Supplementary materials. Multiparametric flow cytometry (MFC) measurable residual disease (MRD) assessment (online only)

Leukemia-associated immunophenotype (LAIP) refers to the identification on the surface of blast cells of surface markers that are not present in normal or recovering bone marrow or blood samples. LAIP can be defined by lack of antigen commonly expressed on healthy precursors, cross-lineage antigen expression (such as lymphoid markers on myeloid blasts), or asynchronous antigen expression (defined as co-expression of mature and immature differentiation antigens). The identification and quantification of LAIPs allows to detect AML residues below the threshold of sensitivity of optical microscopy. To perform such analysis, baseline bone marrow sampling is fundamental to recognize any possible aberrancy in the expression of surface and/or cytoplasmatic cell markers. This approach is applicable in roughly 90% of AML cases, whereas the remaining 10% of patients not meeting this criterion are commonly categorized as no-LAIP and therefore considered not suitable for MRD monitoring through MFC.

In original design of this trial, LAIP identification at baseline represented a critical step for intermediate risk patients, since MRD status (e.g. positivity vs. negativity) after CR achievement guided the transplantation strategy. Each LAIP combination was considered relevant if expressed on at least 50% of blasts. At the established post-consolidation timepoint, bone marrow MFC MRD was determined by a high-sensitivity 8-color MFC assay. Color antigen combinations were selected in each single case to identify immunophenotypes expressed at a frequency <0.01% in normal bone marrow and minimize pitfalls due to "phenotypic switches" that have been described to be occasionally associated with relapses. "Different from normal" antigen expression analysis was also integrated when useful.

To stratify intermediate risk patients to receive either allogeneic (if MRD positive) or autologous (if MRD negative) stem cell transplant, the threshold for discriminating MRD-negative from MRD-positive cases was set at 3.5×10^{-4} (0.035%) residual leukemic cells. This threshold was selected based on retrospective validations in the context of former European Organization for the Research and Treatment of Cancer/GIMEMA protocols.

Supplementary Figure 1. Patients disposition (online only)

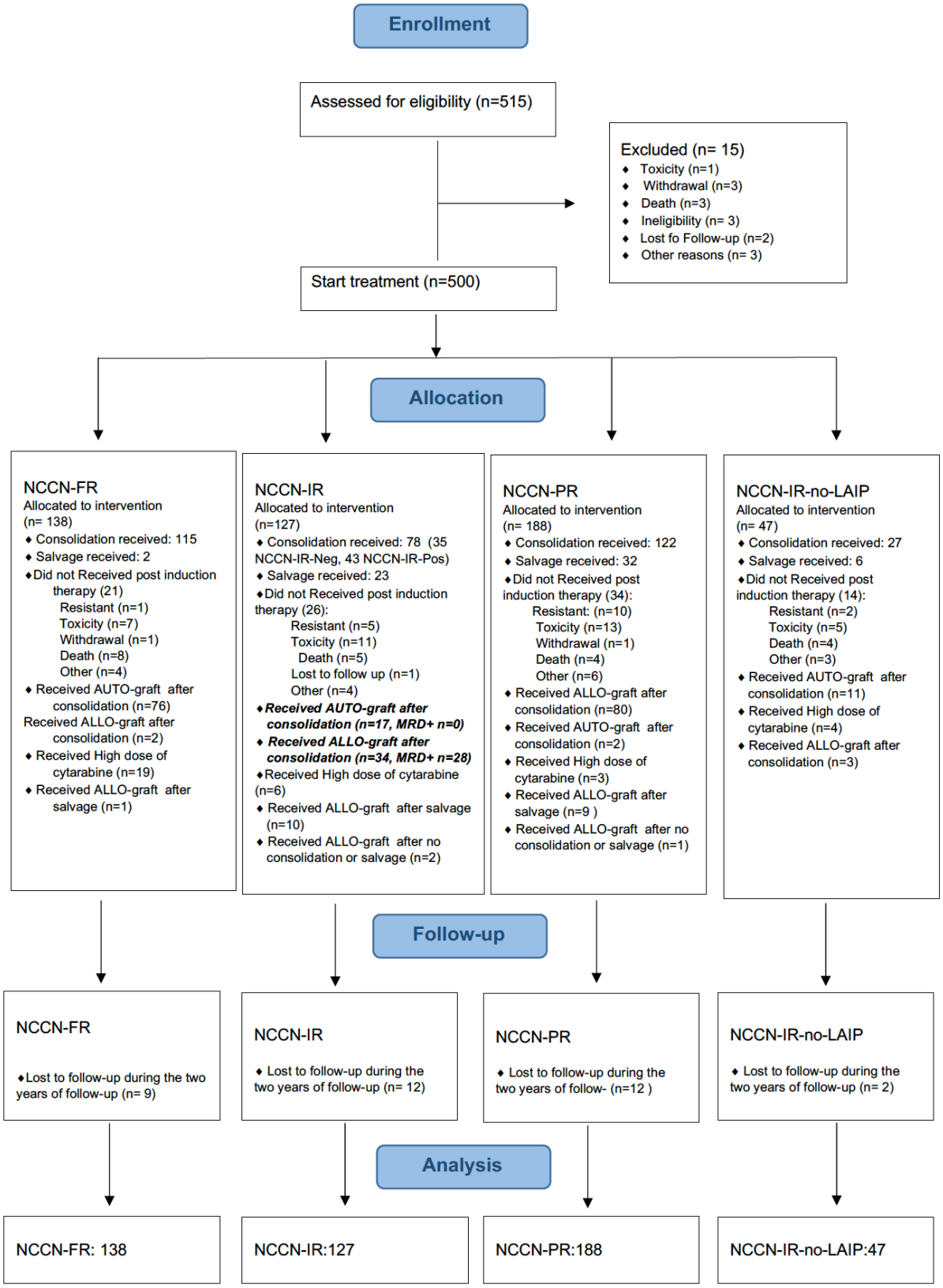
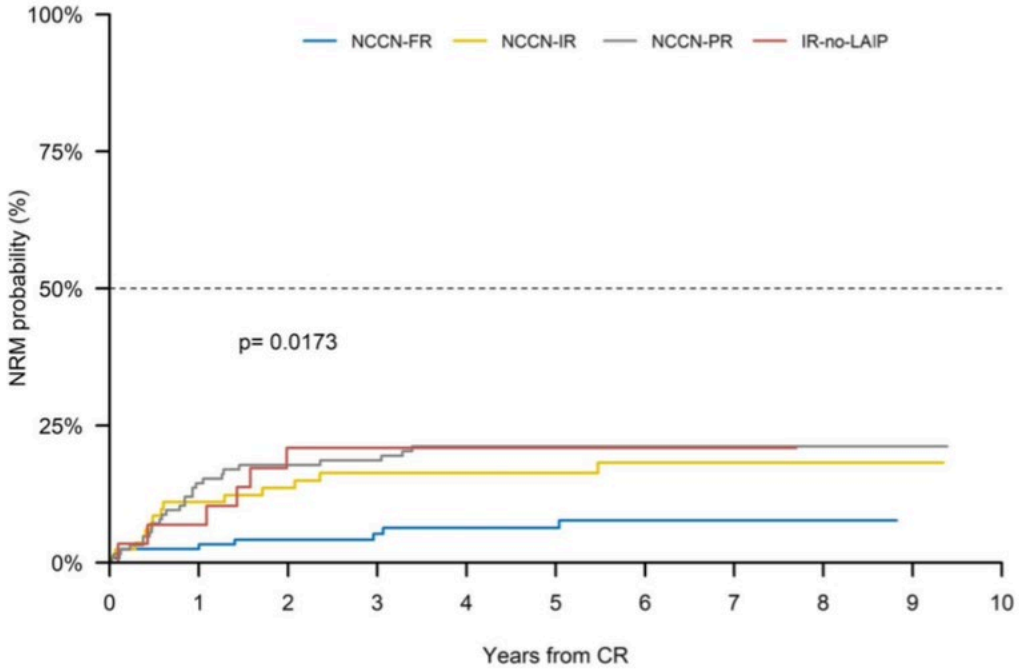


