SUPPLEMENTARY INFORMATION

Supplementary Methods

Study Cohort

129 adult patients, underwent PBSC allografts for AML or high risk MDS between January 2010 and February 2013 at the University Hospital Birmingham. Of these, 101 patients had sufficient bone marrow samples for at least one assay /timepoint to be included in the analysis. We retrospectively evaluated the predictive value of MFC-MRD and MFC-LSC from pre- and post- HCT samples in this cohort of unselected AML patients (Supplementary Figure 1).

Pre transplant data was available from MFC analyses of an immunophenotypic LSC population (MFC-LSC) in 72 patients and standard MRD by LAIP (MFC-MRD) in 66 patients. Post transplant analyses performed within 6 months provided data for MFC-LSC and MFC-MRD in 92 and 69 patients respectively with any subsequent analyses on those patients up to 12 months post transplant also included.

Details of the conditioning regimens used are listed in Supplementary Methods Table 1 below. Recipients of MAC MUD allo-HCT also received alemtuzumab (total dose 50 mg over days -5 to -1). All patients received ciclosporin A (CsA) from day −1 until the institution of CSA taper 60-90 days post-transplant in patients with no evidence of GVHD. Morphological CR was defined as <5% bone marrow blasts.

Chimerism studies were performed on a T-cell purified subset at 90 days post-transplant and then subsequent time-points in patients using fluorescence in situ hybridization (FISH) or variable tandem repeat polymorphism analysis by polymerase chain reaction (PCR) (detailed protocol in Supplementary Methods). Chimerism, cytogenetic data and mutation analysis was performed and reported by the West Midlands Regional Genetics Service. Full donor chimerism was defined as >98% donor cells in either the whole blood or T cell compartment.

All patients were treated on institutional board-approved protocols and gave consent in accordance with the Declaration of Helsinki. Follow-up was current as of September 2013.

Supplementary-Methods Table 1. Conditioning Regimens used

TRANSPLANT CONDITIONING	REGIME	PATIENT NUMBERS
Cyclo/TBI Myeloablative	TBI 14.4Gy in 8 fractions Cyclophosphamide 60mg/kg/day x 2 days	25
Bu/Cy Myeloablative	Busulfan 0.8mg/kg qds over 4 days Cyclophosphamide 60mg/kg x 2 days	5
Flu/Mel/Campath Reduced intensity	Fludarabine 30mg/m2/day for 5 days (d-6 to d-2) Melphalan 140mg/m2/day for 1 day (d-1) Alemtuzunab10mg od IV for 5 days (d-7 to d-3)	60
Flu/Bu/Campath Reduced intensity	Fludarabine 30mg/m2/day for 5 days (d-7 to d-3) Busulphan 3.2mg/kg/day for 2 days (d-5 and d-4) Alemtuzumab30mg od IV for 2 days (d-7 to d-3)	2
FLAMSA Reduced intensity	Fludarabine (30mg/m2) (d-12 to d-9), High-dose AraC (2g/m2) (d-12 to d-9) Amsacrine (100mg/m2)(d -12 to -9) Following 3 days of rest: Busulphan 3.2mg/kg (d-5 to d-3 or if >60yo d-4 to d-3) Busulphan 1.6 mg/kg (d-2) ATG (Rabbit) 1mg/kg (d-3) ATG (Rabbit) 2mg/kg (d-2 and d-1) Fludarabine 30mg/m2 (d-3 to d-2) Cyclosporin (d-1 to d+60) MMF (d-1 to d+35)	2
Reduced intensity cord	Cyclophosphamide 50mg/m2 Day -6 (1 day) Fludarabine 40mg/m2 Days -6 to -2 (5 days) TBI 2 Gy D -1 IV Ciclosporin from Day -3 (trough levels 200-400 mcg/l	6
Flu Bu Cyclo Haploidentical	Fludarabine 30mg/m2 Days -6 to -2 (5 days) Cyclophosphamide 15mg/kg/day x 2 days (D-6 and D-5) TBI 2Gy D-1 Cyclophosphamide 50mg/kg/d (x2 days) D3 and D4 MMF (d-1 to d+35) Tacrolimus (to d180)	1

Multiparameter Flow Cytometry (MFC) Assays:

Bone marrows (BM) were obtained pre-transplant (range 10-90 days pre-transplant) and post-transplant (routinely done 60-90 days and then up to 12 months post-transplant dependent on scheduling directed by clinician) . MFC residual disease (MFC-MRD) was assessed by the reference laboratory as described previously[1] by detection of standard leukemic- aberrant-immunophenotypes (LAIPs) (detailed below in *MFC-MRD analysis*) in parallel with quantification of CD34+ progenitor subsets using the previously characterised LMPP-like subset[2, 3] as the immunophenotypic leukemic stem cell /progenitor population, (MFC-LSC). Fresh BMs were incubated with ammonium chloride to lyse erythrocytes and resulting nucleated cells were labelled with the appropriate 6-8 colour antibody panel in Supplementary Methods Table 2. 500,000 cells were acquired on a FACSCanto II (BD Biosciences) and data was analysed using FACSDiva software (BD Biosciences) followed by FlowJo (FlowJo.com, Tree Star Inc).

MFC-MRD Analysis: Normal antigen profiles for the antibody combinations were established and periodically updated from control bone marrow samples (normal / regenerating marrow). LAIPs were defined as cell populations that deviated from the normal antigen profiles with sufficient detection sensitivity and comprised >10% of leukemic blasts. LAIP percentages were reported as percentage of nucleated cells expressing the identified LAIP. In almost all selected LAIPs the sensitivity threshold was at least 0.1% of total nucleated cells (TNCs) i.e. less than 0.1% of TNCs from the control BMs fell within the defined LAIP gate. LAIPs were identified at presentation and /or relapse. In some patients minor or major immunophenotypic changes from baseline LAIPs were detected. These were considered as MRD if new LAIPs fulfilled criteria for detection sensitivity with less than 0.1% of TNCs from the control BMs fell within the newly defined LAIP gate. If no baseline presentation or relapse sample was available for a patient the “different-from normal” LAIP approach applied to blasts was used to detect MFC-MRD positivity if LAIP was sufficiently specific and sensitive. 500,000 cell events per tube or as many cell events as possible were acquired for follow-up samples. MFC-MRD analysis was not performed on inadequate follow-up samples (defined by <0.2% blasts and/or <100 cell events within the total blast (gated by CD45/SSC plus CD34+ and/or CD117+) gate). Any level of MFC-MRD detected above the sensitivity threshold was considered MRD-positive. Patients were excluded when no LAIP could be identified (15 patients) or there were missing / inadequate samples for MFC monitoring (23 pre, 13 post).

MFC-LSC Analysis: 500,000 or as many as possible fresh bone marrow nucleated cells post ammonium chloride lysis were acquired on a FACSCanto II (BD Biosciences) after labelling with the following antibody combination (Supplementary Methods Table 2B): CD45 RA FITC (5H9), CD45 APC-H7 (2D1), CD34 PerCP (8G12), CD123 PECy7 (7BG) CD38 APC (HB7) CD19 Horizon V450 (*SJ25C1)* – (Becton Dickinson), Post acquisition data was analysed using FACSDiva software (BD Biosciences) followed by FlowJo (FlowJo.com, Tree Star Inc) to quantify CD34+ progenitor compartments that would be predicted to be enriched for leukemic stem cells[2] (referred to as MFC MFC-LSC)**.** CD34+ events were gated based on their CD34, CD45 staining and scatter characteristics. CD19+ B- lymphoid progenitors were excluded from the analysis. The pattern of expression of 38 / CD45RA / CD123 of CD34+CD19- cells was analyzed to identify and quantify the following stem/progenitor compartments (SPC): 1)CD34+CD19-CD38low 2)LMPP-like (CD34+CD19-CD38lowCD45RA-) (Supplementary Figure 2). SPC analysis was not performed on inadequate samples (defined by <0.2% CD34+ blasts and /or <100 cell events within the CD34+ gate. Patients were excluded when there were missing/inadequate samples for MFC-LSC monitoring (pre-HCT=29, post-HCT=7). Detection of LMPP-like SPC was selected as assay for MFC-MFC-LSC detection as this approach has previously been shown to be more sensitive[2] with less potential overlap with normal SPC. LMPP-like SPCs were quantitated as % of total nucleated cells (TNC) with abnormal expansion/ positive when greater than 0.02% (TNC) (mean+1.96xSD of control samples[2] and further validated in 23 more control bone marrow samples (mean+1.96xSD = 0.019% of TNC) during this study (Supplementary Figure 3C).

