Different features of acute myeloid leukemia stem cell quantification in intensively treated patients

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Supplementary materials
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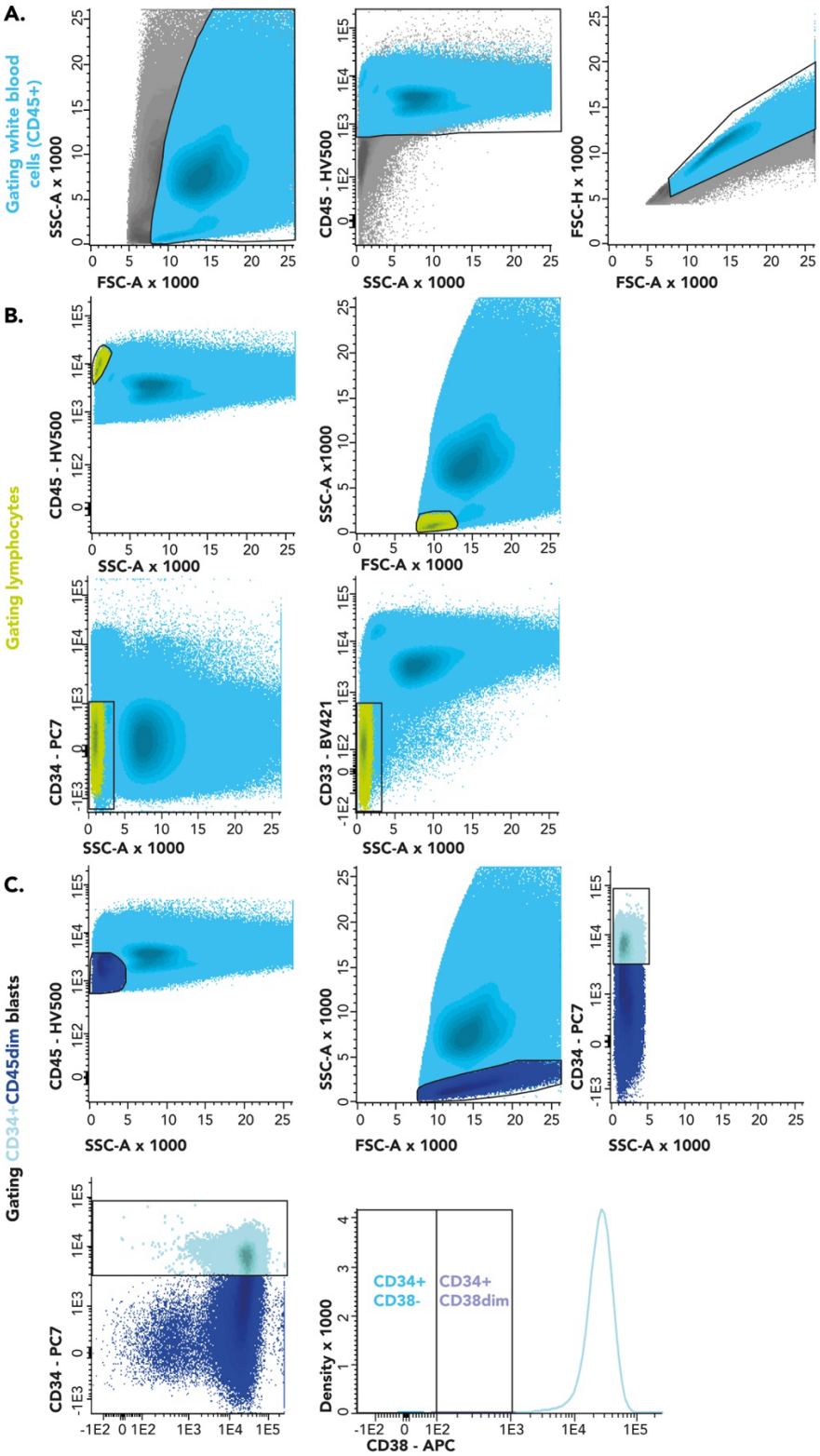
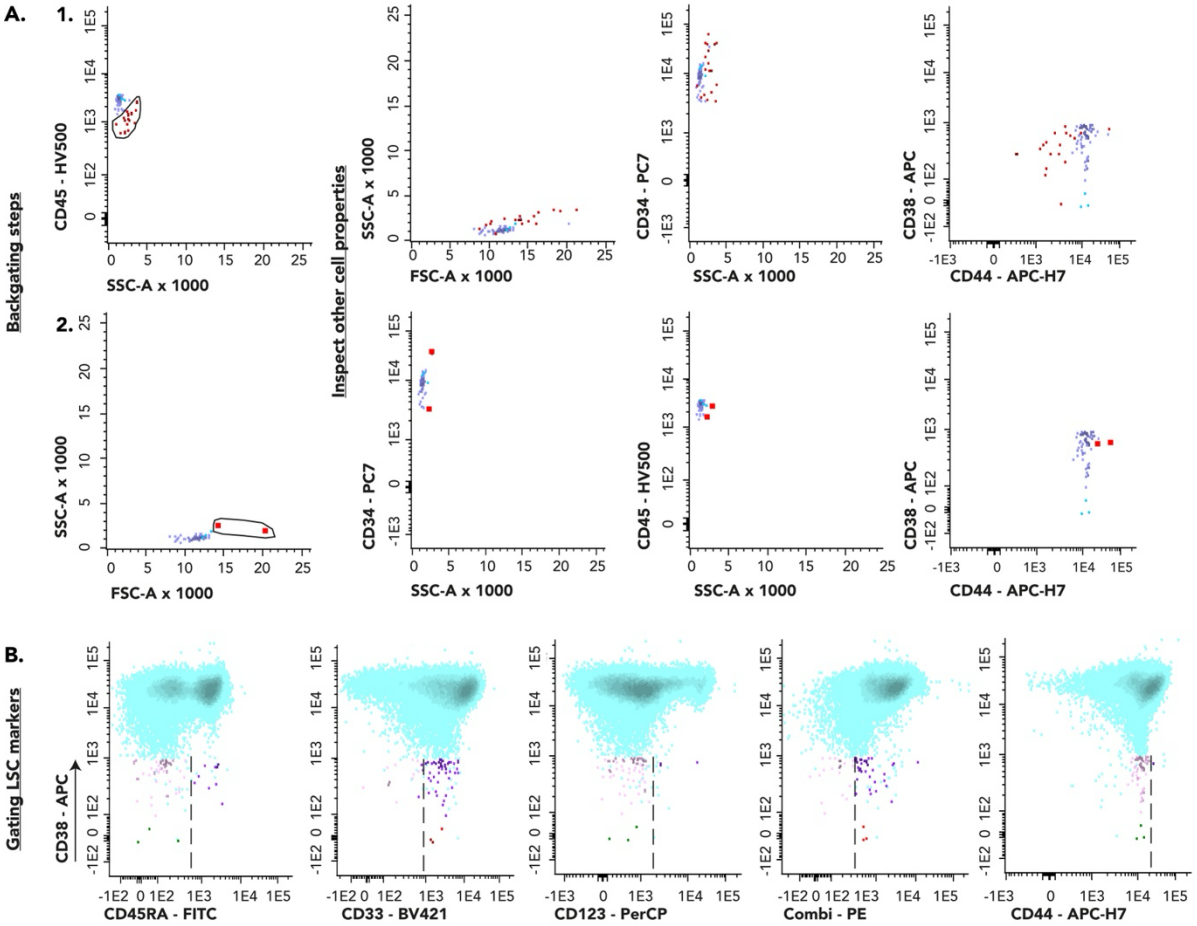
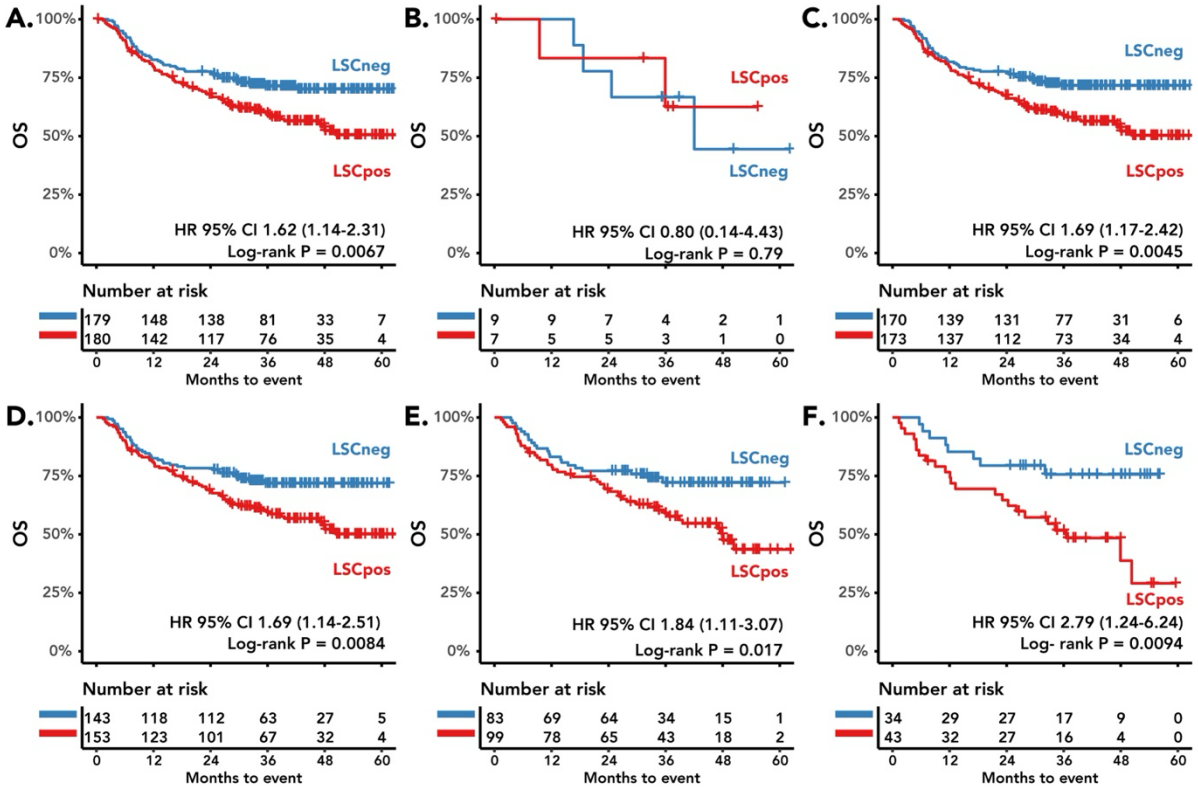


