# Molecular minimal residual disease negativity and decreased stem cell mobilization potential predict excellent outcome after autologous transplant in *NPM1* mutant acute myeloid leukemia

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# Supplemental Material

## Supplemental Tables

**Supplemental Table S1:** Clinical characteristics at diagnosis.

**Supplemental Table S2:** Clinical characteristics at diagnosis of patients with relapse compared to no relapse.

**Supplemental Table S3:** Mobilization of CD34+ cells and MRD[1] status at stem cell collection.

**Supplemental Table S4:** Mobilization of CD34+ cells and MRD status at stem cell collection of patients with relapse compared to no relapse.

**Supplemental Table S5:** Overview on the clinical outcomes.

**Supplemental Table S6:** Clinical characteristics at diagnosis comparing MRD positive and MRD negative patients based on *NPM1* mutation analysis using bone marrow (BM) samples.

**Supplemental Table S7:** Multivariable predictors of progression-free survival and overall survival.

## Supplemental Figures

**Supplemental Figure S1:** Outcome of high versus low CD34+ patients and depending on the MRD status in peripheral blood at stem cell collection.

**Supplemental Table S1:** Clinical characteristics at diagnosis.

| Parameter | All patients (n=42) | High CD34+ (n=21) | Low CD34+ (n=21) | p |
|---|---|---|---|---|
| Gender, male, n (%) | 20 (48) | 7 (33) | 13 (62) | 0.12 |
| Age at diagnosis, (median, range) | 55 (23-70) | 57 (23-70) | 54 (31-66) | 0.41 |
| BMI[1], kg/m$^2$ (median, range) | 25 (19-43) | 25 (20-43) | 25 (19-41) | 0.92 |
| Hb[2], g/L (median, range) | 90 (43-133) | 88 (43-133) | 93 (47-130) | 0.71 |
| Lc[3], G/L (median, range) | 15 (1-160) | 12 (2-160) | 21 (1-144) | 0.82 |
| Tc[4], G/L (median, range) | 103 (9-522) | 114 (28-522) | 81 (9-213) | 0.17 |
| LDH[5], U/L (median, range) | 772 (184-9,134) | 745 (184-2,142) | 1,108 (280-9,134) | 0.10 |
| CRP[6], mg/L (median, range) | 35 (3-342) | 36 (3-342) | 33 (3-224) | 0.88 |
| Albumin, g/L (median, range) | 34 (20-63) | 34 (20-44) | 34 (24-63) | 0.87 |
| Peripheral blasts, % (median, range) | 32 (0-99) | 22 (0-99) | 80 (8-95) | 0.83 |
| Marrow blasts, % (median, range) | 65 (0-95) | 60 (0-95) | 80 (8-95) | 0.73 |
| *De novo* AML[7] | 39 (93%) | 19 (90%) | 20 (95%) | 1.00 |
| Secondary AML from MDS[8], n (%) | 1 (2) | 0 (0) | 1 (5) | 1.00 |
| Therapy-related AML, n (%) | 1 (2) | 1 (5) | 0 (0) | 1.00 |
| Extramedullary AML, n (%) | 1 (2) | 1 (5) | 0 (0) | 1.00 |
| ELN[9] favorable risk, n (%) | 29 (69) | 14 (67) | 15 (71) | 1.00 |
| intermediate risk, n (%) | 13 (31) | 7 (33) | 6 (29) | 1.00 |
| **Molecular mutation profiles** | | | | |
| *NPM1*mut[10] alone, n (%) | 24 (57) | 13 (62) | 11 (52) | 0.76 |
| *NPM1*mut/*FLT3*wt[11], n (%) | 29 (69) | 14 (67) | 15 (71) | 1.00 |
| *NPM1*mut/*FLT3*-ITD[12], n (%) | 13 (31) | 7 (33) | 6 (29) | 1.00 |
| Other associated mutations | | | | |
| *NPM1*mut/*IDH1*[13], n (%) | 2 (5) | 1 (5) | 1 (5) | 1.00 |
| *NPM1*mut/*IDH2*[14], n (%) | 3 (7) | 0 (0) | 3 (15) | 0.16 |
| **Karyotypes** | | | | |
| Normal karyotype, n (%) | 38 (90) | 21 (100) | 17 (81) | 0.11 |
| del(9q), n (%) | 1 (2) | 0 (0) | 1 (5) | 1.00 |
| del(7q), n (%) | 1 (2) | 0 (0) | 1 (5) | 1.00 |
| Trisomy 8, n (%) | 2 (5) | 0 (0) | 2 (10) | 1.00 |

[1] BMI: body mass index; [2] Hb: hemoglobin; [3] Lc: leucocytes; [4] Tc: platelets; [5] LDH: lactate dehydrogenase; [6] CRP: C-reactive protein; [7] AML: acute myeloid leukemia; [8] MDS: myelodysplastic syndrome; [9] ELN: European Leukemia Net classification; [10] *NPM1*mut: mutation in the Nucleophosmin 1 gene; [11] *FLT3wt*: FMS-like tyrosine kinase 3 gene wild-type; [12] *FLT3*-ITD: internal tandem duplication of the *FLT3* gene; [13] *IDH1*mut: mutation of the isocitrate dehydrogenase 1 gene; [14] *IDH*2mut: mutation of the isocitrate dehydrogenase 2 gene.

**Supplemental Table S2:** Clinical characteristics at diagnosis of patients with relapse as compared to no relapse.

| Parameter | All patients (n=42) | Relapse (n=20) | No relapse (n=22) | p |
|---|---|---|---|---|
| Gender, male, n (%) | 20 (48) | 10 (50) | 10 (45) | 1.00 |
| Age at diagnosis, (range) | 55 (23-70) | 56 (31-70) | 55 (23-69) | 0.75 |
| BMI[1], kg/cm2 (range) | 25 (19-43) | 24 (19-32) | 25 (20-43) | 0.06 |
| Hb[2], g/L (range) | 90 (43-133) | 91 (43-130) | 90 (65-133) | 0.34 |
| Lc[3], G/L (range) | 15 (1-160) | 26 (2-160) | 11 (1-145) | 0.32 |
| Tc[4], G/L (range) | 103 (9-522) | 106 (28-522) | 98 (9-213) | 0.67 |
| LDH[5], U/L (range) | 772 (184-9134) | 830 (184-5932) | 756 (280-9134) | 0.55 |
| CRP[6], mg/L (range) | 35 (3-342) | 25 (3-342) | 52 (3-187) | 0.20 |
| Albumin, g/L (range) | 34 (20-63) | 35 (20-63) | 34 (22-41) | 0.23 |
| Peripheral blasts, % (range) | 32 (0-99) | 50 (0-99) | 18 (0-99) | 0.14 |
| Blasts in BM[7], % (range) | 65 (0-95) | 70 (0-95) | 60 (8-95) | 0.47 |
| Deaths, n (%) | 17 (40) | 14 (70) | 3 (15) | **<0.001** |
| PFS[8], months (range) | 13 (1-182) | 5 (1-71) | 50 (2-182) | **0.0005** |
| OS[9], months (range) | 26 (2-182) | 12 (3-73) | 50 (2-182) | **0.049** |
| *De novo* AML | 39 (93%) | 17 (85%) | 22 (100%) | 0.48 |
| Secondary AML[10] from MDS[11], n (%) | 1 (2) | 1 (5) | 0 (0) | 0.48 |
| Therapy-related AML, n (%) | 1 (2) | 1 (5) | 0 (0) | 0.48 |
| Extramedullary (chloroma) AML, n (%) | 1 (2) | 1 (5) | 0 (0) | 0.48 |
| ELN[12] classification favorable risk, n (%) | 29 (69) | 14 (70) | 15 (68) | 1.00 |
| intermediate risk, n (%) | 13 (31) | 6 (30) | 7 (32) | 1.00 |
| Molecular and cytogenetic abnormalities | | | | |
| *NPM1*mut[13] alone, n (%) | 24 (57) | 12 (60) | 12 (55) | 0.56 |
| *NPM1*mut/*FLT3*wt[14], n (%) | 29 (69) | 14 (70) | 15 (68) | 1.00 |
| *NPM1*mut/*FLT3*-ITD[15] n (%) | 13 (31) | 6 (30) | 7 (32) | 1.00 |
| *NPM1*mut/*IDH1*R132mut[16], n (%) | 2 (5) | 2 (10) | 0 (0) | 0.22 |
| *NPM1*mut/del(9)(q12q32) , n (%) | 1 (2) | 0 (0) | 1 (5) | 1.00 |
| *NPM1*mut/del(7), n (%) | 1 (2) | 0 (0) | 1 (5) | 1.00 |
| *NPM1*mut/trisomy 8/-Y, n (%) | 1 (2) | 0 (0) | 1 (5) | 1.00 |
| *NPM1*mut/trisomy 8, n (%) | 1 (2) | 0 (0) | 1 (5) | 1.00 |
| *NPM1*mut/normal karyotype, n (%) | 38 (90) | 19 (95) | 19 (86) | 0.61 |

[1] BMI: body mass index; [2] Hb: hemoglobin; [3] Lc: leukocytes; [4] Tc: thrombocytes; [5] LDH: lactate dehydrogenase; [6] CRP: C-reactive protein; [7] BM: bone marrow; [8] PFS: progression-free survival; [9] OS: overall survival; [10] AML: acute myeloid leukemia; [11] MDS: myelodysplastic syndrome; [12] ELN: European Leukemia Net classification; [13] *NPM1*mut: mutation in the Nucleophosmin 1 gene; [14] *FLT3*wt: FMS-like tyrosine kinase 3 gene wild-type; [15] *FLT3*-ITD: internal tandem duplication of the FLT3 gene; [16] *IDH1*R132mut: R132 mutation of the isocitrate dehydrogenase 1 gene.

**Supplemental Table S3:** Mobilization of CD34+ cells and MRD[1] status at stem cell collection.

| Parameter | All patients (n=42) | High CD34+ (n=21) | Low CD34+ (n=21) | p |
|---|---|---|---|---|
| Day of apheresis after start 2nd chemotherapy cycle, n (range)[2] | 24 (18-83) | 23 (18-54) | 28 (20-83) | **0.01** |
| Single day of apheresis, n (%) | 40 (95) | 21 (100) | 19 (91) | 0.49 |
| Two days of apheresis, n (%) | 2 (5) | 0 (0) | 2 (10) | 0.49 |
| Median peripheral CD34+ cells at day of collection, /μL (range)[3] | 45 (4-1573) | 106 (47-1573) | 21 (4-43) | **<0.0001** |
| Median total yield of collected CD34+ cells, x$10^6$/kg (range) | 8 (3-130) | 12 (6-130) | 5 (3-13) | **<0.0001** |
| Median total amount of transfused CD34+ cells, x$10^6$/kg (range)[4] | 5.0 (2-17) | 5.5 (3-17) | 3.8 (2-7) | **0.0072** |
| MRD-negative in peripheral blood, n (%) | 25 (60) | 10 (48) | 15 (71) | 0.21 |
| MRD-positive in peripheral blood, n (%) | 17 (40) | 11 (52) | 6 (29) | 0.21 |
| MRD-negative in BM[5], n (%) | 20 (48) | 9 (43) | 11 (52) | 0.76 |
| MRD-positive in BM, n (%) | 22 (52) | 12 (57) | 10 (48) | 0.76 |

[1] MRD: minimal residual disease; [2] median number of days since first day of start of the second induction cycle; [3] circulating peripheral CD34+ per microliter; [4] data missing in one patient in the low CD34+ group; [5] BM: bone marrow.

**Supplemental Table S4:** Mobilization of CD34+ cells and MRD status at stem cell collection of patients with relapse compared to no relapse.

| Parameter | All patients (n=42) | Relapse (n=20) | No relapse (n=22) | p |
|---|---|---|---|---|
| Day of apheresis after start second cycle of chemotherapy, n (range) [2] | 24 (18-83) | 23 (18-67) | 27 (20-83) | 0.06 |
| Single day of apheresis, n (%) | 40 (95) | 20 (100) | 20 (91) | 0.49 |
| Two days of apheresis, n (%) | 2 (5) | 0 (0) | 2 (9) | 0.49 |
| Peripheral CD34+ cells at day of collection, x$10^6$/L (range) [3] | 45 (4-1574) | 55 (12-1025) | 36 (4-1574) | 0.23 |
| Median total yield of collected CD34+ cells, x$10^6$/kg (range) | 8 (3-130) | 12 (3-130) | 6 (3-71) | 0.06 |
| Median total amount of transfused CD34+ cells, x$10^6$/kg (range) [4] | 5 (2-17) | 5 (2-17) | 4 (3-7) | 0.2154 |
| MRD-negative in blood, n (%) | 25 (60) | 7 (35) | 18 (82) | **0.004** |
| MRD-positive in blood, n (%) | 17 (40) | 13 (65) | 4 (18) | **0.004** |
| MRD-negative in BM[5], n (%) | 20 (48) | 4 (20) | 16 (73) | **0.0008** |
| MRD-positive in BM, n (%) | 22 (52) | 16 (80) | 6 (27) | **0.0008** |

[1] MRD: minimal residual disease; [2] median number of days since start of second induction cycle; [3] median number of circulating peripheral CD34+ cells per liter; [4] data missing in one patient in the no relapse group; [5] BM: bone marrow.

**Supplemental Table S5:** Overview on clinical outcomes.

| Parameter | All patients (n=42) | High CD34+ (n=21) | Low CD34+ (n=21) | p |
|---|---|---|---|---|
| **Relapse, n (%)** | 20 (48) | 11 (52) | 9 (43) | 0.76 |
| in MRD+[1] bone marrow | 16 (38) | 9 (43) | 7 (33) | 1.00 |
| in MRD- bone marrow | 4 (10) | 2 (10) | 2 (10) | 1.00 |
| in *NPM1*mut[2]/*FLT3*wt[3], n (%) | 14 (33) | 9 (43) | 5 (24) | 0.14 |
| in *NPM1*mut/*FLT3*-ITD[4], n (%) | 6 (14) | 2 (10) | 4 (44) | 0.29 |
| **Death**, n (%) | 17 (40) | 13 (62) | 4 (19) | **0.01** |
| in MRD+ bone marrow | 14 (33) | 10 (48) | 4 (19) | 0.07 |
| in MRD- bone marrow | 3 (7) | 3 (14) | 0 (0) | 0.07 |
| in *NPM1*mut/*FLT3*wt, n (%) | 9 (21) | 8 (38) | 1 (5) | **0.005** |
| in *NPM1*mut/*FLT3*-ITD, n (%) | 8 (19) | 5 (24) | 3 (14) | 0.59 |
| **PFS**[5], median, months (range) | 13 (1-182) | 8 (1-182) | 19 (1-174) | 0.36 |
| **OS**[6], median, months (range) | 26 (2-182) | 23 (2-182) | 39 (3-174) | 0.33 |
| Patients with allo-SCT[7], n (%) | 11 (26) | 4 (19) | 7 (33) | 0.48 |
| Allo-SCT at relapse, n (%) | 10 (24) | 4 (19) | 6 (29) | 0.70 |
| Relapse after allo-SCT, n (%) | 3 (7) | 1 (5) | 2 (10) | 1.00 |

[1] MRD: minimal residual disease; [2] *NPM1*mut: mutation in the Nucleophosmin 1 gene; [3] *FLT3*wt: FMS-like tyrosine kinase 3 gene wild-type; [4] *FLT3*-ITD: internal tandem duplication of the *FLT3* gene; [5] PFS: progression-free survival; [6] OS: overall survival; [7] SCT: stem cell transplantation.

**Supplementary Table S6:** Clinical characteristics at diagnosis comparing MRD positive and negative patients based on *NPM1* mutation analysis from bone marrow (BM).

| Parameter | All patients (n=42) | MRD[1] positive (BM; n=22) | MRD negative (BM; n=20) | p |
|---|---|---|---|---|
| Gender, male, n (%) | 20 (48) | 8 (36) | 12 (60) | 0.22 |
| Age at diagnosis, (median, range) | 55 (23-70) | 58 (31-70) | 54 (23-66) | 0.54 |
| BMI[2], kg/m$^2$ (median, range) | 25 (19-43) | 24 (19-33) | 25 (20-43) | 0.08 |
| Hb[3], g/L (median, range) | 90 (43-133) | 88 (43-130) | 95 (68-133) | 0.11 |
| Lc[4], G/L (median, range) | 14 (1-160) | 28 (2-160) | 8 (1-144) | 0.06 |
| Tc[5], G/L (median, range) | 103 (9-522) | 109 (31-522) | 89 (9-213) | 0.22 |
| LDH[6], U/L (median, range) | 772 (184-9134) | 775 (184-5932) | 767 (280-9134) | 0.63 |
| CRP[7], mg/L (median, range) | 35 (3-342) | 35 (3-342) | 36 (3-187) | 0.51 |
| Albumin, g/L (median, range) | 34 (20-63) | 34 (20-41) | 35 (22-63) | 0.43 |
| Peripheral blasts, % (median, range) | 32 (0-99) | 42 (0-99) | 18 (0-97) | 0.42 |
| Marrow blasts, % (median, range) | 65 (0-95) | 83 (0-95) | 55 (8-95) | 0.15 |
| De novo AML, n (%) | 39 (93%) | 20 (91%) | 19 (95%) | 0.22 |
| Secondary AML[8] from MDS[9], n (%) | 1 (2) | 0 (0) | 1 (5) | 0.48 |
| Therapy-related AML, n (%) | 1 (2) | 1 (5) | 0 (0) | 1.00 |
| Extramedullary (chloroma) AML, n (%) | 1 (2) | 1 (5) | 0 (0) | 1.00 |
| ELN[10] favorable risk, n (%) | 29 (69) | 15 (68) | 14 (70) | 1.00 |
| intermediate risk, n (%) | 13 (31) | 7 (32) | 6 (30) | 1.00 |
| **Mutation profiles** | | | | |
| *NPM1*mut[11] alone, n (%) | 24 (57) | 12 (55) | 12 (60) | 0.76 |
| *NPM1*mut/*FLT3*wt[12], n (%) | 29 (69) | 15 (68) | 14 (70) | 1.00 |
| *NPM1mut*/*FLT3*-ITD[13], n (%) | 13 (31) | 7 (32) | 6 (30) | 1.00 |
| Other associated mutations | | | | |
| *NPM1mut*/*IDH1*[14], n (%) | 2 (5) | 2 (9) | 0 (0) | 0.49 |
| *NPM1mut*/*IDH2*[15], n (%) | 3 (7) | 3 (14) | 0 (0) | 0.12 |
| **Karyotypes** | | | | |
| normal karyotype, n (%) | 38 (90) | 20 (91) | 18 (90) | 1.00 |
| del(9q), n (%) | 1 (2) | 0 (0) | 1 (5) | 0.48 |
| del(7q), n (%) | 1 (2) | 1 (5) | 0 (0) | 1.00 |
| trisomy 8, n (%) | 2 (5) | 1 (5) | 1 (5) | 1.00 |

[1] MRD: minimal residual disease; [2] BMI: body mass index; [3] Hb: hemoglobin; [4] Lc: leucocytes; [5] Tc: platelets; [6] LDH: lactate dehydrogenase; [7] CRP: C-reactive protein; [8] AML: acute myeloid leukemia; [9] MDS: myelodysplastic syndrome; [10] ELN: European Leukemia Net classification; [11] *NPM1*mut: mutation in the Nucleophosmin 1 gene; [12] *FLT3wt*: FMS-like tyrosine kinase 3 gene wild-type; [13] *FLT3*-ITD: internal tandem duplication of the *FLT3* gene; [14] *IDH1*mut: mutation of the isocitrate dehydrogenase 1 gene; [15] *IDH2*mut: mutation of the isocitrate dehydrogenase 2 gene.

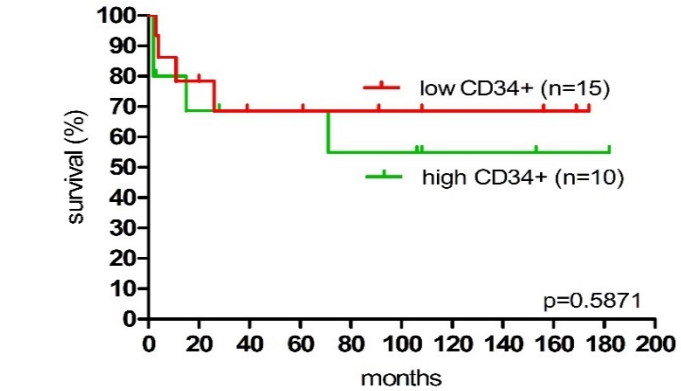
**Supplemental Table S7:** Multivariable predictors of progression-free survival and overall survival.

| Parameter | Progression-free survival | | Overall survival | |
|---|---|---|---|---|
| | **Hazard ratio (95% CI)** | **p** | **Hazard ratio (95% CI)** | **p** |
| MRD-negative [1] | 0.68 (0.56 to 0.81) | **<0.001** | 0.62 (0.49 to 0.78) | **<0.001** |
| CD34+ low [2] | 0.89 (0.67 to 0.96) | **0.022** | 0.84 (0.68 to 0.91) | **0.018** |
| Age [3] | 1.08 (0.88 to 1.21) | 0.682 | 1.10 (0.94 to 1.33) | 0.053 |
| LDH [4] | 1.12 (0.91 to 1.25) | 0.372 | 1.16 (0.87 to 1.41) | 0.317 |
| Leukocytes [5] | 1.21 (0.85 to 1.42) | 0.503 | 1.23 (0.92 to 1.51) | 0.541 |
| ELN risk group [6] | 0.81 (0.60 to 0.95) | **0.027** | 0.68 (0.62 to 0.94) | **0.029** |

[1] *NPM1*mut negativity (versus positivity) assessed by quantitative PCR in the bone marrow after two cycles of induction chemotherapy at peripheral stem cell collection; [2] Low mobilization of CD34+ cells at stem cell collection compared to high CD34 mobilization dichotomized according to the median CD34+ level of the cohort; [3] Age below 60 years compared to age ≥60 years at first diagnosis of AML; [4] normal LDH (ULN: 480 U/L) versus increased levels (>480 U/L) at diagnosis of AML; [5] Peripheral leucocytes at diagnosis of AML below median value (15 G/L) versus above median value; [6] European LeukemiaNet risk category favorable versus intermediate.

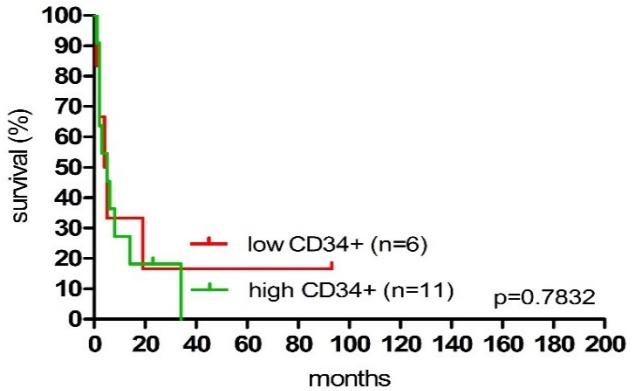
**Supplementary Figure S1:** Outcome of high versus low CD34+ patients and depending on the MRD status in peripheral blood at stem cell collection.

(A) – MRD-negative - PFS - peripheral blood



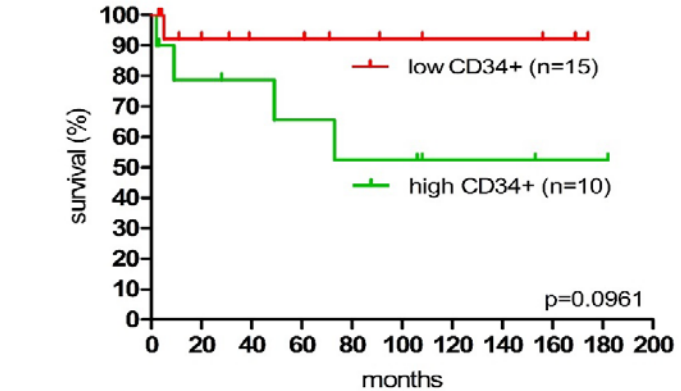

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| low CD34+ | 15 | 9 | 6 | 6 | 5 | 4 | 3 | 3 | 2 | 0 | 0 |
| high CD34+ | 10 | 6 | 5 | 5 | 4 | 4 | 2 | 2 | 1 | 1 | 0 |

(B) – MRD-positive - PFS - peripheral blood



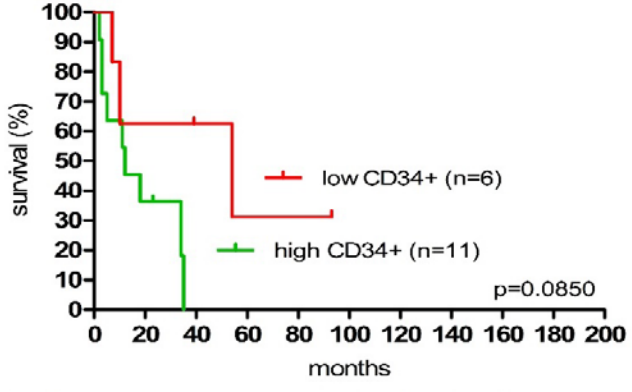

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| low CD34+ | 6 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| high CD34+ | 11 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

(C) – MRD-negative - OS - peripheral blood




| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| low CD34+ | 15 | 10 | 7 | 7 | 5 | 4 | 3 | 3 | 2 | 0 | 0 |
| high CD34+ | 10 | 7 | 6 | 5 | 4 | 4 | 2 | 2 | 1 | 1 | 0 |

(D) – MRD-positive - OS - peripheral blood




| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| low CD34+ | 6 | 3 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| high CD34+ | 11 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |