Supplementary Material

**Supplemental Figure 1a. Distribution of CD34+/CD38-% population according to FAB subtype**

bxp FAB

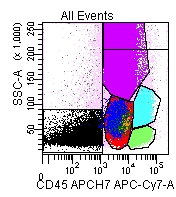
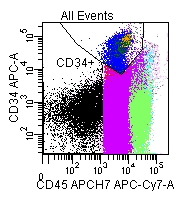
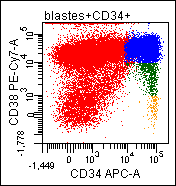
**Supplemental Figure 1b. CD34+CD38-% in relapsed vs no-relapsed and CR1 vs no-CR1 patients**

bxp CR

bxp rel

**Supplemental Figure 1c. Strategy of gating in blasts analysis and CD34+ subpopulations- Multicolour Flow cytometry in normal BM and four examples of AML**

Normal bone marrow

**0,01%**

**%CD34+CD38-**

**%CD34+CD38+lo**

**%CD34+CD38+hi**

**Populations Legend:**

**Red** : immature/blasts CD45+lo/SSClo cells ;

**Blue** : CD34+ cells ;

**Green**: Lymphocytes ;

**Blue cyan** : Monocytes ;

**Violet**: Granulocytes ;

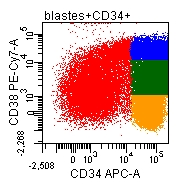
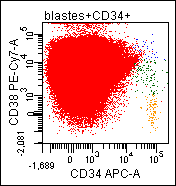
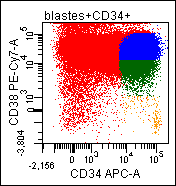
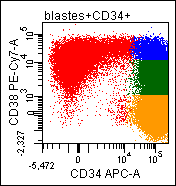
**Black :** CD45 negative cells (erythroblasts).

#3 AML NormalCaryotype, PCR neg

#2 AML NPM1+FLT3ITD-

#4 AML Cx Caryotype, duppMLL+

#1 AML patient NPM1-FLT3ITD+

**48%**

**0,3%**

**35%**

**0,5%**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | All patients | % CD34+CD38- of blasts>1% | % CD34+CD38- of blasts<1% | **p-value** |
| No of patients  (Adults vs children) | 200  (187 vs 13) | 98(49%)  (93 vs5) | 102(51%)  (94 vs 8) | p=0.67 |
| Male/Female,n | 117/83 | 57/41 | 59/43 | p=1 |
| Age at diagnosis, years, mean (range) | 56 (2-89) | 60 (4-87) | 53 (2-89) | **p=0.003** |
| WBC count at diagnosis,  10 9/L, mean (range) | 22,1  (0,5-233) | 19,9  (0,6-152) | 24,2  (0,5-233) | p=0.27 |
| AMLde novo (with MDS),n(%)  AML secondary,n(%)  AML de novo (w/o MDS),n | 56(28%)  64(32%)  80(40%) | 27(28%)  41(42%)  30(30%) | 29(28%)  23(23%)  50(49%) | p=0.19 |
| FAB classification, n  M0/M1/M2/M3/M4/M5/M6/M7/  Not classified | 17/31/31/10/  21/44/4/5/37 | 10/9/15/5/  6/20/1/4/28 | 7/22/16/5/  15/24/3/1/9 | **p=0.0008** |
| Treatment type,n(%)  Intensive chemotherapy  Alternative chemotherapy  Palliatives  APL 2006 | 124(62%)  29(15%)  37(18%)  10(5%) | 51(52%)  20(21%)  22(22%)  5(5%) | 73(72%)  9(9%)  15(15%)  5(5%) | **p=0.002** |
| Cytogenetic risk group,n(%)  Favourable  Intermediate 1  Intermediate 2  Unfavourable  Failed/ND | 190  14(7,4%)  31(16,4%)  58(30,7%)  76(40,2%)  11(5,5%) | 93  5(5%)  11(12%)  25(27%)  47(51%)  5(5%) | 97  9(9%)  20(21%)  33(34%)  29(30%)  6(6%) | **p=0.04** |
| Allogeneic - SCT, n(%) | 41(20,5%) | 21(21%) | 20(20%) | p=0.885 |
| NPM1+  FLT3ITD+  EVI1+ | 29(21,5%)  19(14,2%)  18(13,5%) | 5(8,3%)  8(13,3%)  10(17%) | 24(32%)  11(15%)  8(11%) | **p=0.002**  p=1  p=0.44 |