Detectable CD34+CD19-CD38low SPC were CD45RA+ in most patients and so correlated with LMPP-like SPC expansion. 5 patients had detectable CD34+CD19-CD38low SPC pre or post-HCT that were CD45RA negative and therefore not LMPP-like. Of these, 2 patients relapsed and the other 3 have not. Conventional MFC-MRD analysis included detection of leukemic CD34+CD38low SPC with aberrant markers such as CD7, CD56 or overexpression of CD117 and CD33.

Supplementary-Methods Table 2.

A. MFC-standard MFC-MRD and **B.** MFC-LSC Antibody Panels

Table 2A MFC- Antibody Panel

Tube No.	*FITC*	*PE*	*PerCP*	*PECy7*	*APC*	*APC H7*	*Horizon V450*
1	**HLADR** *L243 (BD)*	**CD13** *L138 (BD)*	**CD34** *8G12 (BD)*	**CD117** *1042D2 (BD)*	**CD33** *P67.6 (BD)*	**CD45** *2D1 (BD)*	
2	**CD38** *HB7 (BD)*	**CD56** *MY31 (BD)*	**CD34**	**CD117**	**CD33**	**CD45**	
3	**CD13** *WM-47 (Dako, Alere)*	**CD11b** *ICRF44 (BD Pharmingen)*	**HLADR** *L243 (BD)*	**CD117**	**CD14** MoP9 *(BD)*	**CD45**	
4	CD38	CD7 *M-T701 (BD)*	CD34	CD117	CD19 *SJ25C1 (BD)*	CD45	
5	**CD38** *HB7 (BD)*	**CD56** *MY31 (BD)*	**CD34**	**CD117**	**CD33**	**CD45**	**CD7** *M-T701 (BD)* **or CD19** *SJ25C1 (BD)*

Table 2B MFC-LSC Antibody Panel

FITC	*PE*	*PerCP*	*PECy7*	*APC*	*APC H7*	*Horizon V450*
CD45RA *HI 1000 (BD)*	**CD117** *1042D2 (BD)*	**CD34** *8G12 (BD)*	**CD123** *7G3(BD)*	**CD38** *HB7 (BD)*	**CD45** *2D1 (BD)*	**CD19** *SJ25C1 (BD)*

BD – Becton Dickinson Biosciences, Oxford, United Kingdom
BD Pharmingen – Becton Dickinson Biosciences - Pharmingen, Oxford, United Kingdom
Dako from Alere Ltd, Stockport, UK

Chimerism analysis: In this study analysis of $CD3^+$ T-lymphocyte chimerism was reported. To obtain purified populations of T-lymphocytes, $CD3^+$ cells were separated from density gradient separated peripheral blood and /or bone marrow mononuclear cells using MACS (Miltenyi Biotec). On FACScan analysis, greater than 95% of cells thus isolated expressed CD3. For sex-matched allografts, DNA was extracted from cell suspensions. The degree of donor/host chimerism was determined by multiplex PCR of microsatellite markers by applying 5 fluorescently labelled primer pairs for the loci MBP (A and B), FGA, D18S391, D18S386 and D13S634. Two microlitres of PCR product was loaded onto a 6% ployacrylamide gel on an ABI-373 gene scanner. Relative heights of donor and host cells in the sample were calculated based on the peak heights and areas of informative alleles (assay sensitivity 1%). Fluorescence in situ hybridization (FISH) was used to monitor chimerism in sex-mismatched allografts. In brief, cell suspensions were fixed using 3:1 ratio methanol:acetic acid fixative and the level of donor/host chimerism was determined by analysis of 250 interphase cells using Vysis CEPXY probe specific for the X centromere and Y heterochromatin (assay sensitivity 1%). Full donor chimerism (FDC) was defined as the presence of >98% cells of donor origin. A lower proportion of donor cells in the allograft recipient was referred to as mixed chimerism (MC).

Statistical Methods:

The prognostic value of MFC-MRD and MFC-LSC positivity was assessed comparing the outcome of those patients in morphological remission who were MRD positive with those without evidence of residual disease. Morphological remission was defined by the local investigator in accredited laboratories. Outcome measures assessed were overall survival (OS) measured from date of HCT until death; relapse free survival (RFS) measured from date of HCT until relapse or death and cumulative incidence of relapse (CIR) measured from date of HCT until relapse, with death as a competing risk; all surviving patients, event free, were censored at the date last known to be alive. Follow-up was complete until September 2013. The Kaplan-Meier method was used to estimate survival probabilities and Cox proportional hazards regression for multivariable analyses. CIR was calculated treating death as a competing risk, however for multivariate analyses, Cox proportional hazard model was applied treating deaths as censored to focus on the underlying hazard of relapse. Following the recommendations of the International Working Group[4] survival outcomes were compared between MFC-MRD (pos vs neg) and MFC-LSC (pos vs neg) using the log rank test and multivariable models adjusting for the following additional known prognostic factors of HCT; cytogenetic risk (adverse vs favourable/intermediate) (as defined by Grimwade et al[5, 6]), disease status (CR vs not CR) and donor type (related vs unrelated). Comparisons of baseline demographics were performed using the Pearson's chi-squared test for categorical

data and two-sample t-tests for continuous variables. All effect sizes are given with 95% confidence intervals (CI), with P<0.05 deemed statistically significant. All statistical analyses were performed using STATA 12 or SAS 9.2.

References

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Supplementary-Results Table 1. Summarised Early Outcomes according to MFC-MRD and MFC-LSC status pre-HCT

	CIR		RFS		OS	
	1-Year CIR (from HCT) % (95% CI)	Unadjusted HR, 95% CI; p-value	1-Year RFS (from HCT) % (95% CI)	Unadjusted HR, 95% CI; p-value	1-Year OS (from HCT) % (95% CI)	Unadjusted HR, 95% CI; p-value
Patient group						
sample≤60 days pre HCT						
(N=59)						
MFC- MRD-	13 (0.04, 0.28)	1	66 (0.48, 0.81)	1	74 (0.54, 0.87)	1
MFC- MRD+	55 (0.33, 0.73)	3.86 (1.47, 10.1) P=0.006	33 (0.15, 0.51)	2.27 (1.08, 4.79) P=0.0308	48 (0.26, 0.67)	2.00 (0.90, 4.44) P=NS
(N=65)						
MFC-LSC-	19 (0.10, 0.33)	1	60 (0.45, 0.72)	1	66 (0.5, 0.77)	1
MFC-LSC+	72 (0.29, 0.91)	11.90 (3.93, 35.98) P<0.0001	10 (0.006, 0.36)	5.84 (2.5, 13.6) P<0.0001	46 (0.16, 0.72)	3.39 (1.36, 8.46) P=0.0088
sample ≤90 days pre HCT						