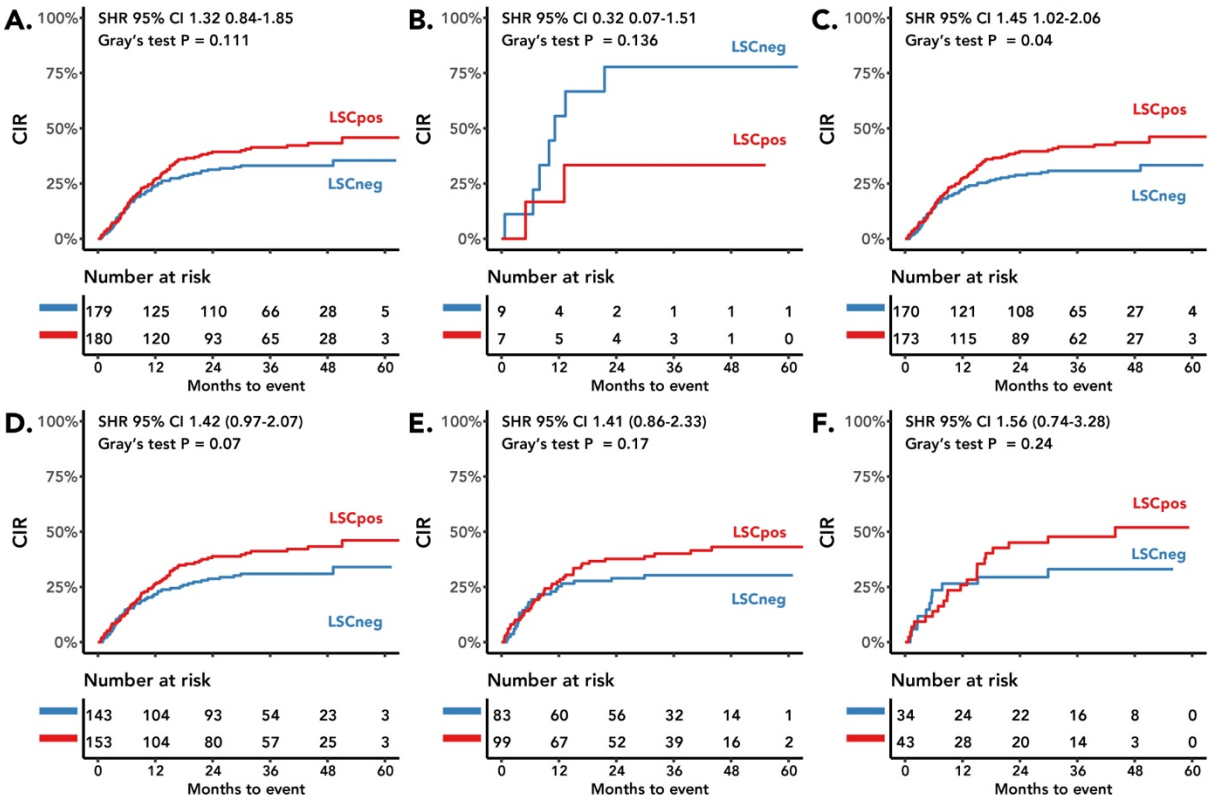
Supplementary Figure S1: Gating strategy LSC after C2. General populations A. gating WBC, debris is gated out by gating out the FSClow and SSClow cells, then only the CD45expressing cells are gated. Afterwards singlets are gating by the FSC-H and FSC-A plot. **B.** Gating lymphocytes**.** These are CD45high cells, SSClow cells, FSClow, CD34neg and CD33neg. **C.** Gating CD34+CD45dim blasts. CD45dim, SSClow-mid, CD34pos. Afterwards the CD38neg (verylow) is gated on 10e2 and CD38dim (10e3).