**Supplemental Table 1. Patient characteristics**

**MDS : myelodysplastic syndrome w/o : without ; SCT : stem cell transplantation ; WBC : white blood cell**

|  |  |  |
| --- | --- | --- |
|  | Median of  %CD34+CD38- in blasts | **p-value** |
| favourable | 0,68  [0,03-5,16] | **<0,001** |
| intermediate 1 | 0,19  [0,04-1,4] |
| intermediate 2 | 0,65  [0,01-56,8] |
| unfavourable | **2,9**  **[0,01-72,2]** |
| Normal karyotype but no molecular biology data | 1,5  [0,04-24,3] |
| NPM1+FLT3ITD-  16/135 | 0,19  [0,02-1,43] | **0,002** |
| NPM1+FLT3ITD+  13/135 | 0,65  [0,07-28,5] |
| NPM1-FLT3ITD+  6/135 | **1,66**  **[0,04-37,5]** |
| NPM1-FLT3ITD-  100/135 | 1,08  [0,01-72,2] |

**Supplemental Table 2. Univariate analyse of % CD34+CD38- levels in different cytogenetic and NPM1/FLT3ITD groups**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Relapsed Patients**  **# 33** | **Sex** | **Age** | **FAB** | **Cytogenetic**  **risk groups\*\*** | **karyotype** | **Molecular profile** | **NPM1/**  **FLT3ITD**  **Groups\*** | **%LSC inCD45 Blastes at diagnosis** | **%LSC inCD45 Blastes at relapse** |
| 1 | F | 59 | M2 | 1 | 45,X,-X,t(8;21)(q22;q22)[20]/46,XX[3] | Wt1+ | 4 | **5,16** | **6,86** |
| 2 | F | 50 | M2 | 1 | 46,XX,t(8;21)[20] | neg | 4 | **1,08** | **1,09** |
| 3 | M | 26 | M4éo | 1 | 46, XY,inv(16)(p13q22)[14]/ 46, XXY6] | Wt1+ | 4 | 0,56 | **3,40** |
| 4 | F | 52 | M4éo | 1 | 46,XX, inv(16)(p13q22) [10] / 46,XX [11]. | Wt1+ | 4 | 0,79 | **1,20** |
| 5 | M | 4 | M1 | 1 | 45,X,-Y,t(8;21)(q22;q22)[10]/ 87-91,XX,-Y,-Y,t(8;21)(q22;q22)x2[cp5]/ 46,XY[5] | Neg  AML1/ETO+ | 4 | **1,81** | **3,27** |
| 6 | F | 11 | M2 | 1 | 46,XX [28] [t(8 ;21) variant] | Neg  AML1/ETO+ | 4 | **3,70** | **3,80** |
| 7 | F | 25 | M1 | 2 | 46,XX[10] | Flt3 mut D835,Wt1RQ, ,NPM1+ | 1 | 0,04 | 0,05 |
| 8 | M | 69 | M(nc) | 3 | 46,XY[20] | neg | 4 | **7,05** | **8,10** |
| 9 | F | 57 | M1 | 3 | 46,XX[25] | Wt1+, Flt3mut+ | 4 | **1,43** | 0,30 |
| 10 | F | 60 | M1 | 3 | 46,XX[20] | neg | 4 | 0,10 | 0,20 |
| 11 | M | 60 | M2 | 3 | 46,XY,t(1;3)(p36;q21)[19]/46,XY[2] | Wt1RQ+ | 4 | **4,42** | **38,95** |
| 12 | F | 62 | M4 | 3 | 46,XX[20] | Wt1RQ+  Flt3RQ | 4 | 0,04 | 0,09 |
| 13 | M | 56 | M4 | 3 | 46,XY[20] | neg | 4 | 0,39 | **1,13** |
| 14 | M | 71 | M7 | 3 | 46,XY[12] | Wt1RQ | 4 | **21,70** | **32,90** |
| 15 | F | 55 | M5 | 3 | 49,XX,+4,+6,+8[20] | Hoxa9-RQ+ Flt3RQ. | 4 | 0,09 | 0,13 |
| 16 | F | 57 | M6 | 3 | echec | Wt1RQ | 4 | 0,16 | 0,33 |
| 17 | M | 65 | M5 | 3 | 46,XY[20] | neg | 4 | 0,08 | ND |
| 18 | F | 60 | M5 | 3 | 46,XX[20] | Flt3ITD+hoxa9+,WT1+ NMP+ | 2 | **28,50** | ND |
| 19 | F | 72 | M0 | 3 | 46,XX[18] | neg | 4 | **56,78** | ND |
| 20 | M | 33 | M(nc) | 5 | 46, XY[15] | ND | ND | **4,26** | **14,25** |
| 21 | F | 60 | M(nc) | 4 | 45,XX,-5,add(7)(q21)?der(7)t(5;7)(q13;q21),-12,del(15)(q?15q23),+mar[11]/ 45,idem,del(6)(q13q15)[9]/46,XX[8] | neg | 4 | 0,48 | **2,25** |
| 22 | M | 55 | M0 | 4 | 46,XY,20q-[15] | neg | 4 | **2,47** | **1,15** |
| 23 | M | 63 | M4 | 4 | 46,XY[20] | EVI1+(dg)/-(relapse) | 4 | **37,26** | **63,47** |
| 24 | M | 51 | M7 | 4 | 43,XY,add(5)(q15),-7,-11,dic(12;18)(p12;p12),-13, add(14)(p11),-15,-16,-17,-18,-21,+8mars[cp20] | Evi1RQ+ | 4 | **4,76** | **2,07** |
| 25 | M | 33 | M0 | 4 | 46,XY,del(5)(q14q34) ish(EGR1-),?t(12;21)(p12;q22)[3]/46,XY[3] | Wt1RQ+Flt3-RQ | 4 | **22,96** | **9,50** |
| 26 | M | 60 | M0 | 4 | 45,XY,-7[17]/45,idem,+mar[3] | Evi1RQ+ Wt1+ | 4 | **10,72** | **7,20** |
| 27 | F | 56 | M1 | 4 | 47,XX,+8,del(20)(q11q13)[20] | Flt3 ITD+. | 3 | **37,57** | **6,39** |
| 28 | M | 15 | M5 | 4 | 46,XY,del(7)(q11q32)[16] | FLT3ITD+ | 3 | **1,53** | 0,29 |
| 29 | M | 64 | M(nc) | 4 | 44,XY,+X,add(3)(q27),add(4)(q23),-6,del(6)(q23q25),-7,-12,del(13)(q14q22),add(15)(p11),i(21)(q10)[19]/46,XY[2] | Evi1+, JAK2- | 4 | 0,62 | ND |
| 30 | M | 70 | M2 | 4 | 48,XY,-1,del(6)(q21q24),+8,+9,-13,-15,+3mars[5]/46,XY[15] | neg | 4 | **6,53** | ND |
| 31 | M | 44 | M5 | 4 | 48,XY,del(1)(?q22q23),dup(1)(q12q21),add(2)(q35),+13,+13[6]/ 96,idemX2,del(5)(q23q31),-7,del(10)(q22q23),-14,-15,+19,+20, +mar(?der(7))[cp10]/ 46,XY[4] | Flt3-RQ+ | 4 | 0,86 | ND |
| 32 | F | 60 | M5 | 4 | 51,XX,+X,+5,+8,+10,+13[15]/46,XX[5] | Flt3 ITD+ Wt1+, Hoxa9+, NPM1+ | 2 | 0,99 | **ND** |
| 33 | M | 56 | M5 | 5 | 46,XY[20] | ND | ND | 0,04 | **0,12** |

**Supplemental Table 3. Relapsed patient’s characteristics**

**\*NPM1/FLT3ITD Groups: 1) NPM1+/FLT3ITD-; 2) NPM1+/FLT3ITD+; 3) NPM1-/FLT3ITD+; 4) NPM1-/FLT3ITD-**

**\*\*Cytogenetic Risk Groups: 1) Favourable; 2) Intermediate1; 3) Intermediate2; 4) Unfavourable; 5)Normal karyotype but no molecular data available.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | All patients | % CD34+CD38- of blasts>1% | % CD34+CD38- of blasts<1% | **p-value** |
| Complete Response,n(%) | 109/163(67%) | 40/76(52%) | 69/87(79%) | **p=0,0005** |
| Relapse,n(%) | 33/109(30%) | 19/40(47%) | 14/69(20%) | **p =0,006** |
| DFS, (Median follow up in months) | 101(10,4) | 12,7 | NA | **p =0,0005** |
| OS, (Median follow up in months) | 153 (7,6) | 14 | NA | **p=0,0001** |

**Supplemental Table 4. Patient outcome according to CD34+/CD38- population**