Normal karyotype is a poor prognostic factor in myeloid leukemia of Down syndrome: a retrospective, international study

Marjolein Blink,[1] Martin Zimmermann,[2] Christine von Neuhoff,[2] Dirk Reinhardt,[2] Valerie de Haas,[3] Henrik Hasle,[4] Maureen M. O'Brien,[5] Batia Stark,[6] Julie Tandonnet,[7] Andrea Pession,[8] Katerina Tousovska,[9] Daniel K.L. Cheuk,[10] Kazuko Kudo,[11] Takashi Taga,[12] Jeffrey E. Rubnitz,[13] Iren Haltrich,[14] Walentyna Balwierz,[15] Rob Pieters,[1,3] Erik Forestier,[16] Bertil Johansson,[17] Marry M. van den Heuvel-Eibrink,[1,3] and C. Michel Zwaan[1,3]

[1]Pediatric Oncology- Hematology, Erasmus MC– Sophia Children's Hospital, Rotterdam, the Netherlands; [2]Acute Myeloid Leukemia Berlin-Frankfurt-Munster Study Group, Department of Pediatric Oncology/ Hematology, Medical School, Hannover, Germany; [3]Dutch Childhood Oncology Group, The Hague, the Netherlands; [4]Nordic Society for Pediatric Hematology and Oncology, Department of Pediatrics, Aarhus University Hospital Skejby, Aarhus, Denmark; [5]Division of Hematology/Oncology, Cincinnati Children's Hospital Medical Center, OH, USA; [6]Hematologic Malignancies Unit, The Center for Pediatric Hematology Oncology, Schneider Children's Medical Center, Petach Tikvah, Israel; [7]Pediatric Oncology and Hematology, Children's Hospital, Bordeaux, France; [8]Pediatric Oncology and Hematology, University of Bologna, Italy; [9]Department of Pediatrics, University Hospital, Hradec Kralové, Czech Republic; [10]Hong Kong Pediatric Hematology Oncology Study Group, Department of Pediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong, China; [11]Division of Hematology and Oncology, Shizuoka Children's Hospital, Japan; [12]Department of Pediatrics, Shiga University of Medical Science, Japan; [13]Leukemia/Lymphoma Division, St. Jude Children's Research Hospital, Memphis, TN, USA; [14]Departments of Pediatrics, Semmelweis University of Medicine, Budapest, Hungary; [15]Department of Pediatric Oncology and Hematology, Polish-American Institute of Pediatrics, Jagiellonian University Medical College, Krakow, Poland; [16]Department of Medical Bioscience, Genetics, University of Umeå, Sweden; [17]Department of Clinical Genetics, University and Regional Laboratories, Skåne University Hospital, Lund University, Lund, Sweden

Supplementary data

Methods

<u>Patients</u>

Data on 451 patients with ML-DS were collected from 13 collaborative study groups participating in the International AML-BFM Study Group, including the Berlin-Frankfurt-Munster Study Group (Germany and Austria; n=122), the Japanese Pediatric Leukemia/ Lymphoma Study Group (Japan; n=96), the Société Française de Lutte contre les Cancers et Leucémies de l'Enfant et de l'Adolescent (France; n=45), the Czech Pediatric Hematology Working Group (Czech Republic; n=6), St. Jude Children's Research Hospital (USA, n=8), the Children's Oncology Group Study, POG 9421 (USA, n=57), the Associazione Italiana di Ematologia ed Oncologia Pediatrica (Italy; n=3), the Nordic Society of Pediatric Haematology and Oncology (NOPHO; Denmark, Finland, Iceland, Norway and Sweden; n=44), the Dutch Childhood Oncology Group (the Netherlands; n=23), the Hong Kong Paediatric Haematology and Oncology Study Group (Hong Kong; n=13), the Polish Paediatric Leukaemia and Lymphoma Study Group (Poland; n= 23), the Israel National Study group for Childhood ALL (Israel; n=6), and the Hungarian Pediatric Oncological Network (Hungary; n=5). For comparison, a reference cohort of non-DS AML patients (n=543) from the same treatment era, kindly provided by the AML-BFM Study Group, was used. This study was approved according to local law and guidelines by the Institutional Review Boards.

 ML-DS patients, including those with a constitutional trisomy 21 (96.4%) or unbalanced Robertsonian translocation (3.6%), were identified by the various study groups. Patients were eligible if diagnosed between January 1, 1995 and January 1, 2005. Patients who were not treated with curative intent from diagnosis were excluded. The collected data at diagnosis comprised karyotype (if considered evaluable and centrally reviewed), sex, age, white blood cell count (WBC), hemoglobin, platelet counts, immunophenotypic data and FAB morphology. In addition, we collected data on treatment, such as therapy protocol (cumulative dosages of drugs), including stem-cell transplantation (SCT), and all events during follow-up (including non-responders, relapse, second malignancy or death). Only patients between 6 months and up to 5 years of age were included in the analyses; TMD-patients were excluded. Patients were treated on national or collaborative group AML trials. The treatment protocols were approved according to local law and guidelines by the Institutional Review Boards of each participating center and/or collaborative group.

