**SUPPLEMENTARY TABLES**

**Supplementary Table S1.** *de novo* AML patient characteristics.

| | ***All patients (n=71)*** | ***JAM-C Low patients (n=30)*** | ***JAM-C High patients (n=41)*** |
|---|---|---|---|
| **Gender, n (%)** | | | |
| Male | 40 (56.34%) | 17 (56.67%) | 23 (56.10%) |
| Female | 31 (43.66%) | 13 (43.33%) | 18 (43.90%) |
| **Age at diagnosis, median [min-max]** | 58 [20-81] | 60 [21-80] | 57 [20-81] |
| **FAB classification, n** | | | |
| M0 | 3 | 2 | 1 |
| M1 | 20 | 11 | 9 |
| M2 | 7 | 2 | 5 |
| M4 | 24 | 10 | 14 |
| M5 | 16 | 5 | 11 |
| M6 | 1 | 0 | 1 |
| **Cytogenetic prognosis, n** | | | |
| Favorable | 11 | 5 | 6 |
| Intermediate | 48 | 19 | 29 |
| Unfavorable | 12 | 6 | 6 |
| **Mutations, positive/total tested** | | | |
| NPM1 | 10/18 | 4/8 | 6/10 |
| FLT3-ITD | 13/34 | 8/16 | 5/18 |
| **WBC count x$10^9$/L at diagnosis, median [min-max]** | 73.2 [1.2-352.6] | 78.25 [16-352.6] | 73 [1.2-187] |
| **Platelet count x$10^9$/L at diagnosis, median [min-max]** | 70 [5-764] | 81 [11-764] | 62 [5-466] |
| **Complete Response, n (%)** | 66 (92.96%) | 27 (90%) | 39 (95.12%) |
| **Relapse, n (%)** | 40 (56.33%) | 14 (46.66%) | 26 (63.41%) |
| **Stem Cell transplantation, n (%)** | | | |
| Autologous | 12 (16.90%) | 4 (13.33%) | 8 (19.51%) |
| Allogenic | 16 (22.53%) | 8 (26.66%) | 8 (19.51%) |

**Supplementary Table S2.** JAM-C expression in monocytic AML.

| | *Classes* | *Statistics* | *All patients (n=71)* | *JAM-C High patients (n=41)* | *JAM-C Low patients (n=30)* | *p-value (Wilcoxon or Khi²)* |
|---|---|---|---|---|---|---|
| FAB classification | M0-M1-M2-M6 | n (%) | 31 (43.66) | 16 (39.02) | 15 (50.00) | 0.3570 |
| | M4-M5-M5a | n (%) | 40 (56.34) | 25 (60.98) | 15 (50.00) | |

**Supplementary Table S3.** Multivariate Cox regression models were used to assess the predictive value of JAM-C-expressing $CD45^{dim}CD34^{+}CD38^{low}CD123^{+}JAM\text{-}C^{Pos}$ cell frequencies.

| | **Multivariate for OS** | | **Multivariate for LFS** | |
|---|---|---|---|---|
| ***Variable*** | ***Hazard Ratio [CI 95%]*** | ***p-value*** | ***Hazard Ratio [CI 95%]*** | ***p-value*** |
| **Age at diagnosis** | | | | |
| > 65 vs ≤ 65 years | 3.39 [1.65-6.99] | 0.0009 | 2.79 [1.35-5.77] | 0.0057 |
| **WBC count at diagnosis** | | | | |
| > 50 vs ≤ 50G/L | 2.22 [1.08-4.58] | 0.0307 | 1.73 [0.87-3.44] | 0.1177 |
| **Cytogenetic prognosis** | | | | |
| Intermediate | 1.06 [0.38-2.92] | 0.9130 | 2.42 [0.70-8.32] | 0.1608 |
| Unfavorable | 2.28 [0.75-6.92] | 0.1441 | 3.20 [0.80-12.7] | 0.0991 |
| **% JAM-C expressing cells** | | | | |
| ***High vs Low*** | ***2.92 [1.40-6.08]*** | ***0.0043*** | ***2.60 [1.27-5.33]*** | ***0.0092*** |

**Supplementary Table S4.** Genes selected for single cell gene expression study.

| Genes | References | Genes | References |
|---|---|---|---|
| ***ACTB*** | Housekeeping gene | ***GORASP2*** | This study |
| ***AAED1*** | This study | ***GPR56*** | Eppert et al; Prashad et al; Ng et al |
| ***ADAM19*** | This study; Eppert et al; Ng et al | ***HLA-DM*** | This study; Eppert et al |
| ***ADRA2C*** | This study; Eppert et al | ***HLA-DPB1*** | This study; Eppert et al; Prashad et al |
| ***ALCAM*** | Eppert et al | ***HLA-DQB1*** | This study; Eppert et al; Prashad et al |
| ***AMACR*** | Eppert et al | ***HLA-DR*** | This study; Eppert et al |
| ***AMICA1*** | This study; Prashad et al; Barreyro et al | ***HOXA2*** | Eppert et al |
| ***AMOT*** | Eppert et al | ***HOXA3*** | This study; Eppert et al; Prashad et al |
| ***BACE2*** | This study; Eppert et al | ***HOXA5*** | This study; Eppert et al; Prashad et al; Ng et al |
| ***BMP1*** | Eppert et al | ***HOXB9*** | Eppert et al; Prashad et al |
| ***C1RL*** | Eppert et al | ***HPGD*** | This study; Eppert et al |
| ***CCL3*** | This study; Eppert et al | ***HPRT1*** | Housekeeping gene |
| ***CD123*** | Marker for identification of subpopulation | ***HSPB1*** | This study; Eppert et al; Prashad et al |
| ***CD226*** | This study; Eppert et al | ***HTRA3*** | This study |
| ***CD244*** | Eppert et al | ***ID3*** | This study; Eppert et al; Prashad et al |
| ***CD300A*** | Eppert et al | ***IL13RA1*** | This study; Eppert et al |
| ***CD302*** | Eppert et al | ***IL15RA*** | This study; Eppert et al |
| ***CD34*** | Marker for identification of subpopulation | ***IL23A*** | Eppert et al |
| ***CD38*** | Marker for identification of subpopulation | ***IQGAP2*** | Eppert et al |
| ***CD47*** | Eppert et al | ***ITGA4*** | Eppert et al |
| ***CD59*** | Eppert et al; Prashad et al | ***ITGA6*** | Eppert et al; Prashad et al |
| ***CDC45*** | This study; Eppert et al; Barreyro et al | ***JAM2*** | This study |
| ***CDKN1A*** | Eppert et al | ***JAM3*** | Marker for identification of subpopulation |
| ***CDKN1C*** | Eppert et al | ***KIF21A*** | This study |
| ***CDKN2A*** | Eppert et al | ***KIT*** | Eppert et al |
| ***CDKN2D*** | Eppert et al | ***LGALS*** | This study; Eppert et al |
| ***CHRNA5*** | This study; Eppert et al; Prashad et al | ***LXN*** | This study; Eppert et al |
| ***CHRNA6*** | This study; Eppert et al | ***MAN1A*** | This study; Eppert et al |
| ***CIITA*** | Eppert et al; Prashad et al | ***MICA*** | Eppert et al |
| ***CLN3*** | Eppert et al | ***NCR3*** | Eppert et al |
| ***CLN5*** | Eppert et al | ***NPR3*** | Thi study ; Eppert et al; Prashad et al |
| ***CNKSR2*** | This study; Eppert et al | ***P2RX7*** | This study; Eppert et al |
| ***CORO2A*** | This study; Eppert et al | ***PAQR5*** | Eppert et al |
| ***CSF1*** | Eppert et al | ***PIK3R5*** | Eppert et al |
| ***CTNNAL1*** | This study; Eppert et al | ***PLA2G6*** | Eppert et al |
| ***CXCR4*** | Eppert et al; Ng et al | ***PMP22*** | Eppert et al |
| ***DNMT3B*** | Eppert et al; Ng et al | ***PTK7*** | This study |
| ***DOCK9*** | This study; Eppert et al | ***PTPRJ*** | This study; Eppert et al |
| ***DUSP5*** | Eppert et al | ***RNASE6*** | Eppert et al |
| ***ESAM*** | This study; Prashad et al | ***SLFN5*** | Prashad et al |
| ***ETS1*** | Eppert et al; Prashad et al | ***SOCS1*** | This study; Eppert et al |
| ***F11R*** | This study | ***SPAG1*** | This study; Eppert et al |
| ***FLT3*** | Eppert et al; Ng et al | ***THY1*** | Eppert et al; Prashad et al |
| ***FOSL1*** | Eppert et al | ***TMEM65*** | This study |
| ***FZD2*** | Eppert et al | ***TNF*** | Eppert et al |
| ***GAPDH*** | Housekeeping gene | ***TRIB2*** | This study; Eppert et al; Barreyro et al |
| ***GAS2L1*** | Eppert et al | ***UGT8*** | Eppert et al |
| ***GORASP1*** | This study | | |