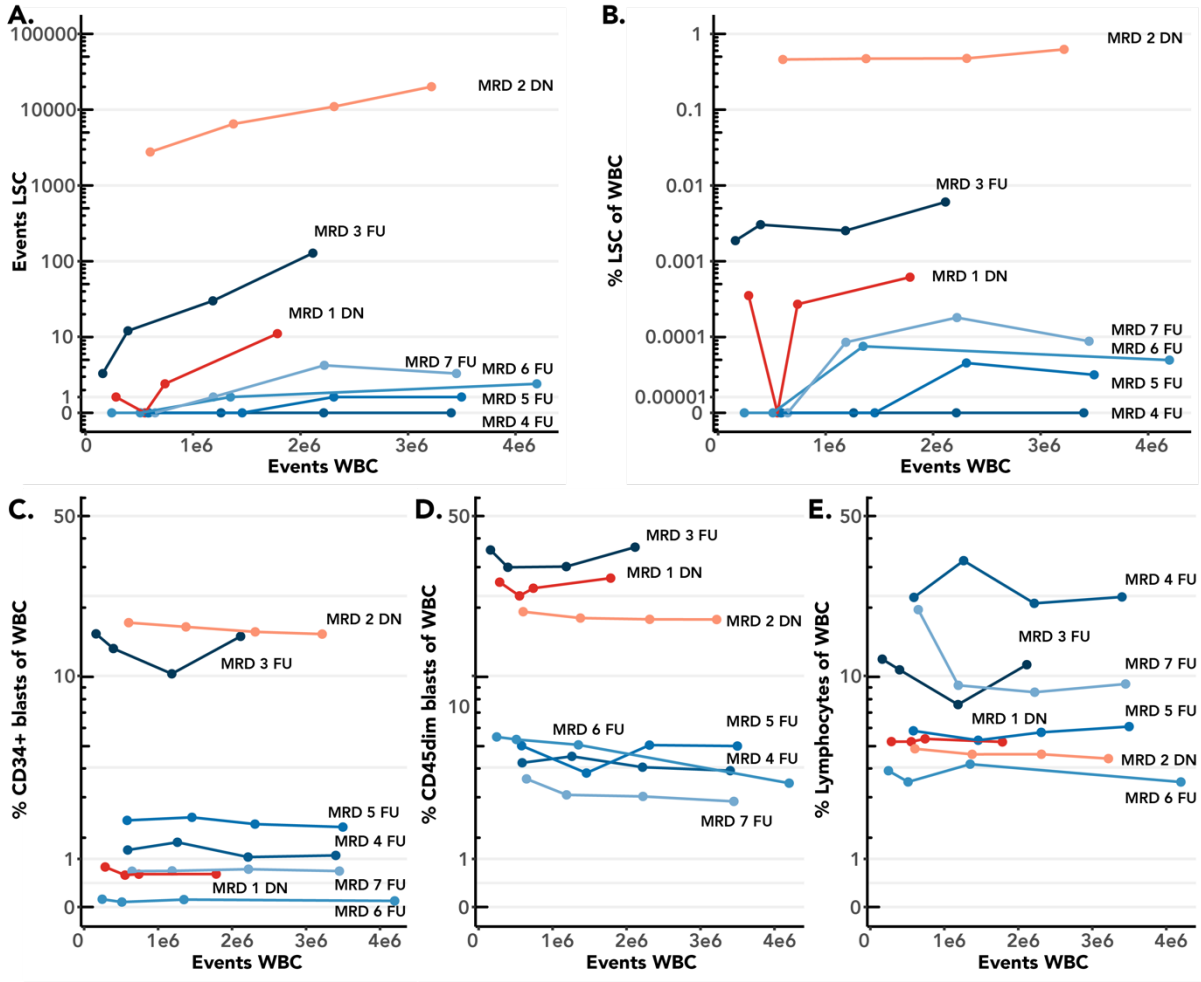
Supplementary Figure S2: Gating strategy after C2. Backgating to exclude aspecific events or noise. A. Gating aspecific events. 1. the cells are outside the population of the other CD34+CD38dim/- events in the CD45/SSC plot (colored red). These cells are inspected based on FSC, CD34 and CD44. These cells have a higher FSC, some events are higher in CD34 expression, and all events have a lower expression of CD44. Because CD44 should be expressed on all CD34+ cells, CD44 can be helpful when deciding if events should be backgated. 2. some outliers in the FSC/SSC plot are left, these have a high or low CD34 expression and are on the border of the CD45/SSC population and are therefore backgated. **B.** Gating LSC markers. For the CD45RA, we use the lymphocytes on their bimodal expression to see where the positive and negative peaks are. For the CD33, CD123 we use the lymphocytes. In the combi, we use the red/dead fraction as negative population. For the CD44, we use the peak of the lymphocyte expression to put the gate. Events that are in the CD38dim or CD38- that are colored light blue, are the backgated events