<u>Cytogenetic results</u>

All karyotypes were provided after review by a national collaborative group, and centrally reviewed by 2 cytogeneticists (EF, BJ). FISH analyses were not standardly performed. Of the 451 cases, karyotypes were available from 358 (79%), comprising 103 (29%) with a normal karyotype (NK; i.e., with the constitutional trisomy 21 only), 55 (15%) with numerical aberrations only, and 120 (34%) with structural aberrations only. Both types of aberrations were found in 80 karyotypes (22%). Typical nonrandom cytogenetic aberrations, such as t(8;21)(q22;q22) and inv(16)(p13q22), frequently found in non-DS pediatric AML were not identified in the DS patients. Only one case had the acute promyelocytic leukemia-associated t(15;17)(q22;q21) and only one had an *MLL* rearrangement – t(9;11)(p21;q23).

As there was no *a priori* knowledge on the prognostic impact of the various cytogenetic groups in ML-DS, the classification of the cases was based on the premise that all groups should be mutually exclusive, i.e. each patient was included only once, although we could not avoid some overlap in additional cytogenetic abnormalities, and sufficiently large (≥5 cases) to allow meaningful statistical analyses.

The numerically largest group included 103 patients (29%) with a normal karyotype (NK). Another entity that was readily delineated consisted of 49 cases with trisomy 8 (14% of all cases), either as a single abnormality (n=16), or with additional cytogenetic aberrations (n=33). The latter group included a) trisomy 8 and gain of chromosome 21 (n=13, ± other additional changes); and b) trisomy 8 and other changes (n=20, excluding chromosome 5/7 aberrations and excluding +21). Next, a group of 82 cases (23%) with losses of chromosome 5/7 material (excluding those with +21) was distinguished. This group could be further subdivided into 50 cases with abnormalities of the p (short) arms only, 13 cases with monosomies 5/7, 10 cases with del(5q)/del(7q), and 9 cases with changes of both the p and q (long) arms of chromosomes 5/7. Other smaller groups consisted of 28 cases (6%) with a gain of chromosome 21 (in addition to +21c); 14 cases (4%) with a duplication of chromosome 1q; and 9 cases (3%) with a deletion of chromosome 16q. Finally, a group of 73 cases (20%) remained, harboring other aberrations that could not be sub-categorized further (Figure 1) (FigureS1) .
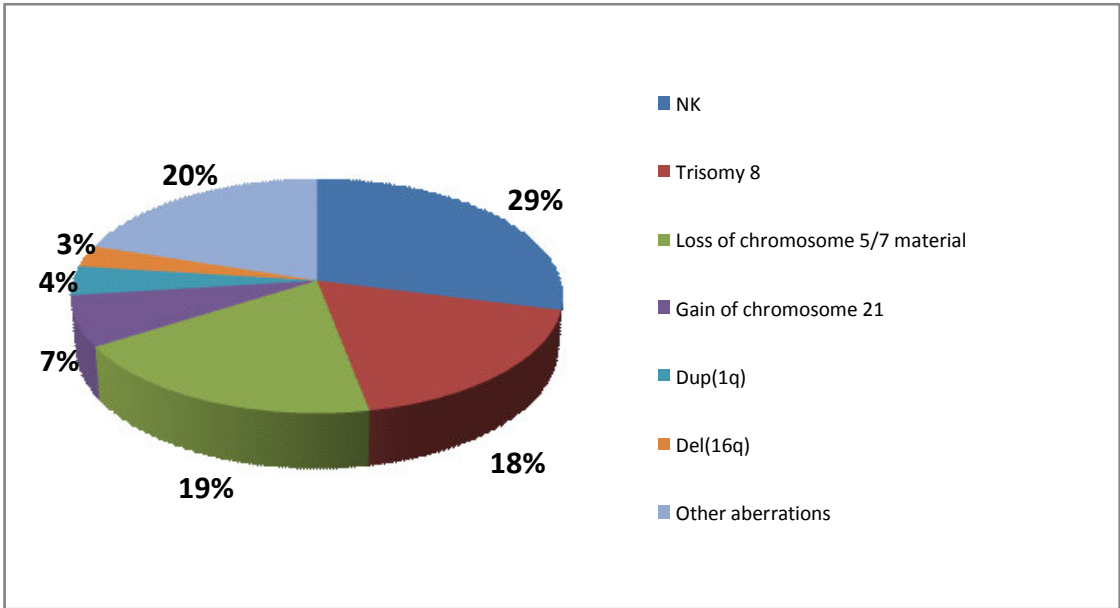
Statistical analyses

Complete remission (CR) was defined as less than 5% blasts in the bone marrow, with regeneration of normal hematopoiesis, and absence of leukemic cells in the cerebrospinal fluid or elsewhere. Patients who failed to achieve CR in time (as specified in the various protocols) were classified as non-responders and considered as failures at day 0. Early death was defined as any death within the first 4-6 weeks of treatment, before evaluation of CR.

Overall survival (OS) was measured from the date of diagnosis to the date of last follow-up or death from any cause. Event-free survival (EFS) was calculated from the date of diagnosis to the first event (non-response, relapse, second malignancy, or death) or to the date of last follow-up. For the OS and EFS analyses, patients who did not experience an event were censored at the time of last follow-up. The Kaplan-Meier method was used to estimate the 7-years probabilities of OS (pOS) and EFS (pEFS), and survival estimates were compared using the log-rank test. Cumulative incidence functions of relapse (with other events and death while in CR as competing event) and cumulative incidence (CI) of toxic death were constructed using the method of Kalbfleisch and Prentice and compared using Gray's test. For multivariate analysis, the Cox proportional-hazard regression model was used. We focused on differences in relapse-free survival (RFS) in order to avoid the influence of non-leukemic events on survival estimates.

