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.
| TRANSPLANT CONDITIONING | REGIME | PATIENT NUMBERS |
|-------------------------|--------|-----------------|
| Cyclo/TBI               | Myeloablative | **Cyclophosphamide 60mg/kg/day x 2 days** |
|                        | TBI 14.4Gy in 8 fractions | 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)** | 60 |
|                        | **Melphalan 140mg/m2/day for 1 day** | |
|                        | **(d-1)** | |
|                        | **Alemtuzumab 10mg od IV for 5 days** | |
|                        | **(d-7 to d-3)** | |
| Flu/Bu/Campath         | Reduced intensity | **Fludarabine 30mg/m2/day for 5 days** |
|                        | **(d-7 to d-3)** | 2 |
|                        | **Busulphan 3.2mg/kg/day for 2 days** | |
|                        | **(d-5 and d-4)** | |
|                        | **Alemtuzumab 30mg od IV for 2 days** | |
|                        | **(d-7 to d-3)** | |
| FLAMSA                 | Reduced intensity | **Fludarabine (30mg/m2)** |
|                        | **(d-12 to d-9)** | 2 |
|                        | **High-dose AraC (2g/m2)** | |
|                        | **(d-12 to d-9)** | |
|                        | **Amsacrine (100mg/m2)** | |
|                        | **(d-12 to d-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)** | |
| 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)** | |
| 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)** | |
**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 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 subset2, 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/inaидеquate 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 CD34+/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 | CD13| CD34  | CD117 | CD33 | CD45   | L243 (BD)    |
|          |       | L138| 8G12  | 1042D2| P67.6| 2D1    | (BD)         |
| 2        | CD38  | CD56| CD34  | CD117 | CD33 | CD45   | HB7 (BD)     |
|          |       | MY31|       |       |      |        | (BD)         |
| 3        | CD13  | CD11b| HLADR | CD117 | CD14 | CD45   | WM-47 (Dako, Alere) |
|          |       | L243|       |       |      |        | (BD)         |
| 4        | CD38  | CD7 | CD34  | CD117 | CD19 | CD45   | M-T701 (BD) |
|          |       | M-T701| |       | SJ25C1|        | (BD)         |
| 5        | CD38  | CD56| CD34  | CD117 | CD33 | CD45   | HB7 (BD)     |
|          |       | MY31|       |       |      |        | (BD)         |

Table 2B MFC-LSC Antibody Panel

| FITC  | PE  | PerCP | PECy7 | APC  | APC H7 | Horizon V450 |
|-------|-----|-------|-------|------|--------|--------------|
| CD45RA| CD117| CD34  | CD123 | CD38 | CD45   | CD19         |
| HI 1000 (BD) | 1042D2 (BD) | 8G12 (BD) | 7G3/BD | HB7 (BD) | 2D1 (BD) | 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% polyacrylamide 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 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 al5, 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

| Patient group | 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 |
|---------------|-----------------------------------|--------------------------------|-----------------------------------|--------------------------------|---------------------------------|--------------------------------|
| **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          |
| **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 ≤60 days pre HCT

(N=59)