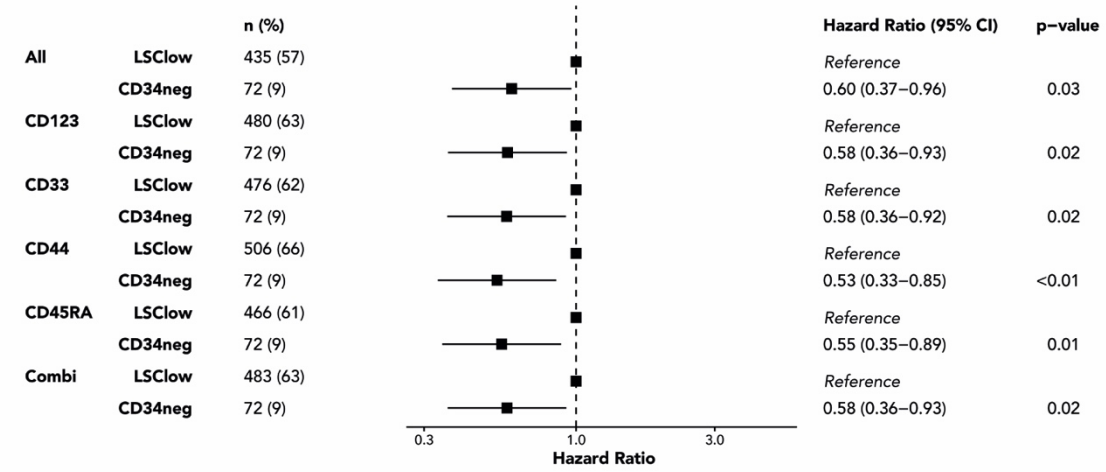
Supplementary Figure S3: Overall Survival of LSC burden after C2 stratified per how many cells are acquired. A. All patients regardless of number of measured cells, **B.** when only selected for patients below 1 million WBC acquired, **C.** when only patients above 1 million WBC acquired. **D.** when only patients above 2 million WBC acquired. **E.** when only patients above 3 million WBC acquired. **F.** when only patients above 4 million WBC acquired.



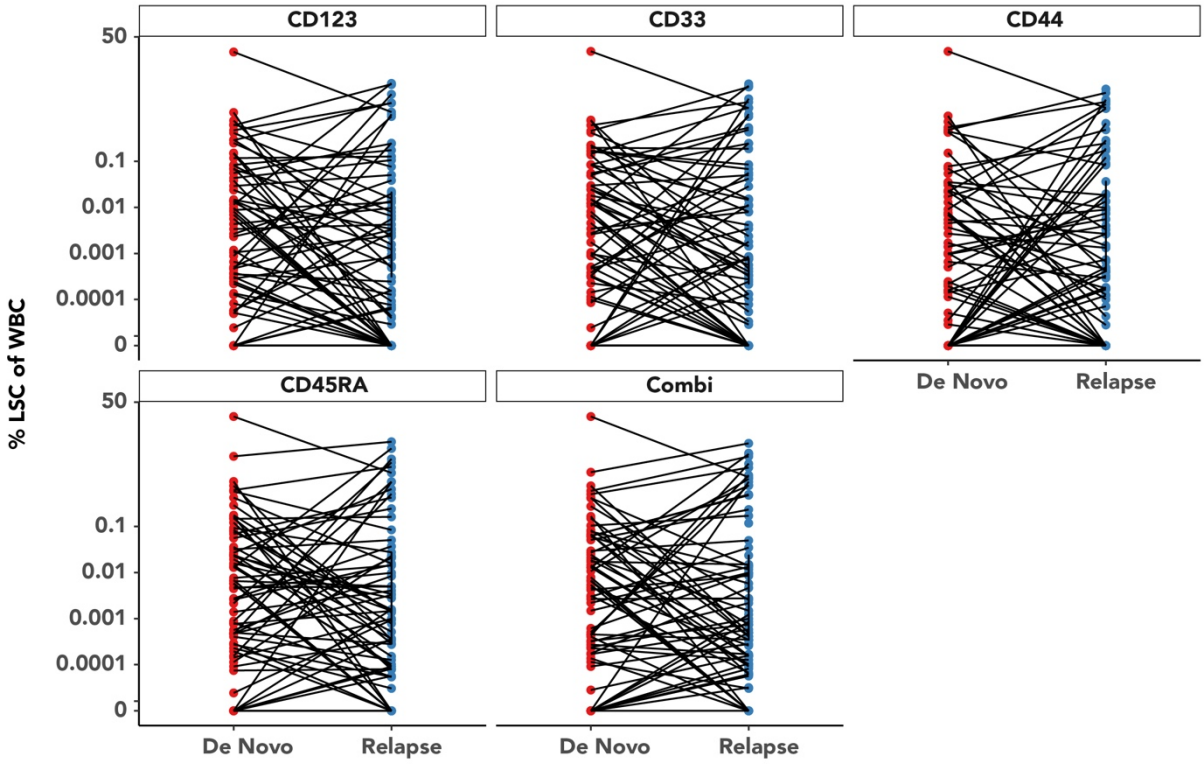
Supplementary Figure S4: Cumulative Incidence of Relapse of LSC burden after C2 stratified per how many cells are acquired. A. CIR All patients regardless of number of measured cells, **B.** CIR when only selected for patients below 1 million WBC acquired, **C.** CIR when only patients above 1 million WBC acquired. **D.** CIR when only patients above 2 million WBC acquired. **E.** CIR when only patients above 3 million WBC acquired. **F.** CIR when only patients above 4 million WBC acquired.



Supplementary Figure S5: Line plots of events LSC, percentage LSC, CD34+ blasts, CD45dim blasts, lymphocytes per acquired amount of WBC. A. Events LSC per acquired WBC, **B.** percentage LSC per acquired WBC, **C.** CD34+blasts, **D.** CD45dim blasts, **E.** Lymphocytes, DN = diagnosis sample, FU = follow up sample; Red indicates samples measured at diagnosis, and blue colored lines show samples post-therapy.