Continuous variables were categorized according to cut-off points; age < or ≥ 3 years, WBC counts < or ≥ 20 x 10^9 and Ara-C <or ≥ 20.000 mg/m^2. The χ2 or Fisher exact test was used to compare discrete variables among groups; the Mann-Whitney U test was used for continuous variables. All *p*-values are descriptive and explorative, and were considered significant if ≤ 0.05. Statistical analyses were performed using SAS software (SAS-PC, Version 9.1).

Figure S1

Distribution of cytogenetic subgroups within ML-DS
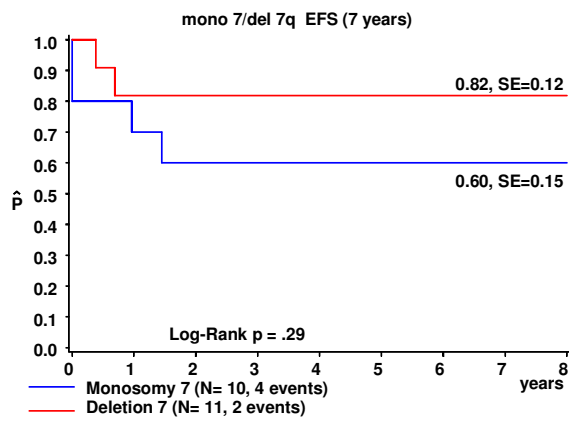

NK= normal karyotype; del= deletion; dupl= duplication
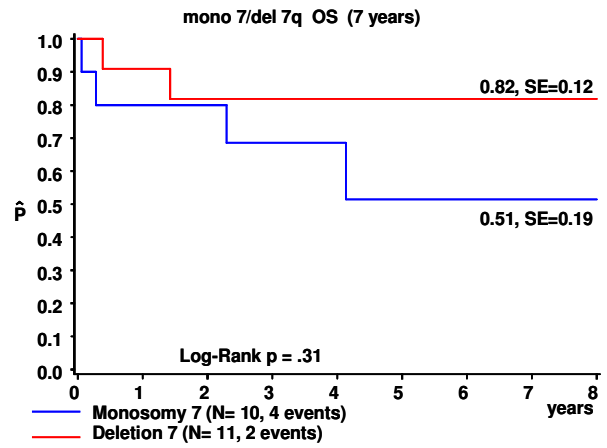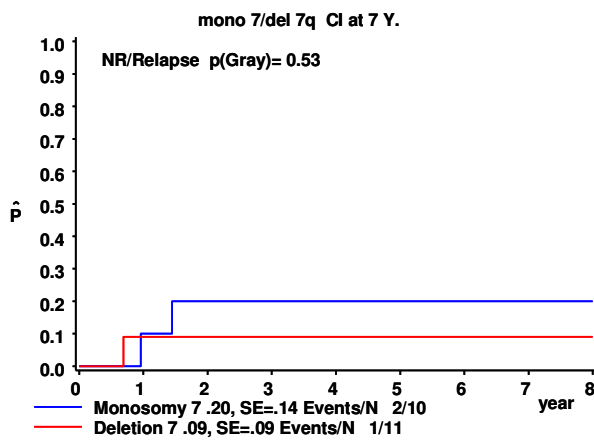
Figure S2A

mono 7/del 7q EFS (7 years)

0.82, SE=0.12

0.60, SE=0.15

Log-Rank p = .29

— Monosomy 7 (N= 10, 4 events)
— Deletion 7 (N= 11, 2 events)

Figure S2B

mono 7/del 7q OS (7 years)

0.82, SE=0.12

0.51, SE=0.19

Log-Rank p = .31

— Monosomy 7 (N= 10, 4 events)
— Deletion 7 (N= 11, 2 events)

Figure S2C

mono 7/del 7q CI at 7 Y.

NR/Relapse p(Gray)= 0.53

— Monosomy 7 .20, SE=.14 Events/N   2/10
— Deletion 7 .09, SE=.09 Events/N   1/11

Figure S2D

mono 7/del 7q CI at 1.5 Y.

Toxic death p(Gray)= 0.45

— Monosomy 7 .20, SE=.13 Events/N   2/10
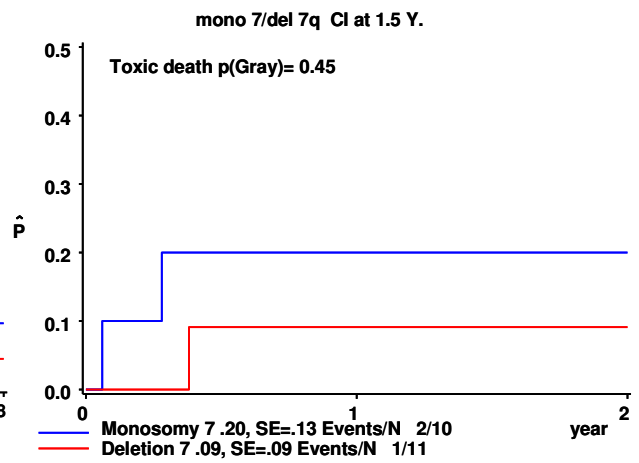— Deletion 7 .09, SE=.09 Events/N   1/11