Table 1. Baseline and demographic characteristics by overall and risk category (online only)

		Risk category				
Characteristic	Overall, N = 500	Favorable Risk, N = 138	Intermediate Risk, N = 127	Poor Risk, N = 188	LAIP not detected, N = 47	p-value[1]
Sex, n (%)						0.68
M	260 (52%)	72 (52%)	70 (55%)	97 (52%)	21 (45%)	
F	240 (48%)	66 (48%)	57 (45%)	91 (48%)	26 (55%)	
Age starting treatment, median (range)	49 (18,61)	51 (18,61)	48 (20,61)	49 (18,61)	51 (19,60)	0.29
WBC, median (range)	14 (0, 353)	18 (1, 186)	7 (0, 154)	27 (0, 353)	2 (1, 144)	**<0.001**
RUNX1/RUNX1T1, n (%)						**0.019**
Negative	472 (95%)	122 (89%)	123 (97%)	181 (96%)	46 (98%)	
Positive	27 (5.4%)	15 (11%)	4 (3.1%)	7 (3.7%)	1 (2.1%)	
CBFbeta/MYH11, n (%)						**<0.001**
Negative	459 (93%)	116 (85%)	116 (93%)	182 (97%)	45 (96%)	
Positive	37 (7.5%)	21 (15%)	9 (7.2%)	5 (2.7%)	2 (4.3%)	
FLT3-ITD, n (%)						**<0.001**
Negative	371 (75%)	137 (100%)	126 (100%)	62 (33%)	46 (100%)	
Positive	126 (25%)	0 (0%)	0 (0%)	126 (67%)	0 (0%)	
FLT3-TKD, n (%)						0.50
Negative	1 (17%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	
Positive	5 (83%)	0 (0%)	3 (100%)	1 (100%)	1 (100%)	
NPM1, n (%)						**<0.001**
Negative	312 (63%)	36 (26%)	124 (98%)	106 (57%)	46 (98%)	
Positive	187 (37%)	102 (74%)	3 (2.4%)	81 (43%)	1 (2.1%)	
Cytogenetic Risk, n (%)						
Favorable Risk	48 (11%)	31	11	4	2	

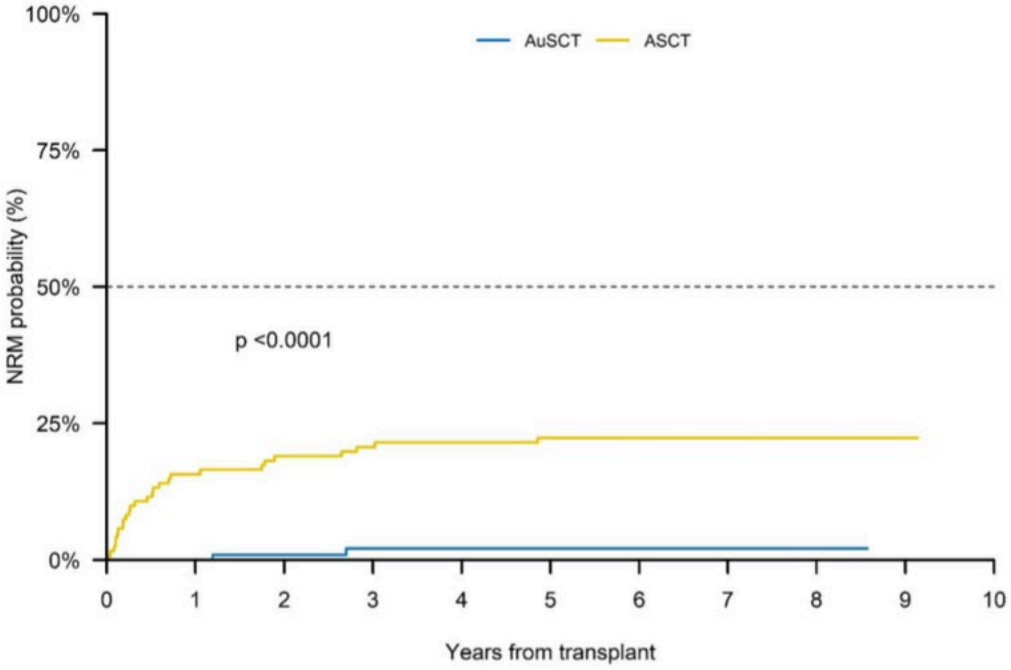
		(24%)	(11%)	(2.5%)	(5.1%)	
Intermediate Risk	316 (73%)	97 (76%)	92 (89%)	90 (56%)	37 (95%)	
Poor Risk	68 (16%)	0 (0%)	0 (0%)	68 (42%)	0 (0%)	

Supplementary figure 2. NRM stratified by risk category (online only).



level	Estimate at 6 years	lower 95%CI	upper 95%CI
NCCN-FR	7.7	2.4	13.0
IR	18.2	9.4	27.1
PR	21.2	13.9	28.5
IR-no-LAIP	20.9	5.6	36.3

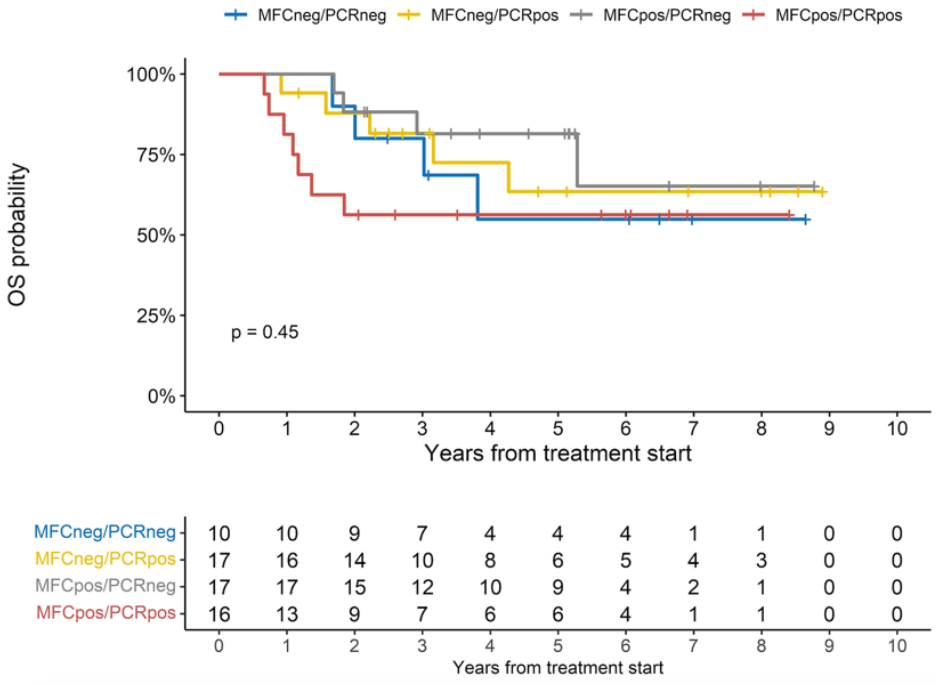
Supplementary figure 3. NRM from transplant stratified by type of transplant (online only).



level	Estimate at 6 years	lower 95%CI	upper 95%CI
AuSCT	2.1	0	5.0
ASCT	22.3	14.9	29.8

Supplementary figure 4. Survival estimates in terms of OS (A) and (DFS) of 60 patients whose MRD was analyzed integrating MFC and RTQ-PCR.

A.



B.