Supplementary Figure S6: Prognostic value of CD34+CD38-LSC+ cells when specifically chosen for sole LSC marker. At diagnosis. LSClow patients are reference**.**



Supplementary Figure S7: Line plots of percentage LSC of WBC at diagnosis and at relapse show that in relapse samples high percentages of LSC per marker are present. Although some do not show LSC at relapse, LSC are mostly present at relapse. Each line represents a patient, however if one patient does not have e.g. CD123 LSC, it still may have CD45RA LSC.

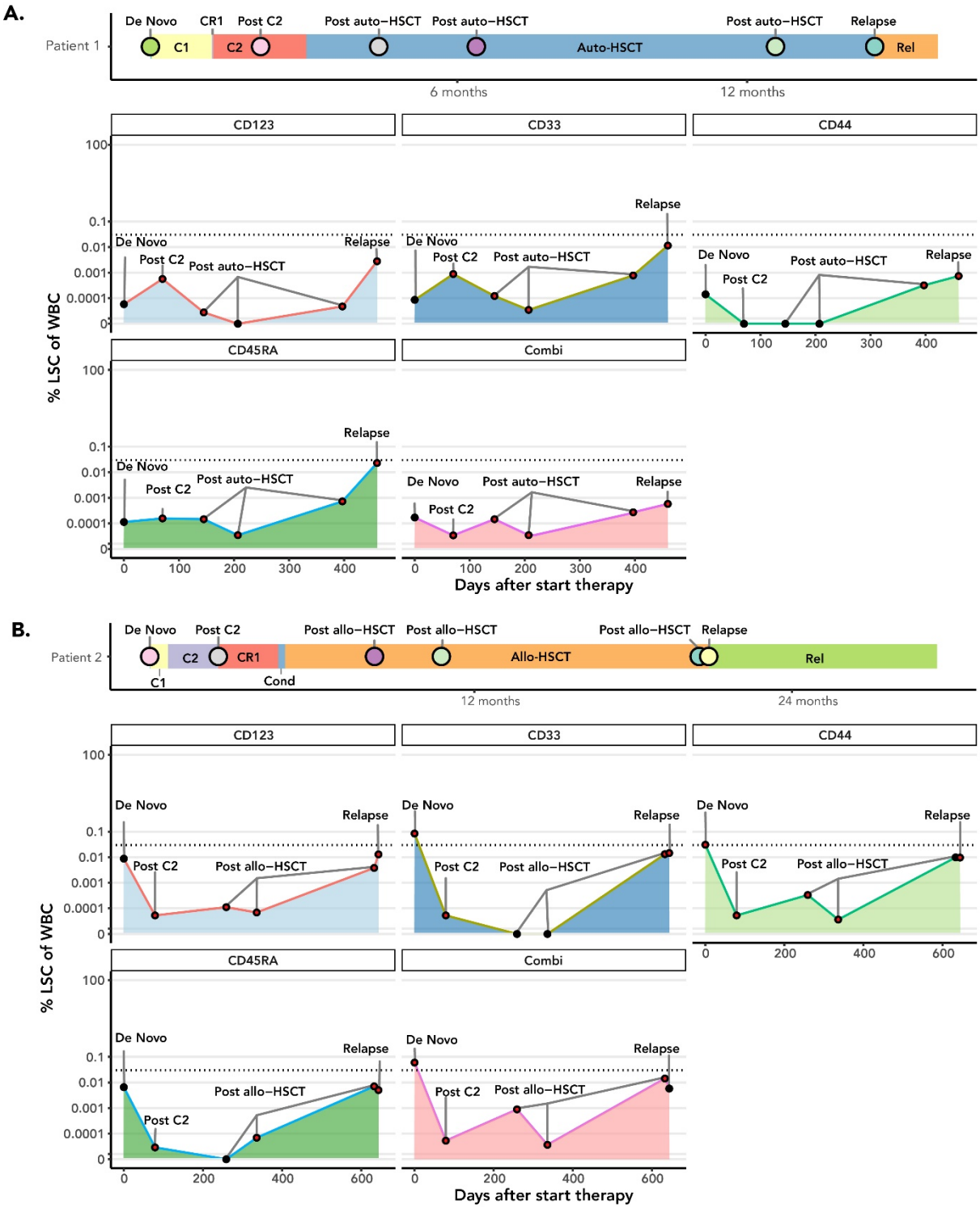


Figure S8. Timeline of two patients showing the LSC burden during the treatment and relapse. A. Patient 1 shows a low amount of LSCs at diagnosis, LSC positivity after C2, and increasing LSC amount until relapse**.** Patterns show that CD123 and CD44 LSCs in samples after autologous stem cell transplantation decrease, while CD33, CD45RA, and Combi LSCs remain. **B.** Patient 2 shows high LSCs at diagnosis, LSC positivity after C2, decrease of CD45RA+ and CD33+LSCs in the post-allo-HSCT samples, while CD123+, CD44+, Combi+ LSCs remain.

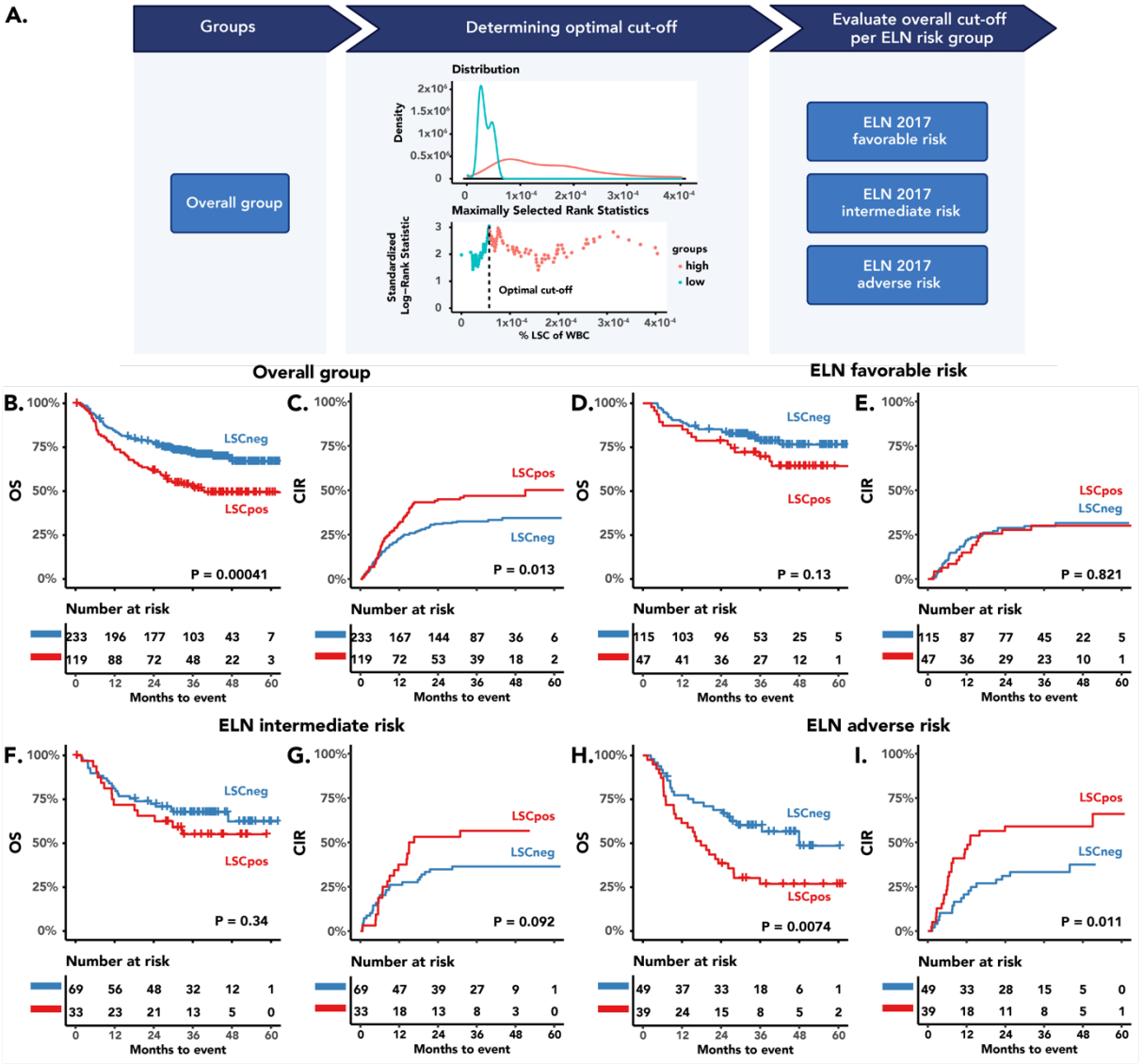
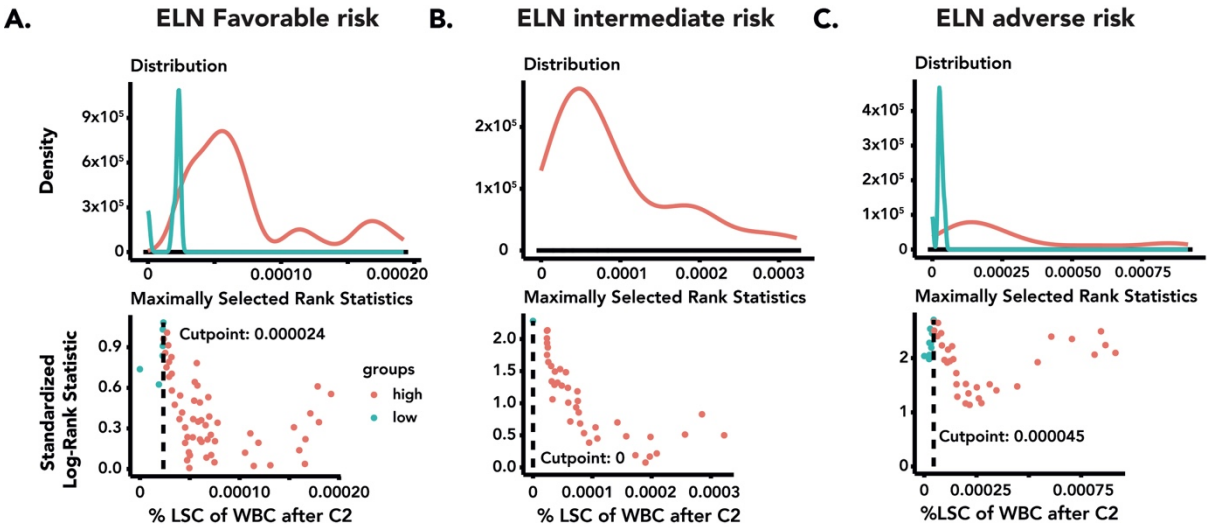
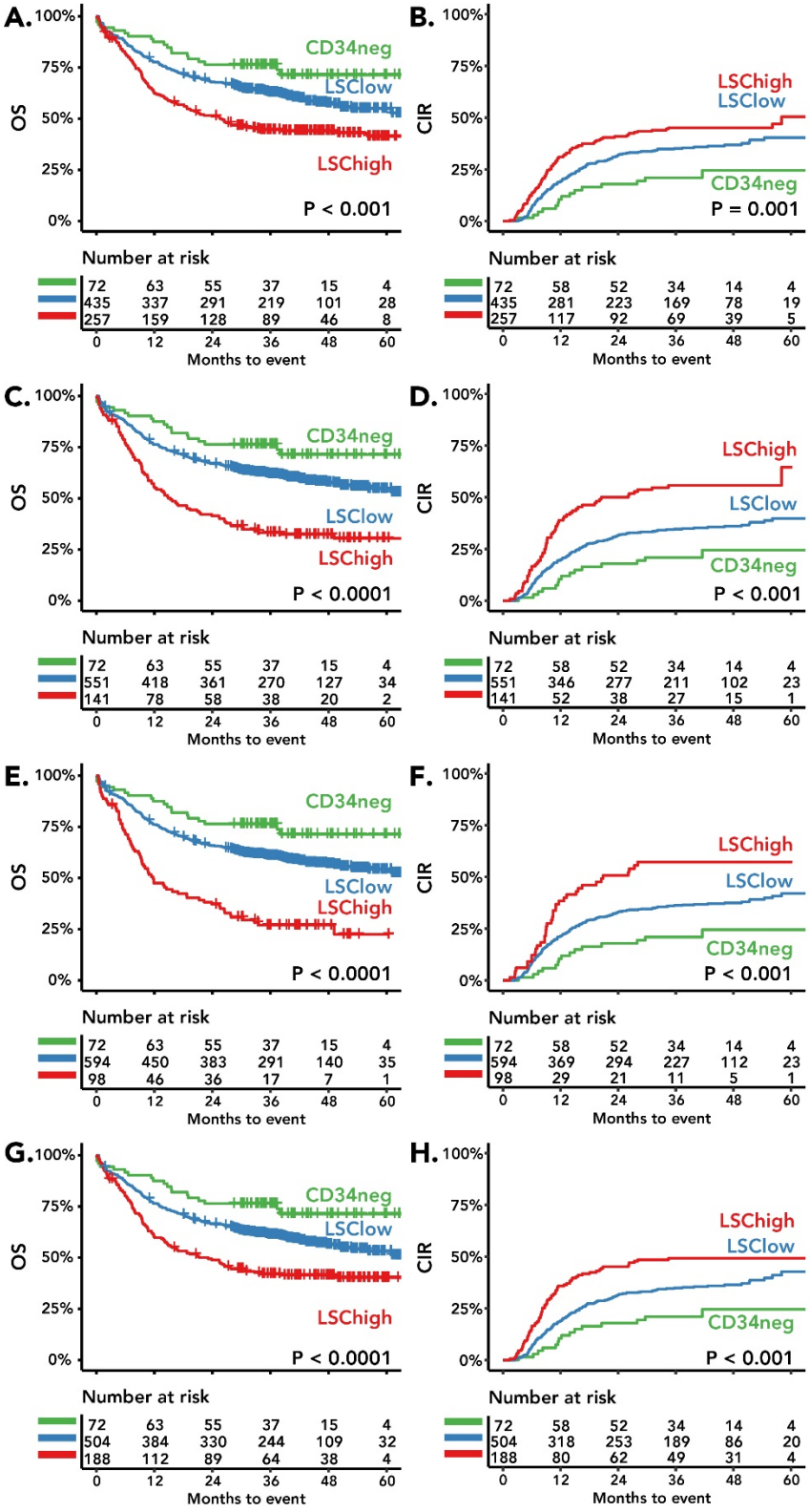