Figure S2: Survival curves of the subgroups of monosomy 7 patients (n=10) and patients with a deletion of 7q (n=11).
   A. Event-free survival curves
   B. Overall survival curves
   C. Cumulative incidence of relapse
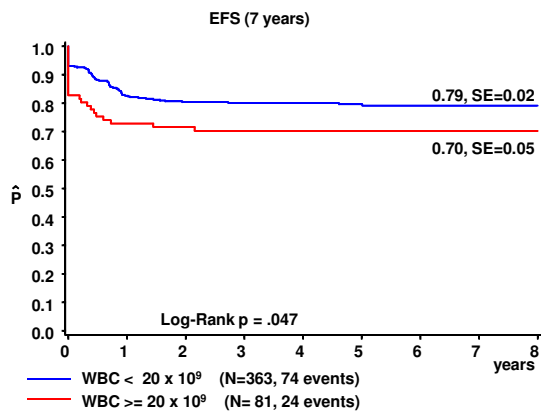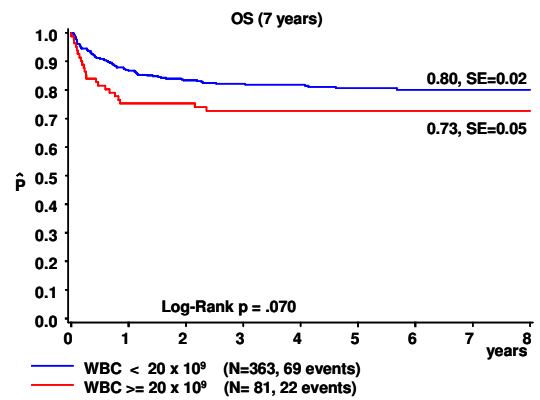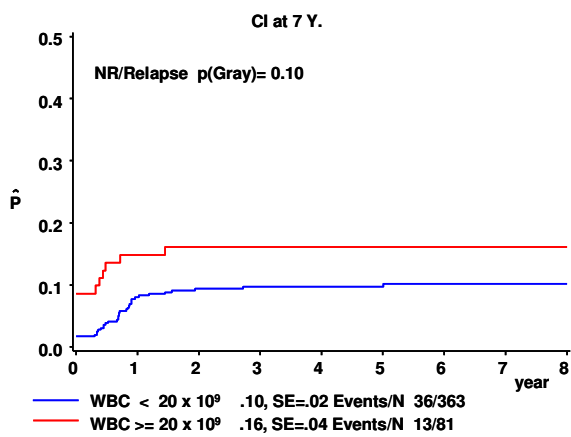   D. Cumulative incidence of toxic death

Figure S3A

EFS (7 years)

0.79, SE=0.02

0.70, SE=0.05

Log-Rank p = .047

years

WBC < 20 x 10⁹ (N=363, 74 events)
WBC >= 20 x 10⁹ (N= 81, 24 events)

Figure S3B

OS (7 years)

0.80, SE=0.02

0.73, SE=0.05

Log-Rank p = .070

years

WBC < 20 x 10⁹ (N=363, 69 events)
WBC >= 20 x 10⁹ (N= 81, 22 events)

Figure S3C

CI at 7 Y.

NR/Relapse p(Gray)= 0.10

year

WBC < 20 x 10⁹ .10, SE=.02 Events/N 36/363
WBC >= 20 x 10⁹ .16, SE=.04 Events/N 13/81

Figure S3D

CI at 1.5 Y.

Toxic death p(Gray)= 0.83

year

WBC < 20 x 10⁹ .07, SE=.01 Events/N 25/363
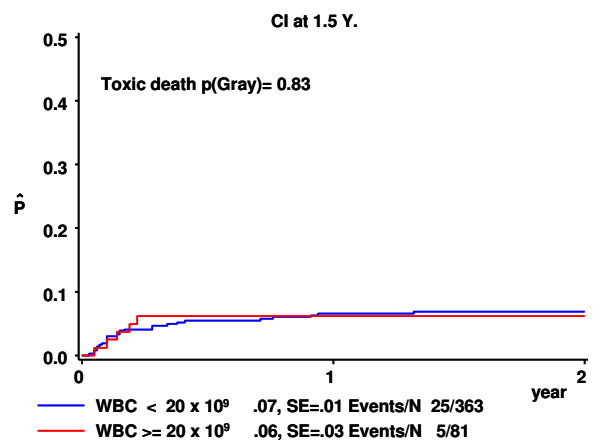WBC >= 20 x 10⁹ .06, SE=.03 Events/N 5/81

Figure S3: Survival curves of ML-DS patients (n=444) according to white blood cell count (WBC).
   A. Event-free survival
   B. Overall survival
   C. Cumulative incidence of relapse
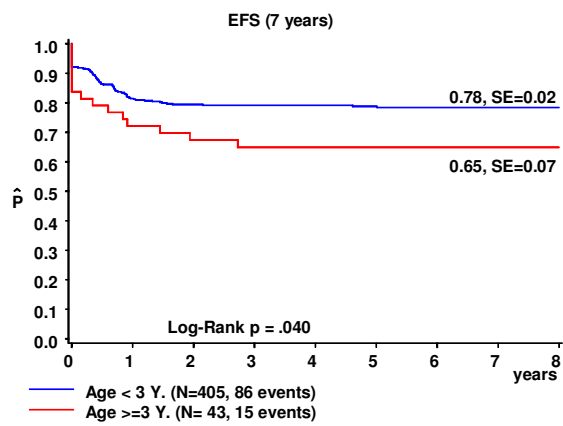   D. Cumulative incidence of toxic death