(N=66)	MFC- MRD-	13 (0.04, 0.27)	1	68 (0.49, 0.82)	1	75 (0.56, 0.87)	1
	MFC- MRD+	44 (0.26, 0.60)	2.79 (1.07, 7.27); *P*=0.036	46 (0.28, 0.62)	1.77 (0.85, 3.68); *P*=NS	59 (0.39, 0.75)	1.57 (0.72, 3.42); *P*=NS
(N=72)	MFC-LSC-	19 (0.09, 0.30)	1	63 (0.48, 0.74)	1	70 (0.56, 0.81)	1
	MFC-LSC+	56 (0.25, 0.78)	5.45 (2.16,13.74); *P*=0.001	22 (0.06, 0.47)	3.44 (1.65, 7.16); *P*=0.001	51 (0.23, 0.73)	2.34 (1.03, 5.34); *P*=0.0415
RIC patients	MFC- MRD-	12 (0.03, 0.27)	1	72 (0.51, 0.86)	1	80 (0.58, 0.91)	1
(N=49)	MFC- MRD+	47 (0.24, 0.67)	2.94 (1.00, 8.64); *P*=0.049	44 (0.22, 0.63)	2.16 (0.92, 5.06); *P*=NS	60 (0.35, 0.78)	2.06 (0.82, 5.15); *P*=NS
(N=51)	MFC-LSC-	16 (0.06, 0.30)	1	66 (0.48, 0.79)	1	74 (0.56, 0.85)	1
	MFC-LSC+	67 (0.12, 0.92)	16.84 (3.98,71.16); *P*=0.0001	-	10.07 (3.66,27.74); *P*<0.001	32 (0.06, 0.63)	4.95 (1.87,13.08); *P*=0.0013
CR1 patients	MFC- MRD-	15 (0.05, 0.31)	1	71 (0.50, 0.84)	1	78 (0.57, 0.90)	1
(N=50) (42 CR1 8 CR1i)	MFC- MRD+	39 (0.18, 0.60)	2.07 (0.72, 5.98); *P*=NS	46 (0.23, 0.66)	1.64 (0.70, 3.88); *P*=NS	58(0.33, 0.77)	1.63 (0.66, 4.027); *P*=NS
(N=52) (43 CR1	MFC-LSC-	20 (0.09, 0.33)	1	61 (0.44, 0.74)	1	68(0.51, 0.80)	1
9 CR1i)	MFC-LSC+	50 (0.11, 0.80)	4.61 (1.38,15.41); *P*=0.0129	28 (0.04, 0.59)	3.19 (1.23, 8.26); *P*=0.0172	53(0.18, 0.80)	2.42 (0.88, 6.68); *P*=NS

Abbreviations: HCT, hematopoietic cell transplantation; RIC, Reduced intensity conditioning; CR1, patients achieving CR after course 1; CR1i, patients achieving CR after course 1 with incomplete count recovery.

MFC-LSC, immunophenotypic leukemic stem cell population; MFC-MRD, standard flow cytometric for MRD.

Supplementary-Results Table 2.

Multivariable Cox Regression model for patients with pretransplant bone marrows within 60 days (n=52)

Variable		Overall Survival Events (n=24)	Relapse Free Survival Events (n=27)	Relapse Rate (CIR) Events (n=18)
Cyto Risk	**Favourable / intermediate**	**1 (Reference)**	1 (Reference)	1 (Reference)
	Adverse	**3.52 (1.22-10.42) p=0.02**	2.22(0.82-6.0) p=NS	2.6 (0.82-5.98) p=NS
Disease status	CR	1 (Reference)	1 (Reference)	1 (Reference)
	Not CR	2.01 (0.18-22.0) p=NS	1.32 (0.19-8.91) p=NS	1.27 (0.194-8.90) p=NS
Donor type	Related	1 (Reference)	1 (Reference)	1 (Reference)
	Unrelated	1.42 (0.58-3.51) p=NS	1.27 (0.53-3.04) p=NS	0.99 (0.529-3.036) p=NS
MRD Pre-SCT	Neg	1 (Reference)	**1 (Reference)**	**1 (Reference)**
	Pos	1.84 (0.78-4.36) p=NS	**2.29 (1.01-5.22) p<0.05**	**4.42(1.01-5.22) p<0.05**
MFC-LSC Pre-SCT	Neg	1 (Reference)	**1 (Reference)**	**1 (Reference)**
	Pos	1.37 (0.40-4.76)p=NS	**3.13 (1.03-9.44) p<0.05**	**6.62 (1.03-9.44) p<0.05**

Significant variables in bold

Supplementary-Results Table 3. MRD (MFC-MRD) and MFC-LSC status post-HCT with other disease markers

Parameter	**MFC-MRD post HCT** (n =69)		**MFC-LSC post HCT (n=92)**		All (N =101)
	MRD+ n=23	**MRD-** n=46	MFC-LSC+ n=16	MFC-LSC- n=76	
Disease status pre HCT					
Not in CR	4	1	4	3	7
Pre HCT MRD+	14	14	10	21	33
Pre HCT MRD-	6	19	4	24	33
Pre HCT MFC-LSC detected	8	5	6	8	15
Pre HCT MFC-LSC not detected	12	27	8	42	57
Routine cytogenetics pre HCT					
Normal karyotype	13	29	10	45	61
Abnormal karyotype	3	6	3	7	10
Missing or inadequate data	7	11	3	24	30
Molecular marker positive pre HCT *(FLT3 ITD, NPM1, JAK2, CBF or NUP98-NSD1* mutant)	4	5	4	6	12
Disease status post HCT					
Routine cytogenetics post HCT					
abnormal karyotype detected in MRD sample- (none detected pre MRD)	5	0	3	1	5 (+ 10 at time of morphological relapse)
Molecular marker detected in MRD sample post SCT *(FLT3 ITD, NPM1, JAK2, CBF or NUP98-NSD1* mutant)	5	1	3	3	6
Post HCT MFC-LSC detected	15	0	NA	NA	16
Post HCT MFC-LSC not detected	7	44	NA	NA	76
Chimerism status day 90 post SCT					
Full donor (myeloablative /reduced intensity)	11 (6/5)	28 (8/20)	6 (2/4)	45 (12/33)	57 (18/39)
Mixed (myeloablative /reduced intensity)	10 (3/7)	17 (1/16)	8 (2/6)	27 (4/23)	37(6/31)
Full recipient	0	0	0	0	0
No data	2	1	1	4	7
Decreasing chimerism pre MRD+	8	NA	5	NA	8
Acute GVHD (grade 2-4)	6 (26%)	13 (28%)	5 (31%)	20 (26%)	28 (28%)

DLI adminstered	3	6	2	6	10

Abbreviations: MRD, minimal residual disease (by MFC-); AML, acute myeloid leukemia; HCT, hematopoietic cell transplantation; ANC, absolute neutrophil count; MFC-LSC, immunophenotypic leukemic stem cell population; DLI, donor lymphocyte infusion

Supplementary-Results Table 4: Detailed Results of Relapsed Patients

Abbreviations:
FDC = Full donor chimerism MC=Mixed chimerism (d=days, m=months)

○ MFC-MRD negative ◔ MFC- MRD positive<0.1% ◑ MFC- MRD positive (0.1-0.5%) ◕ MFC- MRD positive (0.5-1%) ● MFC- MRD positive >1%

IS= Inadequate sample NL= No MFC- LAIP

+=MFC-LSC (LMPP-like) positive - = MFC-LSC (LMPP-like) negative **AbCy**=Abnormal Cytogenetics pre HCT **NCy**=Normal cytogenetics pre HCT

R = Frank relapse/refractory **CyR**=Cytogenetic Relapse R_f =flow sample received at morphologicarelapse

D= Death in remission **DLI**= Donor lymphocyte infusion

Age	Transplant type	Cytogenetics	Status pre	30-90d pre	<30d	Tx	Post 2m	3m	4m	5m	6m	7m	8m	9m	10m	11m	12m	13m	Later
54	RIC Related	Normal	CR1	◔-			MC 95% ○-			MC 93% ○-				MC 92% ○-			MC 93% ◑+ CyR		DLI D_{3y}
60	RIC Unrelated	Normal	CR1		◔-			FDC ◑+		FDC R_f		D							
51	RIC Unrelated	t3:5 FLT3+ NPM1+	CR1	◑- FLT3 neg			MC96% ◑- FLT3+ NPM1+		R_f	D									