sample ≤90 days pre HCT

(N=65)
|                  | MFC- MRD-     | MFC- MRD+    | MFC-LSC- | MFC-LSC+    |
|------------------|---------------|--------------|----------|-------------|
| (N=66)           |               |              |          |             |
| MFC- MRD-        | 13 (0.04, 0.27)| 44 (0.26, 0.60)|          |             |
|                  | 1             | 68 (0.49, 0.82)| 1        | 75 (0.56, 0.87)|
|                  |               | 46 (0.28, 0.62)| 1.77 (0.85, 3.68); | 59 (0.39, 0.75)|
|                  |               | 1           | P=NS     | 1.57 (0.72, 3.42); | P=NS |
|                  |               |             |          |             |
| (N=72)           |               |              |          |             |
| MFC- MRD+        | 19 (0.09, 0.30)| 56 (0.25, 0.78)|          |             |
|                  | 1             | 63 (0.48, 0.74)| 1        | 70 (0.56, 0.81)|
|                  |               | 22 (0.06, 0.47)| 3.44 (1.65, 7.16); | 51 (0.23, 0.73)|
|                  |               |             | P=0.001  | 2.34 (1.03, 5.34); | P=0.0415 |
| MFC-LSC-         |               |              |          |             |
| MFC-LSC+         | 56 (0.25, 0.78)| 44 (0.26, 0.60)|          |             |
|                  | 2.79 (1.07, 7.27); | 46 (0.28, 0.62)| 1.77 (0.85, 3.68); | 59 (0.39, 0.75)|
|                  | P=0.036       | P=NS        | P=NS     | 1.57 (0.72, 3.42); | P=NS |
|                  |               |             |          |             |
| RIC patients     |               |              |          |             |
| (N=49)           |               |              |          |             |
| MFC- MRD-        | 12 (0.03, 0.27)| 47 (0.24, 0.67)|          |             |
|                  | 1             | 72 (0.51, 0.86)| 1        | 80 (0.58, 0.91)|
|                  |               | 44 (0.22, 0.63)| 2.16 (0.92, 5.06); | 60 (0.35, 0.78)|
|                  |               |             | P=NS     | 2.06 (0.82, 5.15); | P=NS |
| (N=51)           |               |              |          |             |
| MFC-LSC-         | 16 (0.06, 0.30)| 67 (0.12, 0.92)|          |             |
| MFC-LSC+         |              | 16.84 (3.98,71.16); | 1        | 74 (0.56, 0.85)|
|                  |               | P=0.001     | 10.07 (3.66,27.74); | 32 (0.06, 0.63)|
|                  |               |             | P<0.001  | 4.95 (1.87,13.08); | P=0.0013 |
| CR1 patients     |               |              |          |             |
| (N=50)           |               |              |          |             |
| (42 CR1 8 CR1i)  | MFC- MRD-     | 15 (0.05, 0.31)|          |             |
|                  | 1             | 71 (0.50, 0.84)| 1        | 78 (0.57, 0.90)|
|                  |               | 46 (0.23, 0.66)| 1.64 (0.70, 3.88); | 58(0.33, 0.77)|
|                  |               |             | P=NS     | 1.63 (0.66, 4.027); | P=NS |
|                  |               |             |          |             |
| (N=52)           |               |              |          |             |
| (43 CR1 9 CR1i)  | MFC- MRD-     | 39 (0.18, 0.60)|          |             |
|                  | 1             | 61 (0.44, 0.74)| 1        | 68(0.51, 0.80)|
|                  |               | 28 (0.04, 0.59)| 3.19 (1.23, 8.26); | 53(0.18, 0.80)|
|                  |               |             | P=0.0129 | 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**    | 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             |
|                  | **Favourable / intermediate**  | **1 (Reference)**                  | **1 (Reference)**                |
|                  | **Adverse**                    | **3.52 (1.22-10.42) p=0.02**       | **2.6 (0.82-5.98) p=NS**          |
| **Disease status**| 1 (Reference)                  | 1 (Reference)                      | 1 (Reference)                    |
|                  | **CR**                         | **2.01 (0.18-22.0) p=NS**          | **1.27 (0.19-8.90) p=NS**         |
|                  | **Not CR**                     | **2.01 (0.18-22.0) p=NS**          | **1.27 (0.19-8.90) p=NS**         |
| **Donor type**   | 1 (Reference)                  | 1 (Reference)                      | 1 (Reference)                    |
|                  | **Related**                    | **1.42 (0.58-3.51) p=NS**          | **0.99 (0.529-3.036) p=NS**       |
|                  | **Unrelated**                  | **1.42 (0.58-3.51) p=NS**          | **0.99 (0.529-3.036) p=NS**       |
| **MRD Pre-SCT**  | 1 (Reference)                  | 1 (Reference)                      | 1 (Reference)                    |
|                  | **Neg**                        | **1.84 (0.78-4.36) p=NS**          | **4.42(1.01-5.22) p<0.05**        |
|                  | **Pos**                        | **1.84 (0.78-4.36) p=NS**          | **4.42(1.01-5.22) p<0.05**        |
| **MFC-LSC Pre-SCT**| 1 (Reference)                  | 1 (Reference)                      | 1 (Reference)                    |
|                  | **Neg**                        | **1.37 (0.40-4.76)p=NS**           | **6.62 (1.03-9.44) p<0.05**       |
|                  | **Pos**                        | **1.37 (0.40-4.76)p=NS**           | **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-                           | 5                        | 0                        | 3             | 1             | 5             |
|    (none detected pre MRD)                                               |                          |                          |              | (+ 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%)      |
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
- IS = Inadequate sample
- NL = No MFC-LAIP
- AbCy = Abnormal Cytogenetics pre HCT
- NCy = Normal cytogenetics pre HCT
- R = Frank relapse/refractory
- CyR = Cytogenetic Relapse
- Rf = flow sample received at morphologic relapse
- D = Death in remission
- DLI = Donor lymphocyte infusion