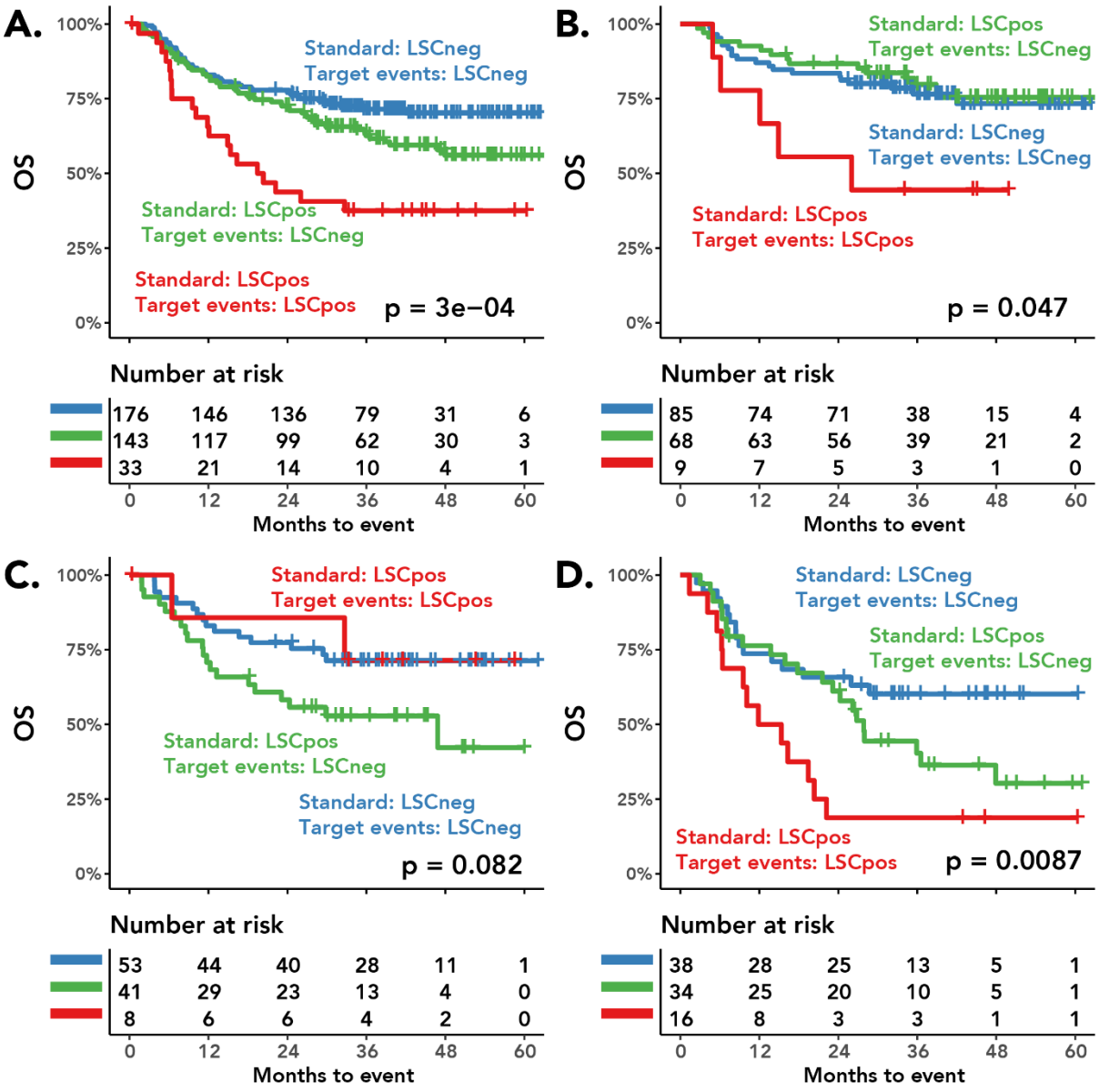
Figure S9: Prognostic value of LSC after C2 when the optimal cut-off is based on outcome of the total group. A. Scheme of the adjustment, for the overall group the optimal cut-off based on EFS was determined with maximally selected rank statistics and this cutoff is evaluated for the overall group and the ELN risk groups. **B and C.** OS and CIR of the whole population prognostic value based on total group. Cut-off 0.000057% LSC of WBC. **D and E.** OS and CIR of the ELN2017 favorable risk patients. **F and G.** OS and CIR of the ELN2017 intermediate-risk patients. **H and I.** OS and CIR of the ELN2017 adverse patients.



Supplementary Figure S10: Maximally selected rank statistics with RFS and determined per ELN2017 risk group. A. 0.000024%, B. 0%, C. 0.000045%



Supplementary Figure S11: Prognostic value of LSC at diagnosis based on variations in LSC analysis. A. & B. OS and CIR of our current standard assay, **C. & D.** OS and CIR with the optimal cut-off with maximally selected rank statistics based on RFS. Cut-off for LSC high 0.3% LSC of WBC. **E. & F.** OS and CIR with the readout of changing the CD38 negative cut-off. Cut-off for LSChigh 3.5% LSC of WBC. **G & H.** OS and CIR with the readout of changing the denominator from WBC to CD34+ blast cells (primitive fraction). Cut-off for LSChigh 1.42% LSC of CD34+ blast cells.



Supplementary Figure S12: Overall survival of patients stratified based on the variation of selecting target events combined with the standard method. A. overall group, **B.** Favorable group. **C.** Intermediate group, **D.** Adverse group

Table S1. Number of WBC acquired during the HO132 trial at diagnosis and after C2[1]

	Diagnosis				*After C2*		
	CD34neg	LSClow	LSChigh	P-value	LSCneg	LSCpos	P-value
n	72	435	257		179	180	
WBC median (min-max)	2,079,870 (29,816-4,765,769)	2,012,398 (49,073-6,178,534)	2,137,489 (53,769-5,684,823)	0.801	2,941,676 (392,399-4,900,207)	3,0983,86 (632,335-5,233,684)	0.122
% of patient four million WBC measured	11.1% (8/72)	10.6% (46/435)	11.3% (29/257)	0.199	19% (34/179)	23.9 % (43/180)	0.319

[1] For the difference in measured cells, the Mann Whitney U test and Kruskal Wallis test was used. For the difference in proportions of patients of which 4 million was measured, the Z test and chi-squared test was performed.

Supplementary table S2. Univariable CIR analysis with subdistribution hazard ratio per LSC marker at diagnosis and after C2

		At diagnosis			*After C2*	
		SHR 95% CI	**P-value**		**SHR 95% CI**	**P-value**
All	CD34neg	0.56 (0.33-0.94)	0.027			
	LSChigh	1.37 (1.05-1.79)	0.022	LSCpos	1.36 (0.97-1.92)	0.076
CD45RA	CD34neg	0.55 (0.32-0.92)	0.022			
	LSChigh	1.34 (1.02-1.77)	0.035	LSCpos	1.59 (1.13-2.22)	0.007
Combi	CD34neg	0.57 (0.37-0.95)	0.032			
	LSChigh	1.54 (1.17-2.04)	0.002	LSCpos	1.40 (1.00-1.96)	0.052
CD123	CD34neg	0.58 (0.34-0.97)	0.038			
	LSChigh	1.64 (1.24-2.16)	0.0004	LSCpos	1.23 (0.85-1.77)	0.270
CD33	CD34neg	0.55 (0.33-0.92)	0.024			
	LSChigh	1.39 (1.05-1.84)	0.020	LSCpos	1.72 (1.23-2.4)	0.002
CD44	CD34neg	0.54 (0.32-0.91)	0.021			
	LSChigh	1.42 (1.07-1.89)	0.002	LSCpos	1.13 (0.76-1.68)	0.540

Supplementary table S3. CIR of possible adjustment for the LSC assay after C2

	All			ELN2017 Favorable risk			ELN2017 Intermediate risk			ELN2017 Adverse risk		
Method for refinement	*SHR (95%CI)*	*LSCneg/ LSCpos*	*P-value*	SHR (95%CI)	LSCneg/ LSCpos	*P-value*	SHR (95%CI)	LSCneg/ LSCpos	*P-value*	SHR (95%CI)	LSCneg/ LSCpos	*P-value*
Standard	1.36 (0.97-1.92)	176/176 (50/50)	0.08	0.75 (0.43-1.31)	85/77 (52/48)	0.31	**1.98 (1.07-3.64)**	**53/49 (52/48)**	**0.03**	1.98 (1.04-3.78)	38/50 (43/57)	0.04
Define the minimum number of target cells	1.77 (1.09-2.88)	319/33 (91/9)	0.02	1.66 (0.60-4.59)	153/9 (94/6)	0.33	1.26 (0.54-2.94)	94/8 (92/8)	0.59	1.81 (0.87-3.76)	72/16 (82/18)	0.11
Optimal cut-off general group	1.55 (1.1-2.18)	233/119 (66/34)	0.01	0.93 (0.51-1.72)	115/47 (71/29)	0.82	1.69 (0.934-3.05)	69/33 (68/32)	0.08	**2.24 (1.21-4.14)**	**49/39 (56/44)**	**0.01**
CD38 cut-off change	**1.85 (1.31-2.6)**	**239/113 (68/32)**	**<0.001**	**1.67 (0.93-3)**	**120/42 (74/26)**	**0.09**	1.57 (0.86-2.86)	67/35 (66/34)	0.14	2.04 (1.11-3.75)	52/36 (59/41)	0.02
PM - LSC (changing the denominator)	1.5 (1.07-2.1)	206/146 (59/41)	0.02	1.06 (0.61-1.86)	100/62 (62/38)	0.83	1.83 (1.01-3.32)	61/41 (60/40)	0.045	1.75 (0.95-3.22)	45/43 (51/49)	0.08
The most optimal cut-off per ELN risk group	1.36 (0.97-1.9)	191/161 (54/46)	0.08	0.67 (0.378-1.18)	91/71 (56/44)	0.17	**1.98 (1.07-3.64)**	**53/49 (52/48)**	**0.03**	**2.25 (1.21-4.19)**	**47/41 (53/47)**	**0.01**

Supplementary table S4. Multivariable OS of possible adjustment for the LSC assay after C2

	All	
Method for refinement	*HR (95%CI)*	*P-value*
Standard	1.76 (1.20-2.58)	0.004
Define the minimum number of target cells	1.97 (1.18-3.28)	0.009
Optimal cut-off general group	1.91 (1.31-2.79)	0.0007
CD38 cut-off change	1.75 (1.20-2.54)	0.003
PM - LSC (changing the denominator)	1.94 (1.34-2.80)	0.0005
The most optimal cut-off per ELN risk group	1.82 (1.24-2.66)	0.002