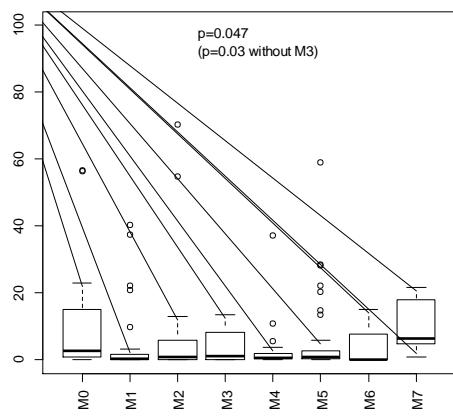
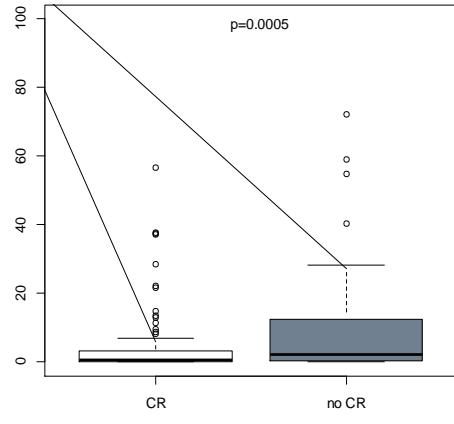
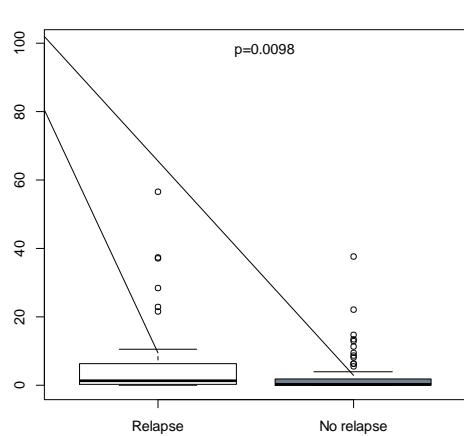


Supplementary Material

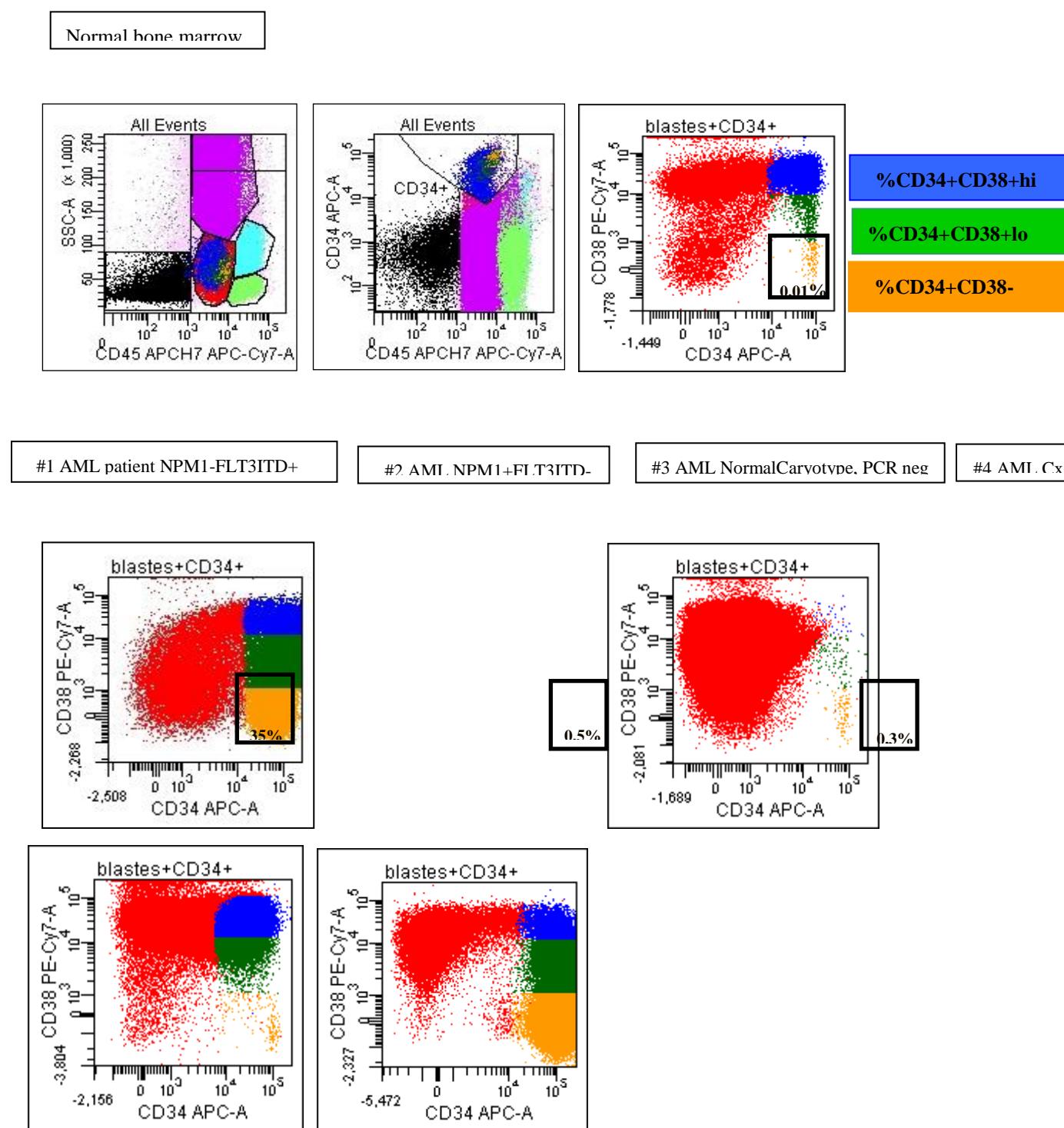
Supplemental Figure 1a. Distribution of CD34+/CD38-% population according to FAB subtype



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



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



| | All patients | % CD34+CD38- of blasts>1% | % CD34+CD38- of blasts<1% |
|--|---|---|--------------------------------------|
| No of patients (Adults vs children) | 200 (187 vs 13) | 98(49%) (93 vs5) | 102(51%) (94 vs 8) |
| Male/Female,n | 117/83 | 57/41 | 59/43 |
| Age at diagnosis, years, mean (range) | 56 (2-89) | 60 (4-87) | 53 (2-89) |
| WBC count at diagnosis, 10 ⁹ /L, mean (range) | 22,1 (0,5-233) | 19,9 (0,6-152) | 24,2 (0,5-233) |
| AMLde novo (with MDS),n(%) | 56(28%) | 27(28%) | 29(28%) |
| AML secondary,n(%) | 64(32%) | 41(42%) | 23(23%) |
| AML de novo (w/o MDS),n | 80(40%) | 30(30%) | 50(49%) |
| FAB classification, n M0/M1/M2/M3/M4/M5/M6/M7/ Not classified | 17/31/31/10 / | 10/9/15/5/ 6/20/1/4/28 21/44/4/5/3 7 | 7/22/16/5/ 15/24/3/1/9 |
| 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%) |
| Cytogenetic risk group,n(%) Favourable Intermediate 1 Intermediate 2 | 190 14(7,4%) 31(16,4%) 58(30,7%) | 93 5(5%) 11(12%) 25(27%) | 97 9(9%) 20(21%) 33(34%) |

| | | | |
|------------------------|-----------|----------|---------|
| Unfavourable | 76(40,2%) | 47(51%) | 29(30%) |
| Failed/ND | 11(5,5%) | 5(5%) | 6(6%) |
| Allogeneic - SCT, n(%) | 41(20,5%) | 21(21%) | 20(20%) |
| NPM1+ | 29(21,5%) | 5(8,3%) | 24(32%) |
| FLT3ITD+ | 19(14,2%) | 8(13,3%) | 11(15%) |
| EVI1+ | 18(13,5%) | 10(17%) | 8(11%) |

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] | |
| intermediate 1 | 0,19 [0,04-1,4] | |
| intermediate 2 | 0,65 [0,01-56,8] | <0,001 |
| 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] | |
| NPM1+FLT3ITD+ 13/135 | 0,65 [0,07-28,5] | |
| NPM1-FLT3ITD+ 6/135 | 1,66 [0,04-37,5] | 0,002 |

| | | |
|---------------|-------------|--|
| NPM1-FLT3ITD- | 1,08 | |
| 100/135 | [0,01-72,2] | |

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

| Relapsing Patients # 33 | Sex | Age | FAB | Cytogenetic risk groups** | karyotype | Molecular profile |
|--|------------|------------|------------|--|--|--------------------------|
| 1 | F | 59 | M2 | 1 | 45,X,-X,t(8;21)(q22;q22)[20]/46,XX[3] | Wt1+ |
| 2 | F | 50 | M2 | 1 | 46,XX,t(8;21)[20] | neg |
| 3 | M | 26 | M4é o | 1 | 46, XY,inv(16)(p13q22)[14]/ 46, XXY6] | Wt1+ |
| 4 | F | 52 | M4é o | 1 | 46,XX, inv(16)(p13q22) [10] / 46,XX [11]. | Wt1+ |
| 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+ |
| 6 | F | 11 | M2 | 1 | 46,XX [28] [t(8 ;21) variant] | Neg AML1/ETO+ |
| 7 | F | 25 | M1 | 2 | 46,XX[10] | Flt3 D835,Wt1RQ, ,NPM |
| 8 | M | 69 | M(n c) | 3 | 46,XY[20] | neg |
| 9 | F | 57 | M1 | 3 | 46,XX[25] | Wt1+, Flt3mut+ |
| 10 | F | 60 | M1 | 3 | 46,XX[20] | neg |
| 11 | M | 60 | M2 | 3 | 46,XY,t(1;3)(p36;q21)[19]/46,XY[2] | Wt1RQ+ |
| 12 | F | 62 | M4 | 3 | 46,XX[20] | Wt1RQ+ Flt3RQ |
| 13 | M | 56 | M4 | 3 | 46,XY[20] | neg |
| 14 | M | 71 | M7 | 3 | 46,XY[12] | Wt1RQ |
| 15 | F | 55 | M5 | 3 | 49,XX,+4,+6,+8[20] | Hoxa9-RQ+ Flt3RQ |

| | | | | | | |
|----|---|----|-----------|---|---|--------------------------|
| 16 | F | 57 | M6 | 3 | echec | Wt1RQ |
| 17 | M | 65 | M5 | 3 | 46,XY[20] | neg |
| 18 | F | 60 | M5 | 3 | 46,XX[20] | Flt3ITD+hoxa9+,W NMP+ |
| 19 | F | 72 | M0 | 3 | 46,XX[18] | neg |
| 20 | M | 33 | M(n c) | 5 | 46, XY[15] | ND |
| 21 | F | 60 | M(n c) | 4 | 45,XX,-5,add(7)(q21)?der(7)t(5;7)(q13;q2 1),-12,del(15)(q?15q23),+mar[11]/ 45,idem,del(6)(q13q15)[9]/46,XX[8] | neg |
| 22 | M | 55 | M0 | 4 | 46,XY,20q-[15] | neg |
| 23 | M | 63 | M4 | 4 | 46,XY[20] | EVI1+(dg)/ -(relaps |
| 24 | M | 51 | M7 | 4 | 43,XY,add(5)(q15),-7,-11,dic(12;18)(p12;p 12),-13, add(14)(p11),-15,-16,-17,-18,-21,+8mars[c p20] | Evi1RQ+ |
| 25 | M | 33 | M0 | 4 | 46,XY,del(5)(q14q34) ish(EGR1-),?t(12;21)(p12;q22)[3]/46,XY[3] | Wt1RQ+Flt3-RQ |
| 26 | M | 60 | M0 | 4 | 45,XY,-7[17]/45,idem,+mar[3] | Evi1RQ+ Wt1+ |
| 27 | F | 56 | M1 | 4 | 47,XX,+8,del(20)(q11q13)[20] | Flt3 ITD+. |
| 28 | M | 15 | M5 | 4 | 46,XY,del(7)(q11q32)[16] | FLT3ITD+ |
| 29 | M | 64 | M(n c) | 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- |
| 30 | M | 70 | M2 | 4 | 48,XY,-1,del(6)(q21q24),+8,+9,-13,-15,+3 mars[5]/46,XY[15] | neg |
| 31 | M | 44 | M5 | 4 | 48,XY,del(1)(?q22q23),dup(1)(q12q21),ad d(2)(q35),+13,+13[6]/ | Flt3-RQ+ |

| | | | | | | |
|----|---|----|----|---|---|------------------------------|
| | | | | | 96,idemX2,del(5)(q23q31),-7,del(10)(q22q23),-14,-15,+19,+20, +mar(?der(7))[cp10]/ 46,XY[4] | |
| 32 | F | 60 | M5 | 4 | 51,XX,+X,+5,+8,+10,+13[15]/46,XX[5] | Flt3 ITD+ W Hoxa9+, NPM1+ |
| 33 | M | 56 | M5 | 5 | 46,XY[20] | ND |

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