| Age | Transplant type | Cytogenetics | Status pre | 30-90d pre | 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% | DLI D3y |
| 60  | RIC Unrelated  | Normal       | CR1        | ⇠          | FDC  ◐■ | FDC  Rf | D     |     |     |     |     |     |     |     |     |      |
| 51  | RIC Unrelated  | L3:5 FLT3+ NPM1+ | CR1 | ⇠          | Rf | D     |     |     |     |     |     |     |     |     |     |      |
| RIC   | Related | Normal | CR1   | FDC  | MC  | MC  | MC  | MC  | RF | D   |
|-------|---------|--------|-------|------|-----|-----|-----|-----|----|-----|
| 66    | Related | Normal | ☜      | ☜+   | ☜+ | ☜+ | ☜+ | ☜+ |    | D   |
| 63    | Unrelated | Trisomy 21 | CR2   | NL-  | FDC | IS- | MC 95% | Rf | D   |
| 50    | Unrelated | Complex | CR1   | ☜+   | MC 82% | ☜+ | Rf |    | D   |
| 56    | Unrelated | Normal | Refractory | R    | FDC | IS- | FDC IS- | Rf | D   |
| 60    | Unrelated | t18:21 JAK2+ | CR1   | ☜- JAK2+ | FDC | FDC | FDC | ☜+ | R  | D   |
| 51    | Related | Normal FLT3+ NPM1+ | CR1   | ☜- FLT3 neg | MC 70% | ☜+ | MC 34% | Rf |    | D   |
| 46    | Unrelated | Normal FLT3+ | CR1   | ☜ FLT3 neg | MC 73% | ☜+ | MC 54% | Rf |    | D   |
| 47    | Unrelated | Monosomy 7 | Refractory | R+ AbCy | MC 7% | Rf |    | D   |
|   | RIC          |                  | CR1 | FLT3       | MC 82% | MC 8% | Rf  | D           |
|---|--------------|------------------|-----|------------|--------|-------|-----|-------------|
| 44| RIC Unrelated| Normal FLT3 +    | CR1 | FLT3 neg   | O-     |       |     |             |
| 57| RIC Related  | Complex including 5q- and 7q- | CR1 | O+         | MC 91% | IS    | MC 82% |             |
| 68| RIC Unrelated| t12:22           | CR1 | AbCy       | FDC O- |       |     | Rf          |
| 59| RIC Unrelated| 3q abn Refractory|     | R+         | FDC    | MC 88% | Rf  |             |
| 61| RIC Unrelated| Normal           | CR1 | O-         | IS=    | FDC   | FDC | R 19m       |
| 41| MA Related   | 7- and 3abn FLT3+| CR2 | AbCy       | MC 34% | D     |     |             |
| 49| MA Related   | Normal FLT3+     | CR1 | FLT3 neg  | FDC 98%| MC 93%| D   |             |
| 28| MA Unrelated | Trisomy 8 t5:15  | CR1 | CyR        | MC 92% | D     |     |             |
| MA Unrelated | t5:11 FLT3+ NUP98/NSD1+ | CR1 | NUP+ | MC 91% | MC 21% RF | D |
| MA Related | MLL rearranged | CR1 | NUP+ | FDC | R F D |
| MA Unrelated | Complex | CR1 | IS- | FDC | FDC RF |
| MA Unrelated | t6:11 FLT3+ | CR1 | FLT3 neg | FDC | FDC RF DLI |
| MA Unrelated | Normal FLT3+ | Refractory | R+ FLT3+ | FDC | RF |
| MA Unrelated | Normal NPM1+ | CR2 | NL- | RF | R3y |
**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 | T x | 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- | FDC |     |    |    |    |    |    |     |     |     |      |
| 65  | RIC Unrelated  | Trisomy 8 FLT3 wt | CR1 | IS | NCy | FDC | FDC | FDC | FDC | FDC 2y |      |     |     |     |     |     |      |
| 57  | RIC Unrelated  | Normal       | CR1        |            |      |     |        | FDC| IS-|    |    |    |    |    |     |     |     |      |
| 34  | RIC Related    | Complex FLT3 + Refractory | R |      |        | FDC | FDC | FDC IS | FDC IS |      | D 2y |     |     |     |     |     |      |
|   | RIC | Trisomy 8 and 12p- | CR1 | CR2 | CR3 | MC (%) | FDC |
|---|-----|-------------------|-----|-----|-----|--------|-----|
| 37 | Related | Trisomy 8 and 12p- | CR1 | IS | FDC | FDC | IS |
| 52 | Unrelated | Normal | CR1 | FDC | IS | MC 92% | O- |
| 62 | Unrelated | Inv 16 (CBFβ/MYH11) | CR1 | IS | FDC | FDC | FDC |
| 51 | Related | Trisomy 11 and 13 | CR2 | N Cy | FDC | FDC | FDC |
| 64 | Unrelated | t11:19 | CR1 | O- | MC 71% | MC 32% | MC 54% | DLI | FDC |
| 66 | Unrelated | Normal | CR1 | O | FDC | FDC | FDC |
| 62 | Unrelated | Normal FLT3+ | CR1 | O- | FDC | FDC | FDC |
| 59 | Related | Normal | CR1 | O- | MC 96% | MC 92% | MC 55% | DLI 16m |
| 55 | Related | Normal | CR2 | O- | MC 91% | MC 92% | MC 92% | O- |
| 66 | Unrelated | Normal FLT3+ | CR1 | O- | IS | FDC | FDC |
| 65 | Related | Normal FLT3+ | CR1 | O- | MC 83% | MC 71% |
| 59 | Unrelated | Normal | CR3 | NL | MC 97% | D |
| RIC    | Transplant Type | FDC | CR1        | MC | CR2        | DLI     | MC |
|--------|----------------|-----|------------|----|------------|---------|----|
| 61     | Unrelated      |     | AbCy       |    |            |         |    |
|        | 5q−            |     |            |    |            |         |    |
| 57     | Unrelated      |     | NL+        |    |            |         |    |
| 48     | Related        |     | ○− FLT3 neg|    | ○−         |         |    |
| 61     | Related        |     | NL− FLT3 neg|    |            |         |    |
| 70     | Unrelated      |     | NL+        |    | D          |         |    |
| 51     | Related        |     | IS−        |    | FDC        | FDC     |    |
| 65     | Unrelated      |     | ○−         |    |            |         |    |
| 60     | Unrelated      |     | ○−         |    | D          |         |    |
| 64     | Unrelated      |     | ○−         |    | FDC        | D       |    |
| 56     | Related        |     | NL         |    |            | DLI 2y  |    |
| 53     | Unrelated      |     |            |    |            | 14m     |    |

**Notes:**
- AbCy: Antibody Cytoplasmic
- FDC: Flow Distribution
- MC: Myeloid Count
- DLI: Donor Lymphocyte Infusion
- D: Death
- NL: Normal
- IS: Internally Stained
- DLI 14m: Donor Lymphocyte Infusion 14 months
- DLI 2y: Donor Lymphocyte Infusion 2 years
|   | RIC     | Status         | CR1                  | CR2                  | MC 60% | MC 63% | DLI | DLI | FDC 1y |
|---|---------|----------------|----------------------|----------------------|--------|--------|--|--|--|--|
| 67| RIC Unrelated | Normal FLT3+  | CR1                  | NL+ FLT3+            | FDC    |        | D  |     |       |
| 58| RIC Unrelated | Monosomy 7 and 21 | CR1                  | NL-                 | FDC    | IS-    | D  |     |       |
| 70| RIC Unrelated | Inv16 CBF/MYH11 | CR2                  | FLT3 neg CBF+       | FDC    |        | D  |     |       |
| 53| RIC Related  | Normal FLT3+ NPM1+ | CR1                  | NL-                 | MC 20% | IS-    |    |     |       |
| 49| RIC Related  | No data        | CR1                  | NL-                 | MC 60% | MC 63% | DLI|     |       |
| 56| RIC Related  | Normal (but dysplasia) | CR1                  | NL-                 | MC 72% |       |    |     |       |
| 61| RIC Unrelated | Trisomy 13     | CR1                  | IS                  | MC 63% | MC 48% | DLI|     |       |
| 47| RIC Unrelated | Normal         | CR2                  | FDC                 |        | FDC    |    |     |       |
| 44| RIC Unrelated | Normal FLT3+   | CR1                  | NL-                 | MC 97% |        |    |     |       |
| 42| RIC Unrelated | Normal         | CR1                  | NL-                 | MC 95% |        |    |     |       |
| RIC | Unrelated | Normal | CR1 | FDC | FDC | 67 |
|-----|-----------|--------|-----|-----|-----|----|
| Related | Trisomy 11 | CR2 | FDC | FDC | 50 |
| Unrelated | Normal | CR1 | FDC | FDC | 64 |
| Related | Normal FLT3+ NPM1+ | CR1 | FDC | FDC | 62 |
| Unrelated | Trisomy 13 | CR1 | FDC | FDC | 61 |
| Unrelated | Isodisomy 13 FLT3 + NPM1 + | CR1 | FDC | FDC | 54 |
| Related | t1:3 FLT3+ | CR1 | FDC | FDC | 61 |
| Unrelated | Trisomy 8 | CR2 | FDC | FDC | 54 |
| Unrelated | Normal FLT3+ NPM1+ | CR1 | FDC | FDC | 62 |
| Unrelated | Normal NPM1+ | CR1 | FDC | FDC | 64 |
|   | RIC       | Complex/Related   | CR1   | CR2   |
|---|-----------|------------------|-------|-------|
| 63| RIC       | Unrelated        | CR1   |       |
|   |           | Complex           |       |       |
|   |           | FLT3+             |       |       |
|   |           | AbCy              |       |       |
|   |           | FDC               |       |       |
| 69| RIC       | Unrelated        | CR1   |       |
|   |           | Del 21q           |       |       |
|   |           | (Loss of RUNX1)   |       |       |
|   |           | N Cy              |       |       |
|   |           | MC 68%            |       |       |
| 56| RIC       | Related           | CR1   |       |
|   |           | Normal            |       |       |
|   |           | NPM1+             |       |       |
| 53| RIC       | Related           | CR1   |       |
|   |           | Normal            |       |       |
|   |           | MC 92%            |       |       |
|   |           | MC 68%            |       |       |
| 47| RIC       | Related           | CR1   |       |
|   |           | Complex           |       |       |
|   |           | including 7q-     |       |       |
|   |           | AbCy              |       |       |
|   |           | MC 86%            |       |       |
| 55| RIC       | Unrelated        | CR2   |       |
|   |           | Normal            |       |       |
|   |           | NL-               |       |       |
| 47| MA        | Related           | CR1   |       |
|   |           | T(3;12)           |       |       |
|   |           | EVI1/ETV6         |       |       |
|   |           | IS-               |       |       |
| 42| MA        | Unrelated        | CR1   |       |
|   |           | t12:17            |       |       |
|   |           | FLT3+             |       |       |
|   |           | IS-               |       |       |
|   |           | N Cy              |       |       |
|   |           | FLT3 neg          |       |       |
|   |           | AbCy              |       |       |
|   |           | PCR+              |       |       |
|   |           | FDC               |       |       |
| 29| MA        | Unrelated        | CR2   |       |
|   |           | Normal            |       |       |
|   |           | IS-               |       |       |
|   |           | FDC               |       |       |
| 21| MA        | Related           | CR1   |       |
|   |           | 7-                |       |       |
|   |           | IS+               |       |       |
| 26| MA        | Related           | CR2   |       |
|   |           | Inv 16            |       |       |
|   |           | (CBFB/MYH1)       |       |       |
|   |           | IS                |       |       |
|   |           | CBF neg           |       |       |
|   |           | FDC               |       |       |
|   |           | CBF neg           |       |       |
|   |           | FDC               |       |       |
| MA     | Related/Unrelated | t(B:21) (RUNx1/1T1) | Related/Unrelated | t(8:21) | Unrelated | Complex | Complex | Complex | Complex |
|--------|-------------------|---------------------|-------------------|---------|-----------|---------|---------|---------|---------|
| 27     | Related           | CR2                 | CBF +             | MC 97%  | IS - CBF neg | FDC     | D       |         |         |
| 35     | Unrelated         | CR2                 | NL -              | MC 91%  | NL -      |         |         |         |         |
| 39     | Related           | Normal              | CR1               | NL -    | FDC       | FDC     | FDC     | FDC     | FDC     |
| 22     | Unrelated         | Inv 16              | CR2               | ○ - CBF+ | D         |         |         |         |         |
| 34     | Unrelated         | Complex             | CR1               | ○ - N Cy | FDC       | IS      | D       |         |         |
| 18     | Unrelated         | 5q- and near tetraploid | CR1         | ○ - N Cy | FDC       | ○ -     |         |         |         |
| 23     | Unrelated         | Complex             | CR2               | NL -    | D         |         |         |         |         |
| 44     | Related           | Normal              | CR1               | ○ -     | D         |         |         |         |         |
| 24     | Related           | Inv 16 and FLT3+    | CR2               | CBF+    | FDC       | ○ - CBF neg |         |         |
| 46     | Related           | Inv 16              | CR2               | CBF+    | FDC       | ○ CBF   |         |         |
|   | MA  | Unrelated | Normal FLT3+ | CR1 | neg | FDC |  
|---|-----|-----------|--------------|-----|-----|-----|  
| 18 |     |           |              |     |     |     |  
| 44 | MA  | Unrelated | Monosomy 7   | CR1 |     |     |  
| 44 | MA  | Related   | Normal FLT3+ | CR1 |     |     |  
| 37 | MA  | Unrelated | Normal       | CR1 |     |     |  
| 20 | MA  | Related   | MLL          | CR2 | R+  |     |  
| 26 | MA  | Related   | Normal FLT3+ | CR2 |     |     |  

- **neg**: Negative
- **○-**: Negative
- **○+:** Positive
- **FDC**: Follow-up for clinical decision
- **FLT3 neg**: FLT3 negative
- **CEBP A neg**: CEBP A negative
- **Morph**: Morphology
- **MC**: Myelocyte
- **91%**: Percentage
- **NL-FLT3 neg**: Normal FLT3 negative
- **R+**: Reverse signal

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### 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

Patients allografted for AML or High risk MDS between Jan 2010 and Feb 2013 (n=129)

Patients with no samples sent to reference laboratory (n=28)

Patients included in the analysis (N=101) (had pre and/or post transplant sample data)

MFC-MRD analysis (n=66)

Excluded for MFC-MRD analysis (n=16) no LAIP (n=9), insufficient sample (n=7)

MFC-LSC analysis (n=72)

Excluded for MFC-LSC analysis as insufficient sample (n=10)

Patients with pre-transplant samples sent (n=82)

Patients with no samples sent to reference laboratory (n=28)

Patients with post transplant samples sent (n=95)

Total samples processed (n=149) sent ≤3 months post (n=88)

Patients sent <60 days pre transplant (n=74)

Patients sent <90 days pre transplant (n=82)

MFC-MRD analysis (n=69)

Excluded for MFC-MRD analysis (n=26) no LAIP (n=15), insufficient sample (n=11)

MFC-LSC analysis n=92

Excluded for MFC-LSC analysis as insufficient sample (n=3)
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) mononuclear 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:

A Patient sample → Ammonium chloride red cell lysis → Stain total nucleated cells

Gate SPC in CD34+CD19- compartment

Enumerate SPC as % of TNC
| 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   | 0.007    |
| ± SD   | ±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 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**

|                  | Post course 1 | Post course 2 | Relapse 7 months later | Control |
|------------------|---------------|---------------|------------------------|---------|
| **LAIP CD7+CD33+** | Freq. of Total: 0.025 | Freq. of Total: 0.005 | Freq. of Total: 17.284 | Freq. of Total: 0.002 |
| **MFC-LSC LMPP gate (CD45RA+ /CD123)** | Freq. of Total: 0.003 | Freq. of Total: 0.059 | Freq. of Total: 0.424 | Freq. of Total: 0.001 |
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:

![Graph A: Cumulative incidence of relapse (CIR) according to post-HCT residual disease status (at any time point) by MFC-LSC](image1)

B:

![Graph B: Cumulative incidence of relapse (CIR) according to post-HCT residual disease status (at any time point) by MFC-MRD](image2)