**Supplementary Table S5.** Statistical analysis of genes shown in violin plots for patient #4.

| | pvalue | Ratio (PCA Cluster1/PCA Cluster2) |
|---|---|---|
| ***PTK7*** | $1.21 \times 10^{-15}$ | 13.176 |
| ***PMP22*** | $2.43^{-9}$ | 14.715 |
| ***ALCAM*** | $9.37^{-7}$ | 6.322 |
| ***F11R*** | $6.99^{-6}$ | 5.538 |
| ***ESAM*** | $1.11^{-5}$ | 11.280 |
| ***CD47*** | $6.31^{-7}$ | 6.494 |
| ***CD302*** | 0.0432 | 4.179 |
| ***ITGA6*** | 0.0001 | 3.488 |
| ***ITGA4*** | 0.0100 | 3.487 |
| ***CTNNAL1*** | 0.0071 | 6.347 |
| ***CLN3*** | $6.63^{-5}$ | 8.694 |
| ***HPGD*** | 0.0020 | 8.312 |
| ***CHRNA5*** | 0.0198 | 4.779 |

**Supplementary Table S6.** Limit Dilution Assay was performed by plating increasing numbers of indicated fraction of sorted leukemic cells from samples of patients #4, #7 and #8. The number of wells demonstrating the presence of at least one CAFC, as well as the number of wells that have been plated is indicated for each cell dose. LTC-IC frequency, as well as the upper and lower estimates for each sample is reported. ($\chi^2$ test 42.3 and $p=7.85 \times 10^{-11}$).

| Dose | Wells Tested | Wells with at least one colony | Group | Confidence intervals for 1/stem cell frequency [lower-upper] |
|---|---|---|---|---|
| 30 | 24 | 6 | ***$CD45^{dim}CD34^{+}CD38^{low}CD123^{+}CD41^{-}$ JAM-$C^{Neg}$*** | ***1/49.2 [68.8-35.2]*** |
| 15 | 36 | 4 | | |
| 10 | 36 | 8 | | |
| 5 | 60 | 11 | | |
| 1 | 36 | 7 | | |
| 30 | 24 | 14 | ***$CD45^{dim}CD34^{+}CD38^{low}CD123^{+}CD41^{-}$ JAM-$C^{Pos}$*** | ***1/14.9 [18.6-11.9]*** |
| 15 | 36 | 22 | | |
| 10 | 36 | 17 | | |
| 5 | 60 | 23 | | |
| 1 | 36 | 16 | | |

**Supplementary Table S7.** Limit Dilution Assay was performed by injecting increasing numbers of indicated fraction of sorted leukemic cells from sample of patient #8. The number of mice demonstrating the presence of at least 5% of human leukemic cells at time of harvest, as well as the number of transplanted mice is indicated for each cell dose. LIC frequency, as well as the upper and lower estimates for each sample is reported. ($\chi^2$ test 11.9 and p= 0.000558).

| Dose | Mice Tested | Mice Engrafted (in BM hCD45 cells>5%) | Group | Confidence intervals for 1/stem cell frequency [lower-upper] |
|---|---|---|---|---|
| 350 | 3 | 1 | ***Patient #8*** ***$CD45^{dim}CD34^{+}CD38^{low}CD123^{+}CD41^{-}$*** ***JAM-$C^{Neg}$*** | ***1/599.6*** ***[1907-188.8]*** |
| 200 | 3 | 1 | | |
| 100 | 3 | 0 | | |
| 50 | 3 | 1 | | |
| 10 | 2 | 0 | | |
| 350 | 3 | 3 | ***Patient #8*** ***$CD45^{dim}CD34^{+}CD38^{low}CD123^{+}CD41^{-}$*** ***JAM-$C^{Pos}$*** | ***1/62.9*** ***[151-26.4]*** |
| 200 | 3 | 3 | | |
| 100 | 2 | 1 | | |
| 50 | 3 | 1 | | |
| 10 | 3 | 2 | | |

**Supplementary Table S8.** Frequencies of human and mouse cells in the engrafted mice.

| Mice | | %hCD45 in BM | %mCD45 in BM |
|---|---|---|---|
| **KG1/JAM-C$^{Neg}$ into NSG wt :** | **1** | 0.0016 | 99.9984 |
| | **2** | 0.0008 | 99.9992 |
| | **3** | 0.0037 | 99.9963 |
| | **4** | 0.0080 | 99.9920 |
| | **5** | 0.0046 | 99.9954 |
| **KG1/JAM-C$^{Pos}$ into NSG wt :** | **1** | **0.8356** | 99.1644 |
| | **2** | **0.1149** | 99.8851 |
| | **3** | **0.0159** | 99.8410 |
| | **4** | **0.0507** | 99.9493 |
| | **5** | **0.1038** | 99.8962 |
| **KG1/ JAM-C$^{Pos}$ into NSG- JAMBKO :** | **1** | **0.0581** | 99.9419 |
| | **2** | 0.0004 | 99.9996 |
| | **3** | 0.0049 | 99.9951 |
| | **4** | **1.5218** | 98.4782 |
| | **5** | 0.0073 | 99.9927 |