66	RIC Related	Normal	CR1	●	◔-			FDC ◑+			MC 96% ◕+		MC 92%		MC 78% R_f			D	
63	RIC Unrelated	Trisomy 21	CR2	NL-				FDC IS-			MC 95% NL-			R_f			D		
50	RIC Unrelated	Complex	CR1	○+				MC 82% ◑+		R_f					D				
56	RIC Unrelated	Normal	Refractory		R			FDC IS-			FDC IS-				R_f		D		
60	RIC Unrelated	t18:21 JAK2+	CR1		○- **JAK2+**			FDC			FDC		FDC			●+		R	D_{14m}
51	RIC Related	Normal FLT3+ NPM1+	CR1	◔- **FLT3 neg**				MC 70% ◔+			MC 34% R_f **FLT3+ NPM1+**					D			
46	RIC Unrelated	Normal FLT3 +	CR1		○ **FLT3 neg**			MC 73% ●+ **FLT3+**		MC 54% R_f									
47	RIC Unrelated	Monosomy 7	Refractory	R+ **AbCy**			MC 7% R_f	D											

44	RIC Unrelated	Normal FLT3 +	CR1	**FLT3 neg**				MC 82% ○-		MC 8%		R_f **FLT3+**		D				
57	RIC Related	Complex including 5q- and 7q-	CR1		○+					MC 91%	IS		MC 82% ◑ **CyR**					
68	RIC Unrelated	t12:22	CR1		◑+ **AbCy**			FDC ○-				R_f						
59	RIC Unrelated	3q abn	Refrac tory		R+ **AbCy**			FDC ●+ **CyR**	MC 88% R_f									
61	RIC Unrelated	Normal	CR1	○-			IS-	FDC			FDC						FDC		R 19m
41	MA Related	7- and 3abn FLT3+	CR2	○ **AbCy**	●+		MC 34% R_f	D										
49	MA Related	Normal FLT3+	CR1		◑+ **FLT3 neg**		FDC 98% ●+	MC 93% R_f **CyR**			D							
28	MA Unrelated	Trisomy 8 t5:15	CR1						MC 92% ●- **CyR FLT3+**		D							

19	MA Unrelated	t5:11 FLT3+ NUP98/NSD 1+	CR1	◔- **NUP+**				MC 91% ●+ **NUP+**	MC 21% R_f						D				
21	MA Related	MLL rearranged	CR1	◔-					FDC ◔-						R		D		
41	MA Unrelated	Complex	CR1		IS-			FDC ○-			FDC ◑+				MC 39%	R_f			
44	MA Unrelated	t6:11 FLT3 +	CR1	○- **FLT3 neg**				FDC				FDC ●+ **CyR**	**DLI**		FDC R_f D				
23	MA Unrelated	Normal FLT3+	Refractory		R+ **FLT3+**			FDC ○+ **FLT3+**		◑+			R_f						
40	MA Unrelated	Normal NPM1 +	CR2					NL-											R_{3y}

Supplementary-Results Table 5: Detailed Results of Non Relapsed Patients

Abbreviations:
FDC = Full donor chimerism MC=Mixed chimerism (d=days, m=months)

○ MFC-MRD negative ◔ MFC- MRD positive<0.1% ◑ MFC- MRD positive (0.1-0.5%) ◕ MFC- MRD positive (0.5-1%) ● MFC- MRD positive >1%

IS= Inadequate sample NL= No MFC- LAIP

+=MFC-LSC (LMPP-like) positive - = MFC-LSC (LMPP-like) negative **AbCy**=Abnormal Cytogenetics pre HCT **NCy**=Normal cytogenetics pre HCT

R = Frank relapse/refractory **CyR**=Cytogenetic Relapse Rf =flow sample received at relapse

D= Death in remission **DLI**= Donor lymphocyte infusion

Age	Transplant type	Cytogenetics	Status pre	30-90d pre	<30d	Tx	Post 2m	3m	4m	5m	6m	7m	8m	9m	10m	11m	12m	Later
66	RIC Unrelated	Normal	CR3					FDC IS-										
61	RIC Related	Normal (FLT3 wt)	CR1	IS-			FDC		FDC ○-	D								
55	RIC Unrelated	Normal (FLT3 wt)	CR2	NL-				MC 93% IS						MC 82% NL-				
65	RIC Unrelated	Trisomy 8 FLT3 wt	CR1	IS	**N Cy**			FDC ○-			FDC			FDC				FDC 2y
57	RIC Unrelated	Normal	CR1					FDC IS-				FDC						
34	RIC Related	Complex FLT3 +	Refractory	R				FDC IS			FDC IS -				FDC ○-			D2y

37	RIC Related	Trisomy 8 and 12p-	CR1		○-		IS	FDC			FDC IS						
52	RIC Unrelated	Normal	CR1					FDC IS-			MC 92% ○-		○-				
62	RIC Unrelated	Inv 16 (CBFB/MYH11)	CR2	◑- **CBF neg**				FDC ○-			FDC						FDC	
51	RIC Related	Trisomy 11 and 13	CR2		**N Cy**			FDC IS-			FDC						FDC	
64	RIC Unrelated	t11:19	CR1		○-			MC 71% ○-			MC 32%				MC 54%	**DLI**	FDC	
66	RIC Unrelated	Normal	CR1		○			FDC ○-			FDC						FDC	
62	RIC Unrelated	Normal FLT3+	CR1	○- **FLT3 neg**				FDC ○-			FDC						FDC	
59	RIC Related	Normal	CR1		○-			○-	MC 96%			MC 92% ○-					MC 55% ○-	**DLI 16m**
55	RIC Related	Normal	CR2		○-			MC 91% ○-				MC 92% ○-			MC 92% ○-		○-	
66	RIC Unrelated	Normal FLT3 +	CR1		○-		IS-	FDC				FDC						
65	RIC Related	Normal FLT3+	CR1		○- **FLT3 neg**			MC 83% ○-	MC 68%								MC 71%	
59	RIC Unrelated	Normal	CR3					NL-	MC 97%		D							

61	RIC Unrelated	5q-	CR1	● AbCy				FDC ○-	MC 66%		○-					**DLI**	MC 96%	**DLI 14m**
57	RIC Unrelated	Normal	CR2						NL+								NL+	
48	RIC Related	Normal FLT3 + NPM1+	CR1		○- **FLT3 neg**			MC 81% ○-		MC 49%			MC 76%		○-		MC 90% ○-	
61	RIC Related	Normal FLT3+	CR1		NL- **FLT3 neg**			MC 92% IS-			MC 84%			MC 90%		D		
70	RIC Unrelated	Normal	CR1	NL+				MC 93% NL-	D									
51	RIC Related	Normal FLT3+ NPM1+	CR1	IS-				FDC NL-			FDC							
65	RIC Unrelated	Normal	CR1		○-			MC 82%	○-		MC 72%				MC 60%			
60	RIC Unrelated	Normal FLT3+ NPM1+	CR1		● **FLT3 neg**			FDC			FDC NL-							
64	RIC Unrelated	Normal FLT3+ NPM1+	CR1	○-				FDC	D									
56	RIC Related	Normal	CR1	NL				MC 68% NL-	MC 49%						MC 57%			**DLI 2y**
53	RIC Unrelated	No data	CR2					MC 94% IS-			MC 87% ○		NL		MC 93%			14m ○-

67	RIC Unrelated	Normal FLT3+	CR1		NL+ **FLT3+**		NL⁻	FDC			MC 86% NL⁻		**D**				
58	RIC Unrelated	Monosomy 7 and 21	CR1	◑- **N Cy**	◔-			FDC ○-		IS⁻	FDC						**D** (12m)
70	RIC Unrelated	Inv16 CBFB/MYH11	CR2		○- **CBF+**			FDC ○- **CBF neg**			FDC						
53	RIC Related	Normal FLT3+ NPM1+	CR1	○- **FLT3 neg NPM1 neg**				MC 20% IS⁻			MC 30%						
49	RIC Related	No data	CR1		○-			○	MC 60%		MC 60%	MC 63%					**DLI**
56	RIC Related	Normal	CR1 (but dysplasia)	●				MC 72% ◑-				MC81% ○-					
61	RIC Unrelated	Trisomy 13	CR1	IS				MC 63% ○-			MC 48%		**DLI**	MC 33%		**DLI**		FDC 1y
47	RIC Unrelated	Normal	CR2					FDC ○-			FDC			FDC			
44	RIC Unrelated	Normal FLT3+	CR1		○- **FLT3 neg**			FDC ◔-			MC 97%						
42	RIC Unrelated	Normal	CR1	○-				FDC ○-			MC 95%						

67	RIC Unrelated	Normal	CR1					FDC NL-			FDC							
50	RIC Related	Trisomy 11	CR2		◑-			FDC ○-			FDC							
64	RIC Unrelated	Normal	CR1					FDC ○-			FDC							
62	RIC Related	Normal FLT3+ NPM1+	CR1	○- FLT3 neg				MC 97% ○-			MC 84%		MC 25%				**DLI**	
61	RIC Unrelated	Trisomy 13	CR1	◑- Ab Cy				FDC ○-			FDC	○-			○-			
54	RIC Unrelated	Isodisomy 13 FLT3 + NPM1 +	CR1		◑+ FLT3+ NPM1+		FDC ○-					**NPM1+**	D					

61	RIC Unrelated	t1:3 FLT3+	CR1		○-			FDC ○-		D								
54	RIC Unrelated	Trisomy 8	CR2	○-				MC 90% ○-										
62	RIC Unrelated	Normal FLT3+ NPM1+	CR1		○-		**NPM1 neg**	FDC ○- **NPM1 neg**										
64	RIC Unrelated	Normal NPM1+	CR1				◑-	MC 78% ◔-	◔- **NPM1+**					**DLI**			○- **NPM1 neg**	

63	RIC Unrelated	Complex FLT3+	CR1		◔− AbCy			FDC ○−										
69	RIC Unrelated	Del 21q (Loss of RUNX1)	CR1		○− N Cy			MC 68% ○−										
56	RIC Related	Normal NPM1+	CR1					○−										
53	RIC Related	Normal	CR1		◑−			MC 92% ○−	MC 68%									

47	RIC Related	Complex including 7q-	CR1		◔+ AbCy				MC 86% ○−									
55	RIC Unrelated	Normal	CR2		NL−			IS										
47	MA Related	T(3;12) EVI1/ETV6	CR1		◔− AbCy PCR+			FDC	IS−		FDC						FDC	
42	MA Unrelated	t12:17 FLT3+	CR1	IS− N Cy FLT3 neg				FDC ○−							○−			
29	MA Unrelated	Normal	CR2	◑−				FDC			FDC						FDC	
21	MA Related	7-	CR1	◑+ N Cy				FDC ◑−			FDC						FDC	
26	MA Related	Inv 16 (CBFB/MYH1)	CR2	IS CBF neg				FDC ○−			FDC					CBF neg	FDC	

								CBF neg										
27	MA Related	t8:21 (RUNx1/1T1)	CR2		**CBF +**			MC 97% IS**-** **CBF neg**			FDC IS**-** **CBF neg**	D						
35	MA Unrelated	Normal FLT3 TKD+ NPM1+	CR2		NL**-**			MC 91% NL**-**										

39	MA Related	Normal	CR1	NL**-**				FDC NL**-**			FDC						FDC	
22	MA Unrelated	Inv 16	CR2	○**-** **CBF+**			D											
34	MA Unrelated	Complex	CR1	◑**-** **N Cy**				FDC IS				D						
18	MA Unrelated	5q- and near tetraploid	CR1	◔**-** **N Cy**				FDC ○**-**										
23	MA Unrelated	Complex	CR2					NL**-**										
44	MA Related	Normal	CR1	○**-**				D										
24	MA Related	Inv 16 and FLT3+	CR2	**CBF+**				FDC ○**-** **CBF neg**										
46	MA Related	Inv 16	CR2	**CBF+**				FDC ○ **CBF**										

								neg										
18	MA Unrelated	Normal FLT3+	CR1	○-				FDC ○-										
44	MA Unrelated	Monosomy 7 FLT3+	CR1		○+			FDC ○-										

44	MA Related	Normal FLT3+	CR1		○- **FLT3 neg**			FDC ○-						FDC		**FLT3 neg**		
37	MA Unrelated	Normal Biallelic CEBPA	CR1		◑- **CEBPA neg**			FDC ◔-			FDC						FDC	
20	MA Related	MLL rearranged 11q deletion	CR2	R+	Morph CR **NCy**			MC 91% ○-						FDC				
26	MA Related	Normal FLT3+	CR2	○- **FLT3 neg**				FDC NL- **FLT3 neg**			FDC						FDC	

Comparative analysis of pretransplant MFC-MRD and MFC-LSC levels in CRi patients versus non CRi patients

MFC-MRD

Statistic	CRi	Not CRi
N	15	46
Mean	0.23	0.105
SD	0.66	0.24
Median	0.0009	0.01
Range	0, 2.6	0, 1.4
IQR	0, 0.15	0, 0.12

P value of 0.8384 using Wilcoxon non parametric test to assess the difference in MFC-MRD level between groups suggesting no significant difference between the CRi and Not CRi groups. (CRi=1 for CRi patients and CRi=0 for Not CRi patients).

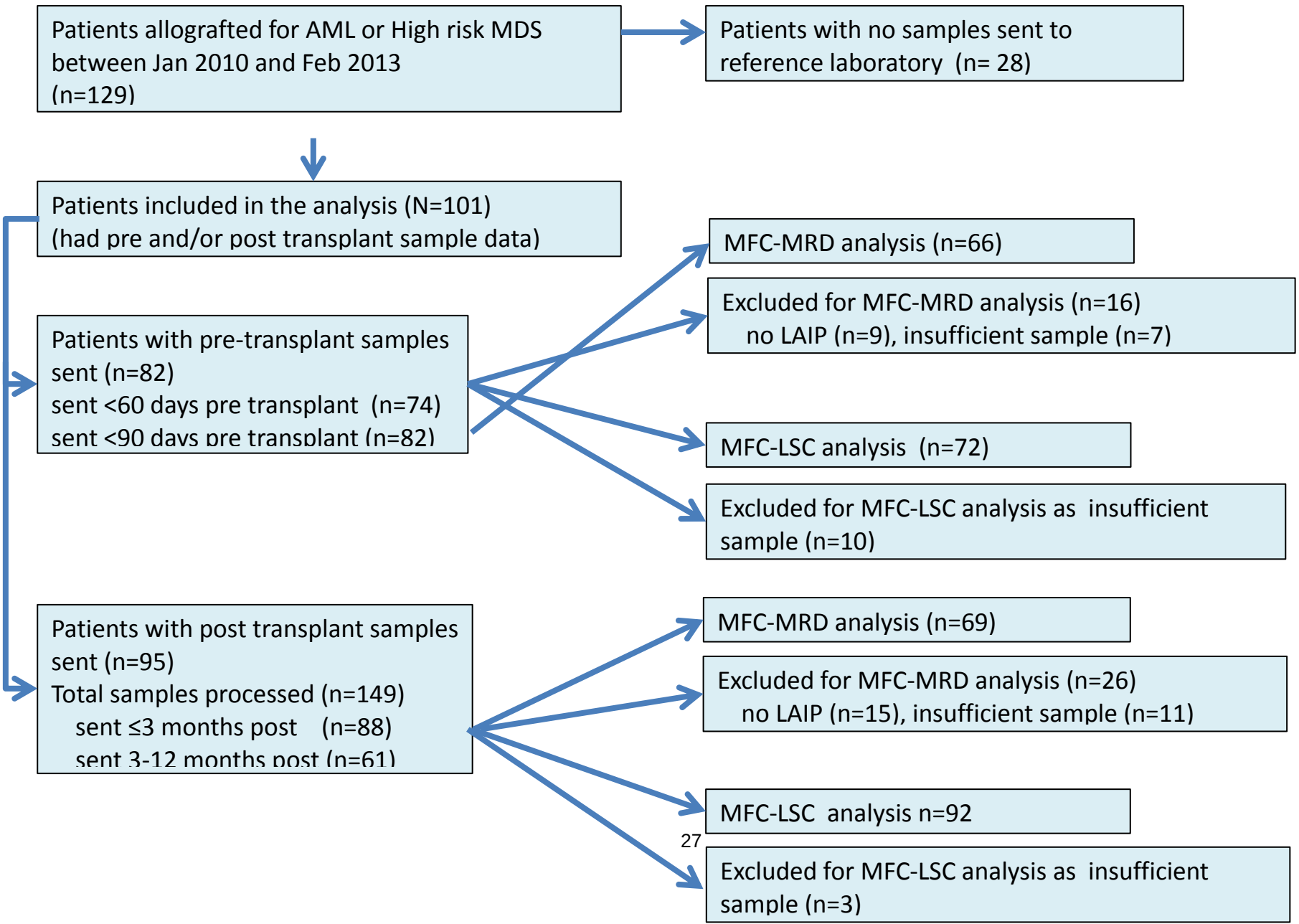
MFC-LSC

Statistic	CRi	Not CRi
N	16	50
Mean	0.112	0.0436
SD	0.31	0.16
Median	0.01	0.01
Range	0, 1.23	0, 1.0
IQR	0, 0.045	0, 0.02

P-value of 0.9372 using Wilcoxon non parametric test to assess the difference in MFC-MRD level between groups suggesting no significant difference between the CRi and Not CRi groups. (CRi=1 for CRi patients and CRi=0 for Not CRi patients).

However for both these analyses an effect of sample hemodilution on MFC-MRD and MFC-LSC cannot be excluded.

Supplementary Figure 1: Outline of Study patients with samples pre- and post- HCT analysed for either standard flow cytometric detection (MFC-MRD) or by immunophenotypic assay of LSC populations (MFC-LSC); LAIP, leukemia-associated immunophenotype



Supplementary Figure 2: Strategy for immunophenotyping analysis of marrow stem /progenitor populations

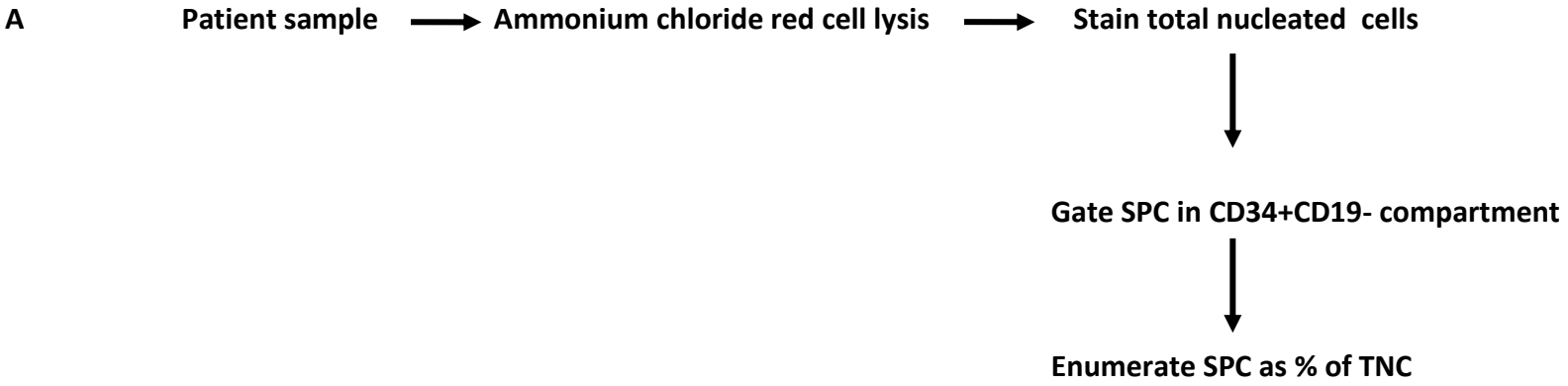
(A) Schematic representation of how control samples and AML study samples were processed to quantitate marrow stem/progenitor populations (SPC) with immunophenotyping panel (MFC-LSC Antibody panel -Supplementary Table 2B).
MFC-LSC populations (MFC-LSC) were detected by an abnormal increase in LMPP-like SPC (ie > 0.02% of TNC)
(0.02 =mean+1.96xSD of control samples in this study as previously)

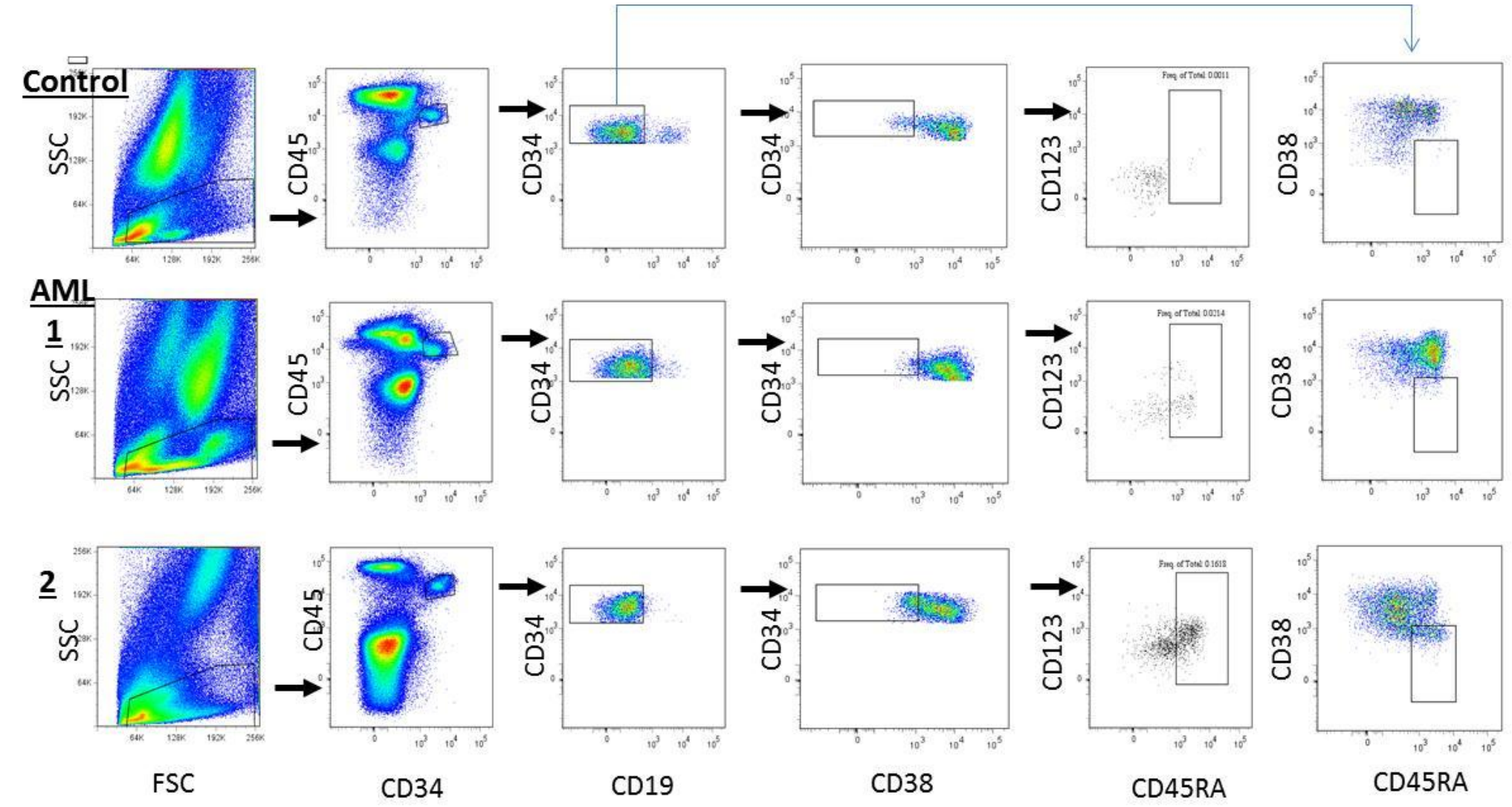
(B) Representative set of plots showing gating strategy to enumerate LMPP-like populations (defined as CD34+CD19-CD38lowCD45RA+) .
(i) monuclear gate applied to ungated cells (FSC/SSC) *(ii)* CD34+ gating (CD34/CD45) *(iii)* CD34+CD19- gating (CD34/CD19)
(iv) CD34+CD19-CD38low gating (CD34/CD38) ***(v)*** **LMPP gate** (CD45RA+) applied to CD34+CD19-CD38low (+CD123 expression)
(vi) LMPP gate (CD38lowCD45RA+) check by applying to CD34+CD19- population

Control – example of control sample
AML -example of AML patient monitoring samples (1 & 2).
Both AML patient samples had expanded LMPP-like SPC and therefore were MFC-LSC –positive.

(C) The % of LMPP-like cells within marrow TNC in each of 23 controls stained and analysed with MFC-LSC antibody panel confirming threshold of > 0.02% of TNC (mean+ 1.96xSD) established previously (Craddock et al 2013).

Supplementary Figure 2:




B
(i)
(ii)
(iii)
(iv)
(v)
(vi)
Control
AML
1
2
SSC
CD45
CD34
CD123
CD38
Freq. of Total 0.0011
Freq. of Total 0.0214
Freq. of Total 0.1613
FSC
CD34
CD19
CD38
CD45RA
CD45RA

C

Sample	MFC-LMPP
N1	0.005
N2	0.016
N3	0.005
N4	0.010
N5	0.002
N6	0.004
N7	0.002
N8	0.002
N9	0.002
N10	0.024
N11	0.009
N12	0.010
N13	0.003
N14	0.013
N15	0.018
N16	0.008
N17	0.008
N18	0.014
N19	0.001
N20	0.007
N21	0.001
N22	0.002
N23	0.001
Mean **± SD**	0.007 ±0.006
Mean + **1.96xSD**	0.0188

Supplementary Figure 3: Example of MFC-LSC analysis applied to a patient with no prior diagnostic flow cytometric data but post course 1 and post course 2 samples.

Standard MFC-MRD was applied. Although post course 1 and course 2 there were a few blasts (defined by defined by gating using CD34+ /CD117+ /CD45/ SSC / FSC parameters) with an aberrant phenotype of CD7+CD33+, this was below the detection threshold particularly without any diagnostic LAIP data. However this LAIP emerged at relapse 7 months later. There was no other LAIP detected.

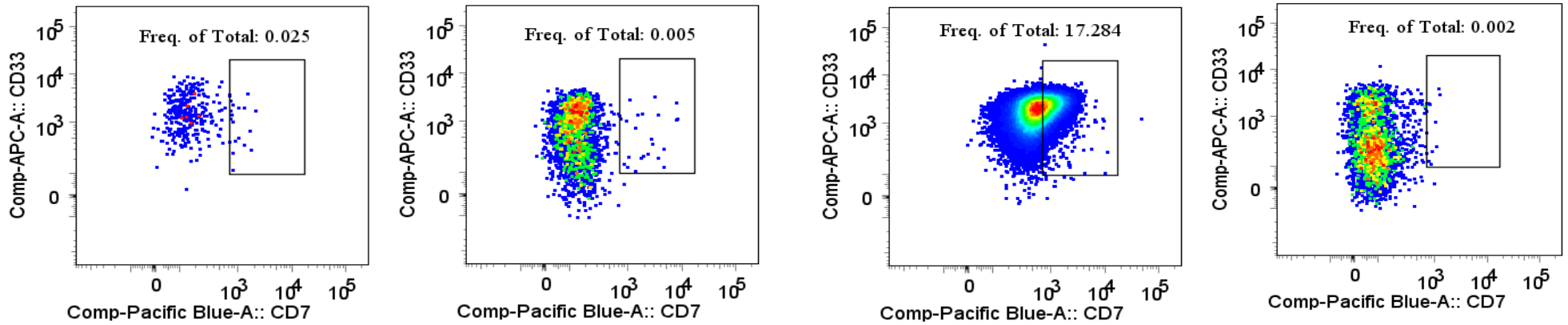
MFC-LSC were also monitored in this patient (by gating strategy in Supplementary Figure 1, detection threshold 0.02%). MFC-LSC plots are of CD34+CD19-CD38- SPCs with LMPP-gate (CD45RA+ / CD123) applied. Although post course 1 patient was MFC-LSC-negative, post course 2 there was a clear MFC-LSC population (0.059%) as well as other CD45RA- CD34+CD38-SPC populations including some CD45- cells with high CD123. MFC-LSC-positivity preceded relapse by 7 months. Interestingly, the LMPP-like MFC-LSC were the only CD34+CD38- SPC population at relapse.

Supplementary Figure 3:

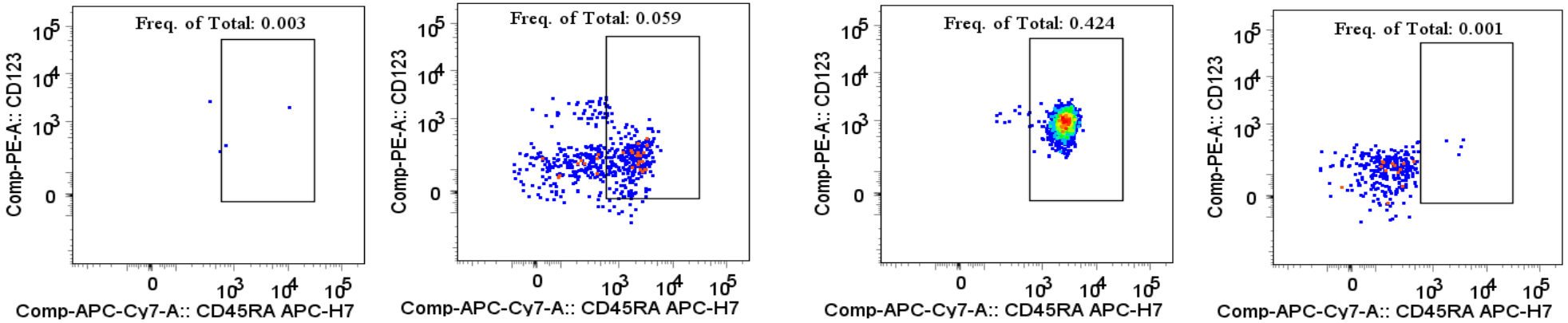
AML Patient **Control**

Post course 1 Post course 2 Relapse 7 months later

LAIP CD7+CD33+ (LAIP gate applied to plots of CD34+CD117+ blasts)

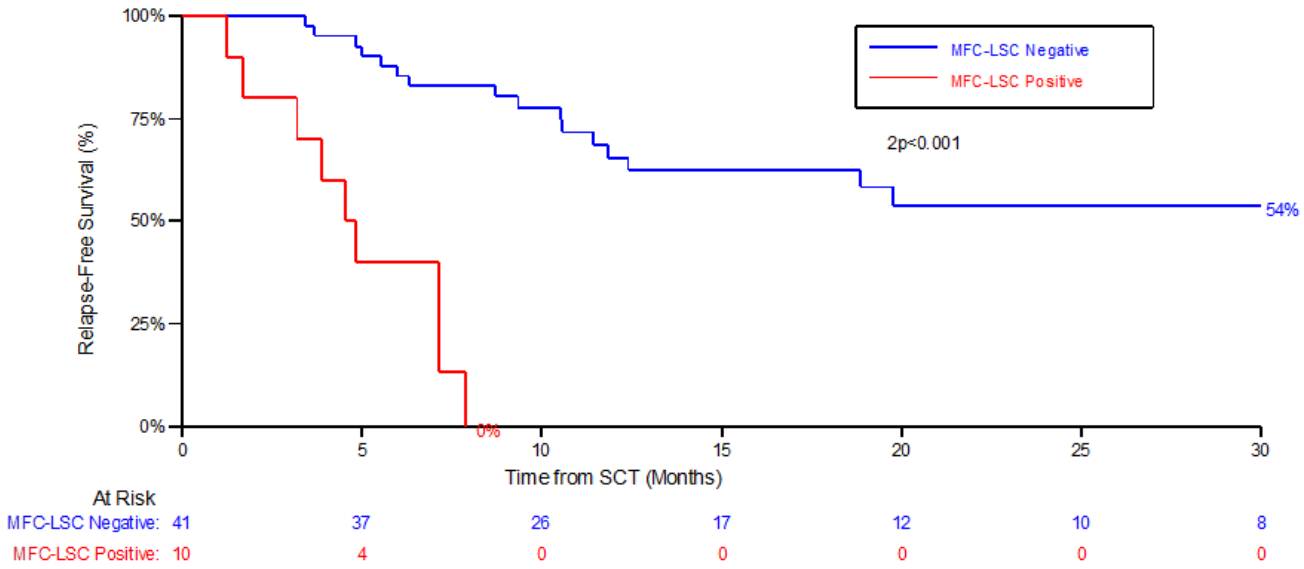


MFC-LSC LMPP gate (CD45RA+ /CD123) applied to plots of CD34+CD19-CD38low gated cells

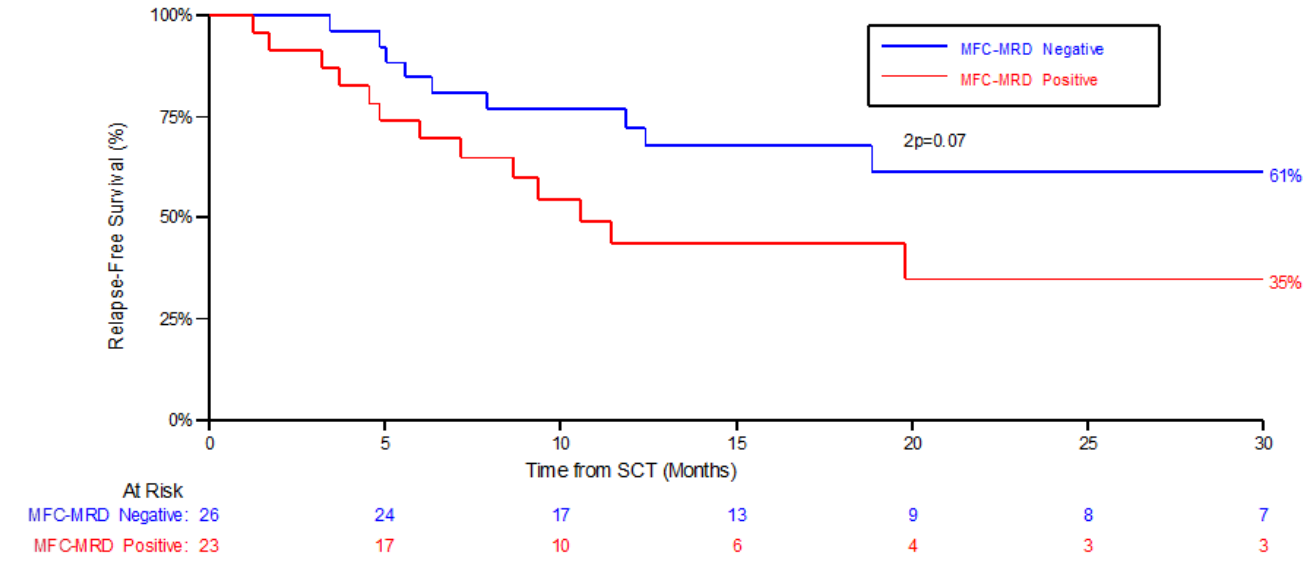


Supplementary Figure 4: Relapse-Free-Survival (RFS) in RIC patients only according to pre-HCT residual disease status by either **A:** immunophenotypic assay of LSC populations (MFC-LSC) or **B:** standard flow cytometric detection (MFC-MRD)

A:

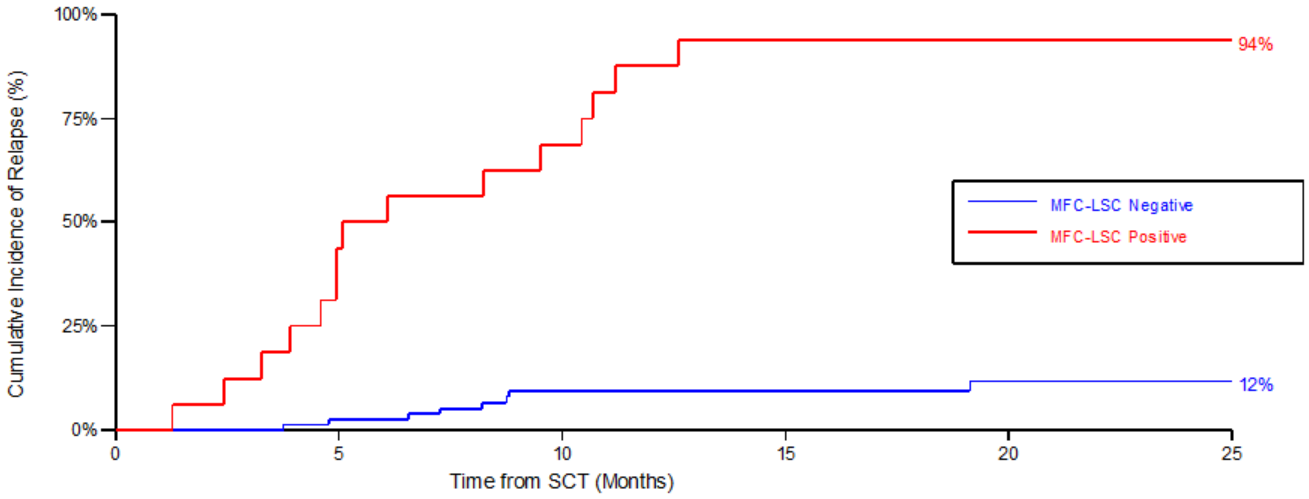


B:



Supplementary Figure 5: Cumulative incidence of relapse (CIR) according to post-HCT residual disease status (at any time point) by either **A:** immunophenotypic assay of LSC populations (MFC-LSC) or **B:** standard flow cytometric detection (MFC-MRD)

A:



B: