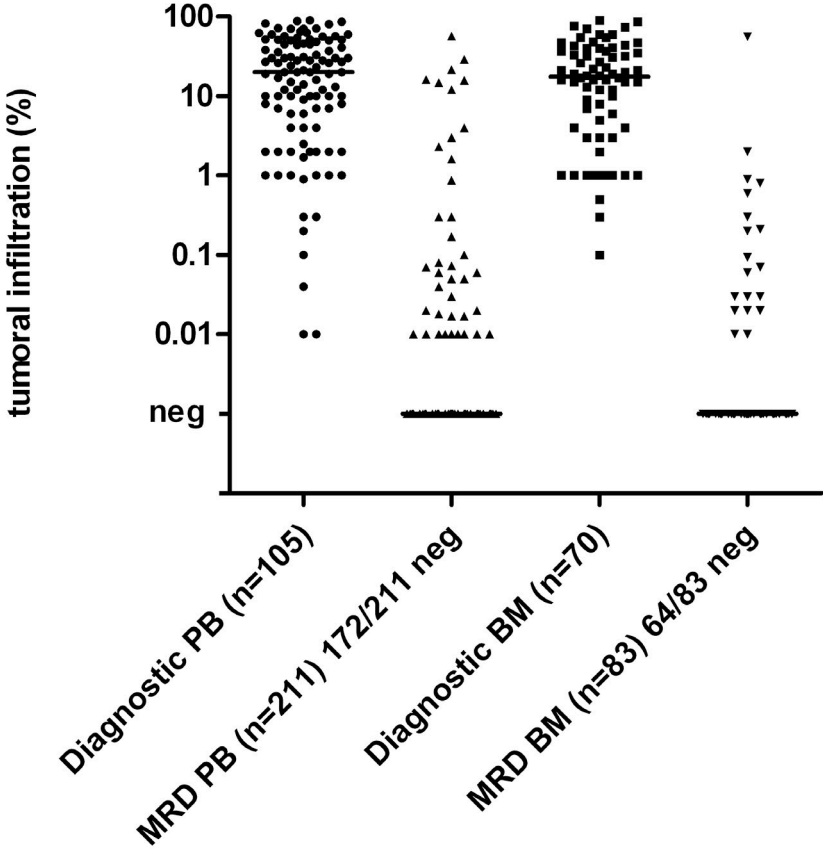


Figure S3. Correlation between infiltration evaluated by MFC and RQ-PCR Ct value from a 1 log dilution for 100 diagnostic PB or BM samples with semi-logarithmic (A) or logarithmic (B) expression. The 90% confidence interval or prediction is given in C. Six samples with low level cycle threshold (Ct) values within the 90% confidence limits are indicated in red in A and B and the 4 samples with Ct values above the confidence limits are shown in orange.

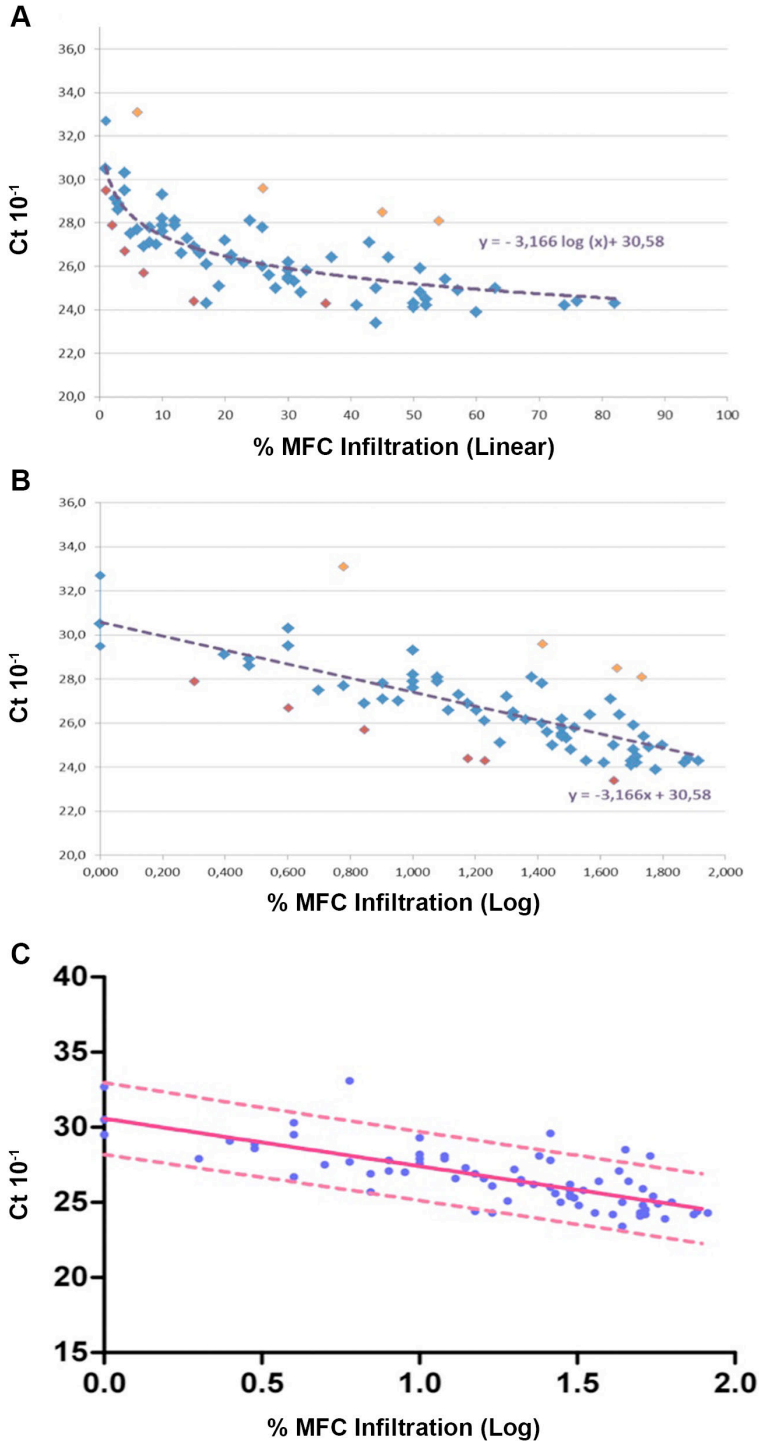


Figure S4. Agreement plot for MRD positivity as defined by the MFC and RQ-PCR methods. The figure shows the corresponding Cohen's kappa agreement charts for 3 scenarios: positive RQ-PCR including all BQR, positive RQ-PCR including BQR with 2-3/3 positive triplicates and positive RQ-PCR $\geq 0.01\%$.

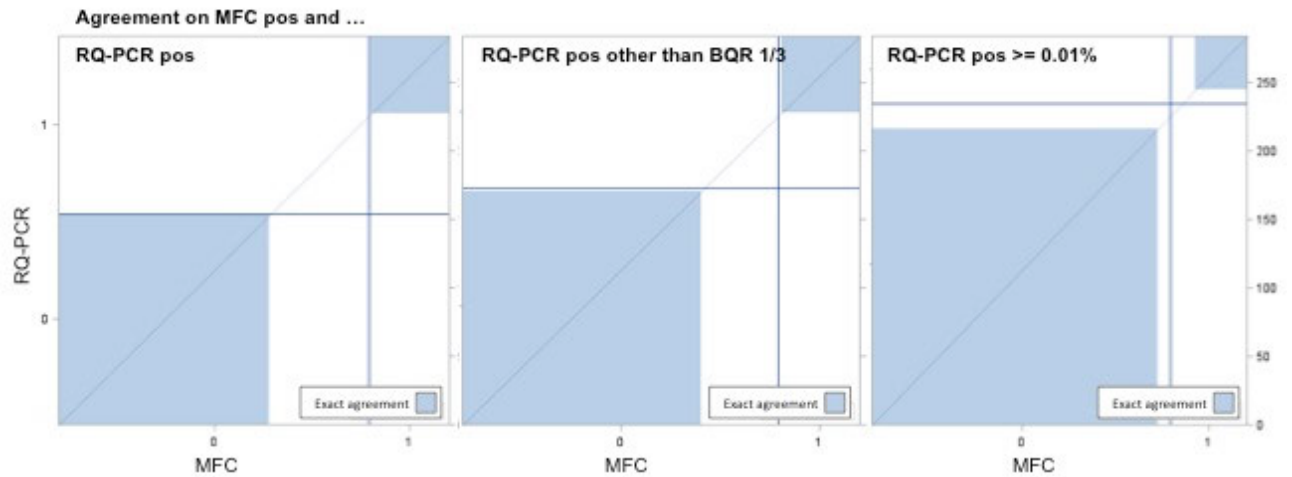


Figure S5. Comparison of MRD results obtained by MFC and RQ-PCR by Bland–Altman analysis. The differences and the mean were calculated from logarithmically transformed proportional MRD results. Analysis was restricted to positive MRD results.

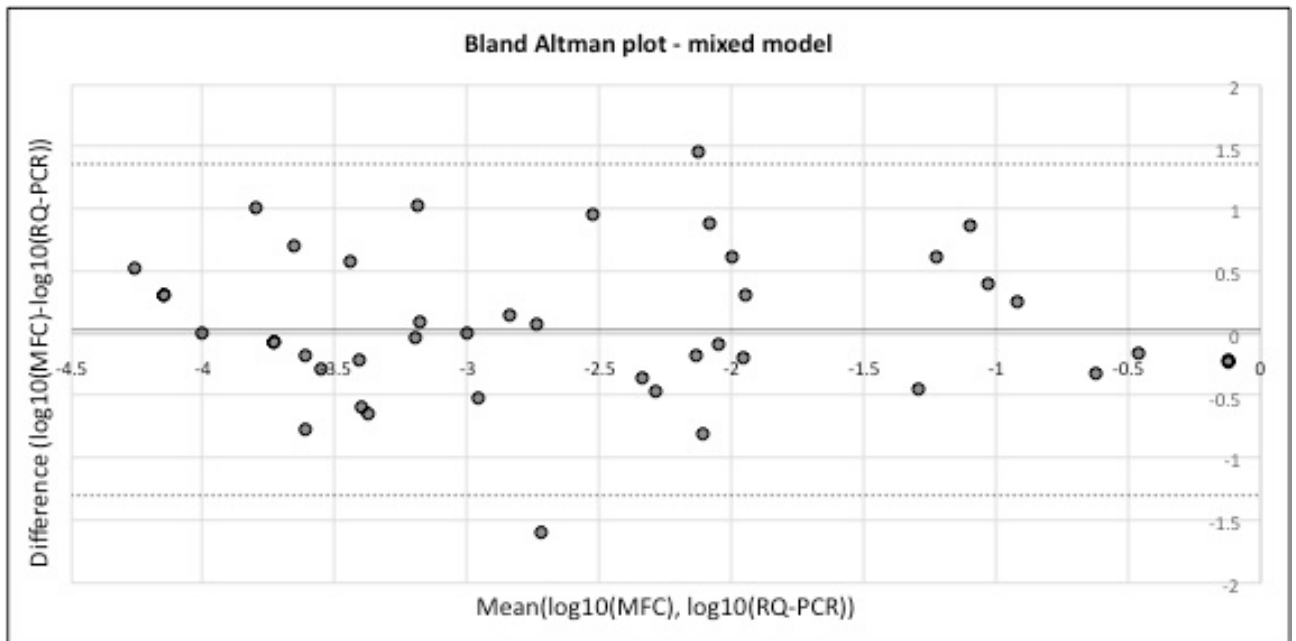


Figure S6. Comparison of IgH and BCL1-IgH PCR quantification for 11 patients. The numbers refer to the number of samples represented by each line.

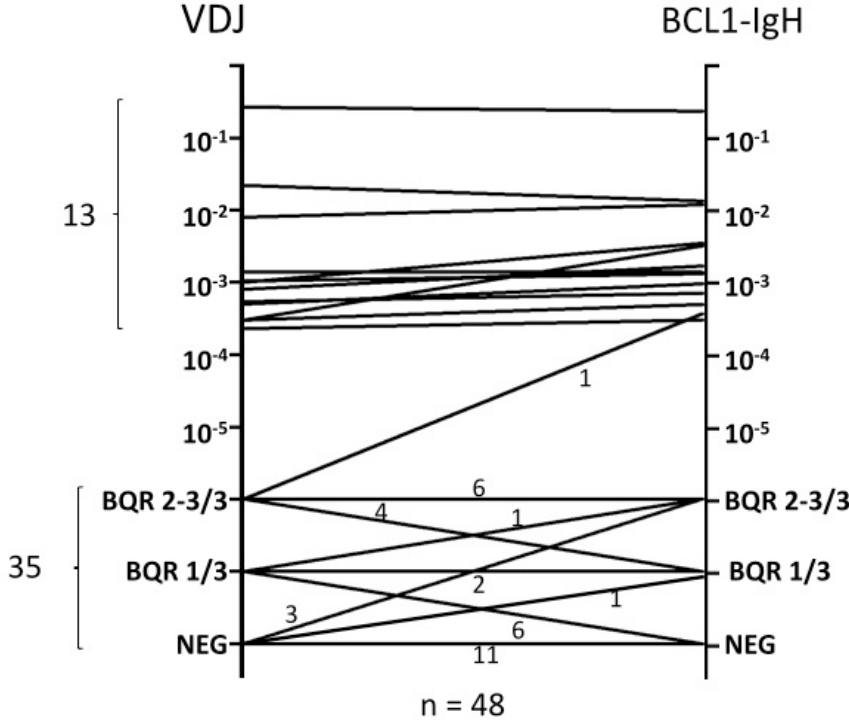


Table S3. Comparison of a 1Ct and a 3Ct Q-PCR cut-off above background for evaluation of positivity by RQ-PCR.

The Euro-MRD group defined two criteria for definition of BQR positivity, with either 3Ct or 1Ct difference from first background positivity. In the former setting the aim is to avoid false positive MRD results, whereas the latter aims to avoid false negative results. The difference is based on the minimal difference between the highest Ct value considered to represent MRD positivity and the lowest Ct value of the polyclonal PBLs used to assess specificity. Under maximum sensitivity conditions, as used here, the Ct value of at least one of the triplicates must be ≥ 1 Ct below the lowest Ct of background, whereas under therapy intensification conditions which aim to reduce false positive results, the Ct value of at least one of the triplicates must be ≥ 3 Ct below the lowest Ct of background.

In order to assess the impact of these different cut-offs, we compared the 68 BQR samples using 3Ct and 1Ct cut-offs. As shown in Table S1, the former led to 15 BQR samples being reclassified as negative, including 1 which were previously positive by MFC (at 0.01%). The number of BQR samples diminished from 68 to 53, with no significant change in the incidence of MFC positivity in the different RQ-PCR categories. A more restrictive definition of low level PCR positivity therefore led to a diminution of BQR samples from 68/284 (24%) to 53/284 (19%).

RQ-PCR samples (n)	Pos > QR			Pos BQR			Neg	TOTAL
	$\geq 0.1\%$	$< 0.1\%$ $\geq 0.01\%$	$< 0.01\%$	BQR 3/3	BQR 2/3	BQR 1/3		
3Ct cut-off	26	23	13	19	18	16	169	284
% Pos MFC/RQ-PCR	96%	61%	31%	37%	28%	6%	1%	20%
1Ct cut-off	26	23	13	23	26	19	154	284
% Pos MFC/RQ-PCR	96%	61%	31%	30%	23%	5%	1%	20%

Table S4. Latency between first positive MRD (PB) and first clinical relapse in 10 patients. RQ-PCR pos represent positive results with at least 2 positive triplicates. BQR means that MRD remained positive below the quantifiable range after the end of the treatment until clinical relapse.

Patient N°	RQ-PCR pos	RQ-PCR > 0.01%	MFC pos	Sensitivity	QR
514-201	7	2	2	1.00E-05	1.00E-05
367-203	24	5	5	1.00E-05	1.00E-05
489-203	BQR (43)	9	18	1.00E-05	1.00E-04
274-208	1	0	0	1.00E-04	5.00E-04
527-220	21	9	17	3.00E-05	3.00E-05
567-202	24	11	ND	3.00E-05	1.00E-04
274-209	BQR (15)	0	4	1.00E-05	5.00E-05
274-210	BQR (24)	5	5	1.00E-05	2.00E-04
247-235	BQR (4)	0	0	3.00E-05	3.00E-05
247-220	48	11	ND	1.00E-04	5.00E-04
Median (mths)	22.5	5	4.5		