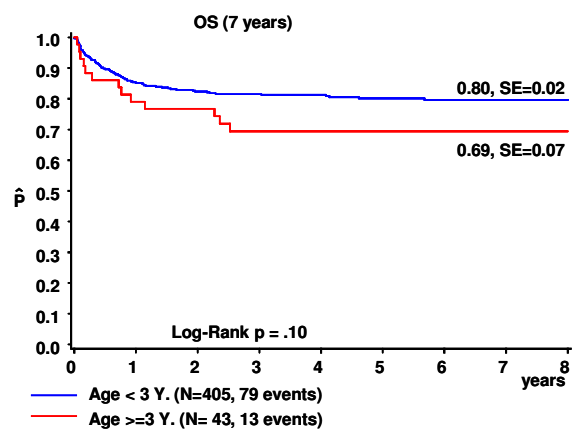**Figure S4A**
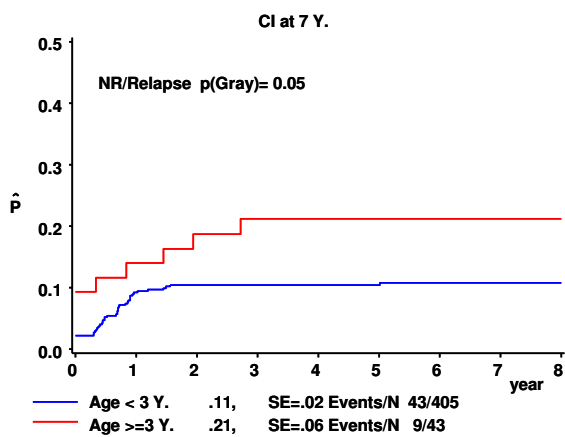
EFS (7 years)

0.78, SE=0.02

0.65, SE=0.07

Log-Rank p = .040

Age < 3 Y. (N=405, 86 events)
Age >=3 Y. (N= 43, 15 events)

Figure S4B

OS (7 years)

0.80, SE=0.02

0.69, SE=0.07

Log-Rank p = .10

Age < 3 Y. (N=405, 79 events)
Age >=3 Y. (N= 43, 13 events)

Figure S4C

CI at 7 Y.

NR/Relapse p(Gray)= 0.05

Age < 3 Y.   .11,   SE=.02 Events/N 43/405
Age >=3 Y.   .21,   SE=.06 Events/N   9/43

Figure S4D

CI at 1.5 Y.

Toxic death p(Gray)= 0.58

Age < 3 Y.   .07,   SE=.01 Events/N 28/405
Age >=3 Y.   .05,   SE=.03 Events/N 2/43
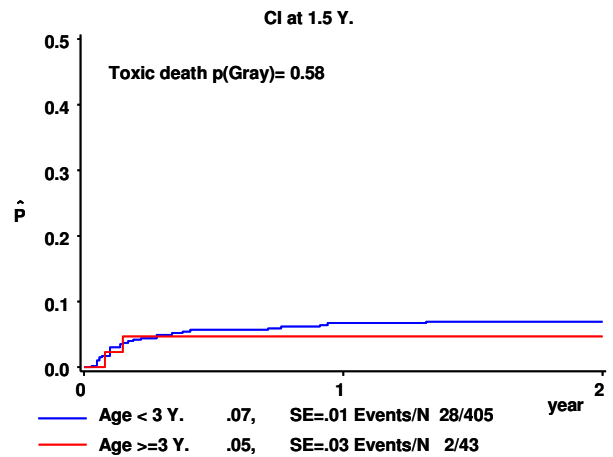
Figure S4: Survival curves for ML-DS (n= 448) patients according to age.
   A. Event-free survival
   B. Overall survival
   C. Cumulative incidence of relapse
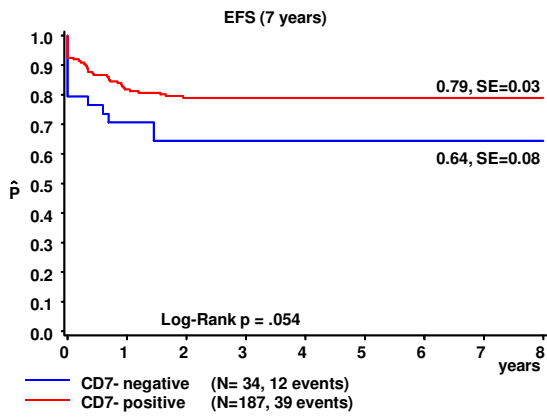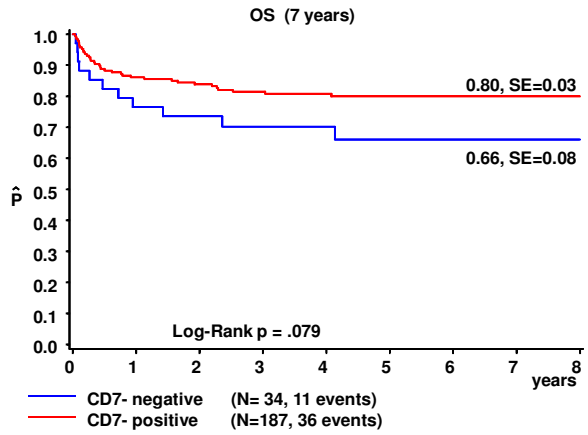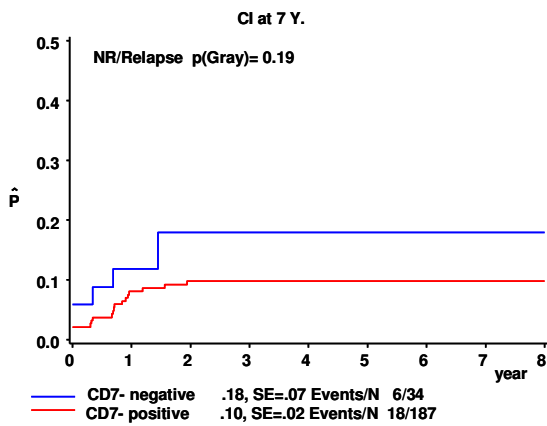   D. Cumulative incidence of relapse

Figure S5A

EFS (7 years)

0.79, SE=0.03

0.64, SE=0.08

Log-Rank p = .054

CD7- negative (N= 34, 12 events)
CD7- positive   (N=187, 39 events)

Figure S5B

OS (7 years)

0.80, SE=0.03

0.66, SE=0.08

Log-Rank p = .079

CD7- negative   (N= 34, 11 events)
CD7- positive   (N=187, 36 events)

Figure S5C

CI at 7 Y.

NR/Relapse p(Gray)= 0.19

CD7- negative   .18, SE=.07 Events/N 6/34
CD7- positive   .10, SE=.02 Events/N 18/187

Figure S5D

CI at 1.5 Y.

Toxic death p(Gray)= 0.61

CD7- negative   .09, SE=.05 Events/N 3/34
CD7- positive   .06, SE=.02 Events/N 12/187
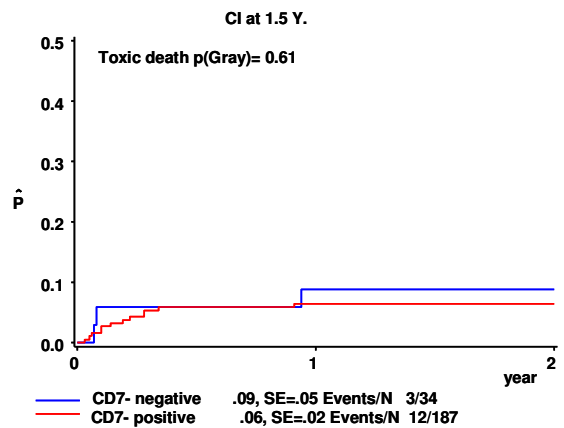
Figure S5: Survival curves for ML-DS patients (n=221), positive and negative for CD7

    A.   Event-free survival curves
    B.   Overall survival curves
    C.   Cumulative incidence of relapse
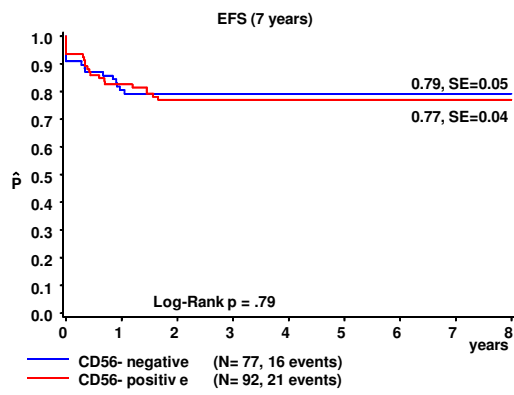   D.   Cumulative incidence of toxic death
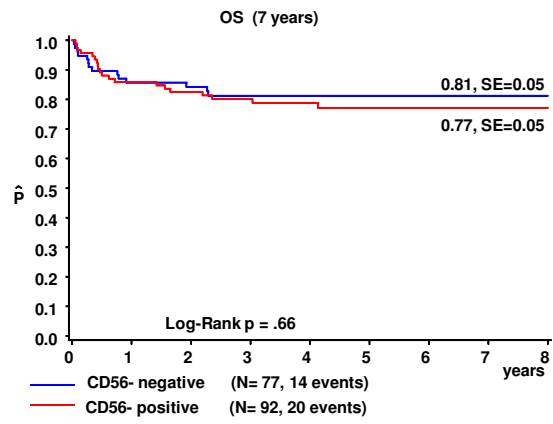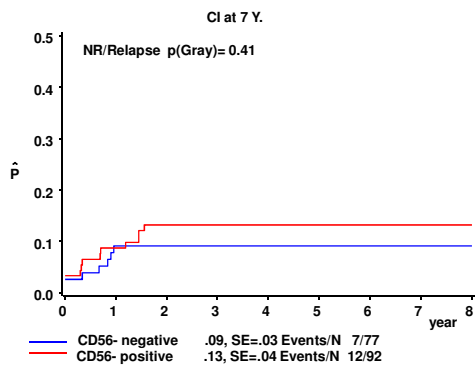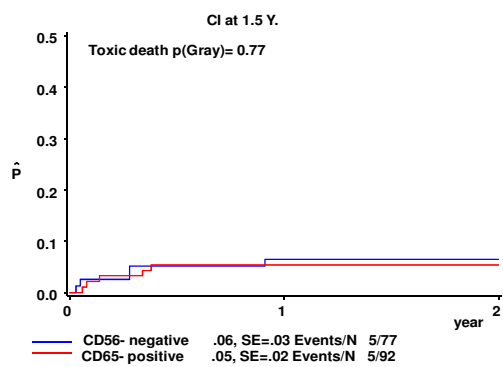
Figure S6A

Figure S6B

EFS (7 years)

0.79, SE=0.05

0.77, SE=0.04

Log-Rank p = .79

CD56- negative   (N= 77, 16 events)
CD56- positiv e   (N= 92, 21 events)

OS (7 years)

0.81, SE=0.05

0.77, SE=0.05

Log-Rank p = .66

CD56- negative   (N= 77, 14 events)
CD56- positive   (N= 92, 20 events)

Figure S6C

Figure S6C

CI at 7 Y.

NR/Relapse p(Gray)= 0.41

CD56- negative   .09, SE=.03 Events/N 7/77
CD56- positive   .13, SE=.04 Events/N 12/92

CI at 1.5 Y.

Toxic death p(Gray)= 0.77

CD56- negative   .06, SE=.03 Events/N 5/77
CD65- positive   .05, SE=.02 Events/N 5/92

Figure S6: Survival curves for ML-DS patients (n=169), positive and negative for CD56

 A. Event-free survival curves
 B. Overall survival curves
 C. Cumulative incidence of relapse
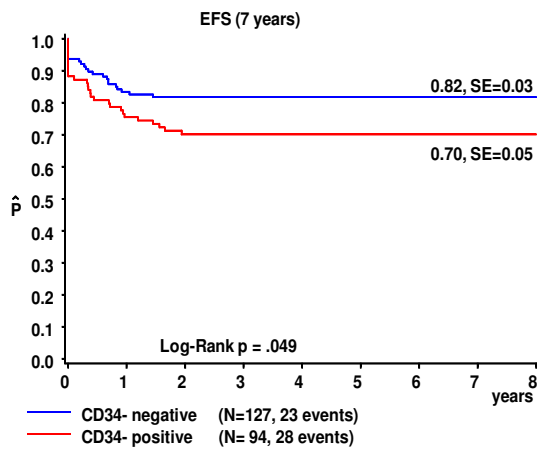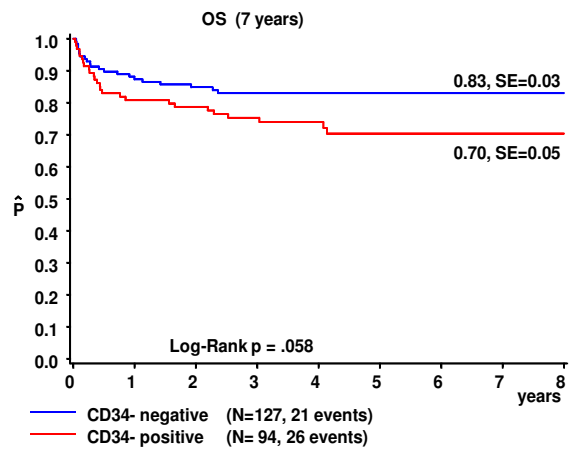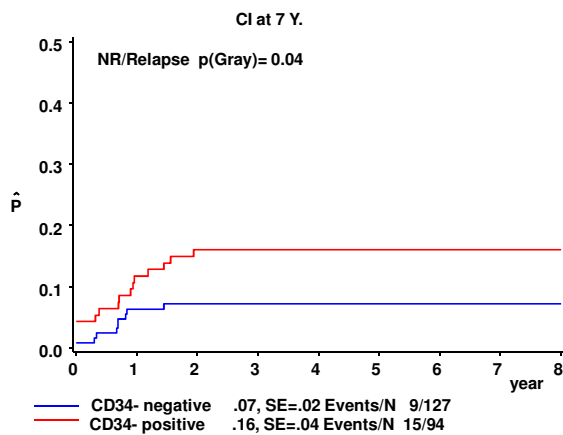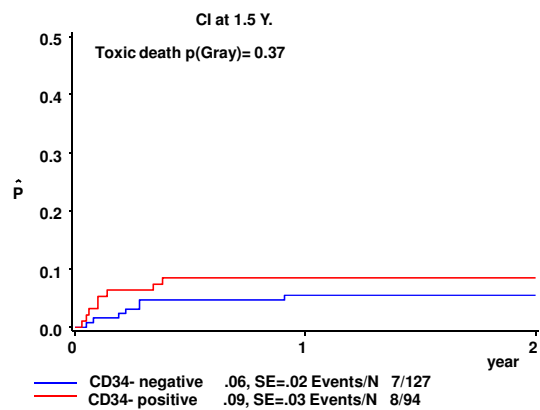 D. Cumulative incidence of toxic death

Figure S7A

EFS (7 years)

0.82, SE=0.03

0.70, SE=0.05

Log-Rank p = .049

— CD34- negative   (N=127, 23 events)
— CD34- positive   (N= 94, 28 events)

Figure S7B

OS (7 years)

0.83, SE=0.03

0.70, SE=0.05

Log-Rank p = .058

— CD34- negative   (N=127, 21 events)
— CD34- positive   (N= 94, 26 events)

Figure S7C

CI at 7 Y.

NR/Relapse p(Gray)= 0.04

— CD34- negative   .07, SE=.02 Events/N   9/127
— CD34- positive   .16, SE=.04 Events/N 15/94

Figure S7D

CI at 1.5 Y.

Toxic death p(Gray)= 0.37

— CD34- negative   .06, SE=.02 Events/N   7/127
— CD34- positive   .09, SE=.03 Events/N   8/94

Figure S7: Survival curves for ML-DS patients (n=221), positive and negative for CD34

    A.   Event-free survival curves
    B.   Overall survival curves
    C.   Cumulative incidence of relapse
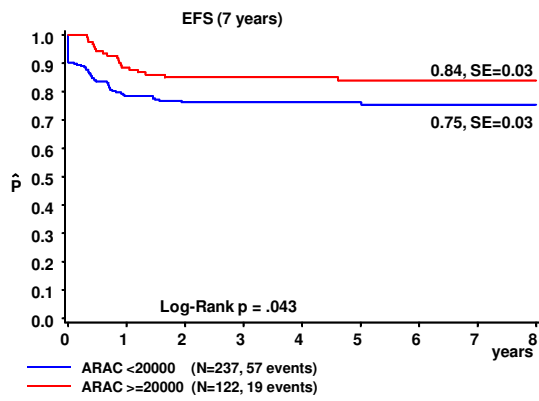   D.   Cumulative incidence of toxic death
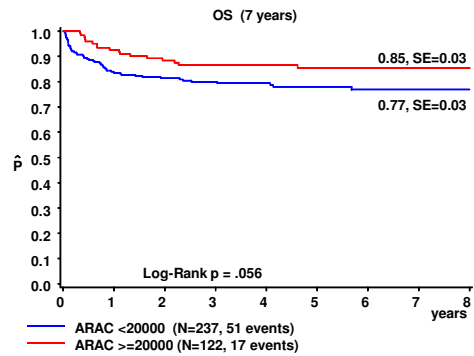
Figure S8A
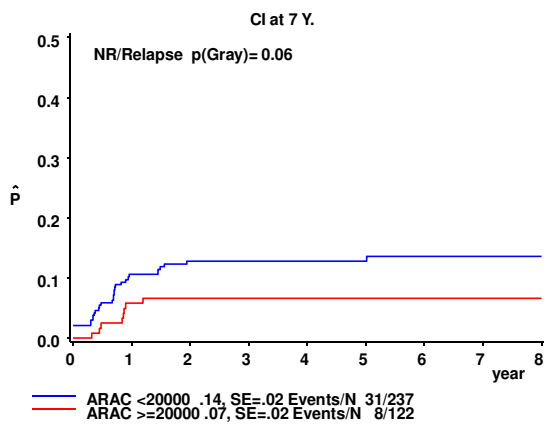

Figure S8B

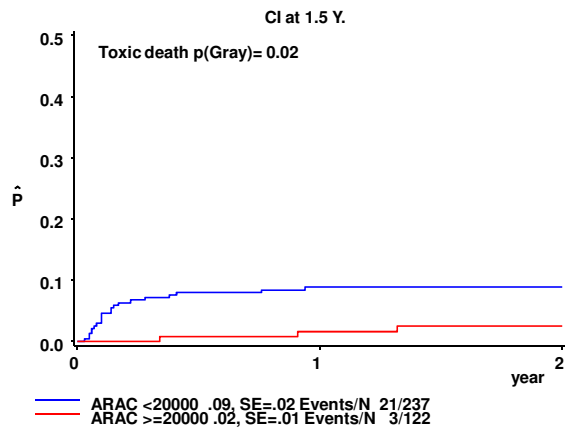**Figure S8C**

Figure S8D

Figure S8: Survival curves for ML-DS patients (n=359) treated with different dosages of cytarabine (Ara-C)
   A.   Event-free survival curves
   B.   Overall survival curves
   C.   Cumulative incidence of relapse
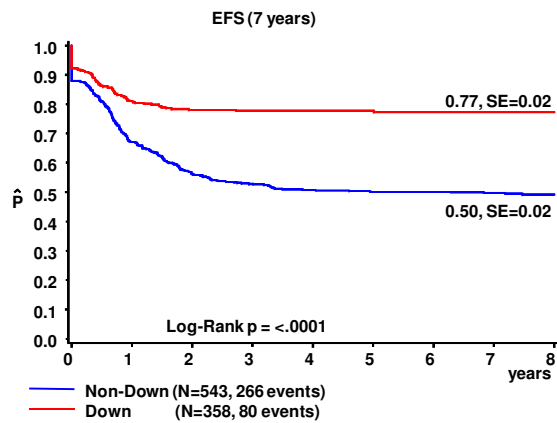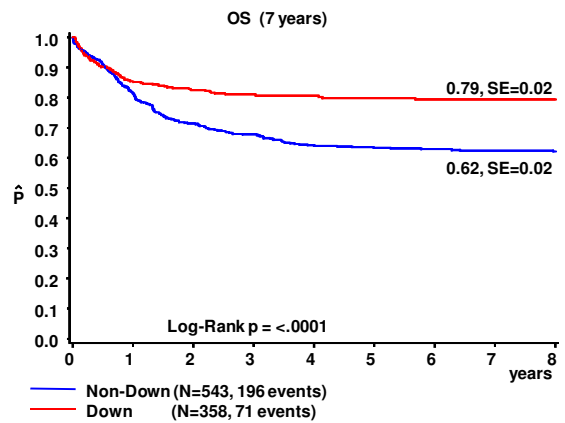   D.   Cumulative incidence of toxic death

Figure S9A

EFS (7 years)

0.77, SE=0.02

0.50, SE=0.02

Log-Rank p = <.0001

Non-Down (N=543, 266 events)
Down   (N=358, 80 events)

Figure S9B

OS (7 years)

0.79, SE=0.02

0.62, SE=0.02

Log-Rank p = <.0001

Non-Down (N=543, 196 events)
Down   (N=358, 71 events)

Figure S9C

CI at 7 Y.

NR/Relapse p(Gray) <.0001

Non-Down NR/Relapse .42, SE=.02 Events/N 222/543
Down   NR/Relapse .13, SE=.02 Events/N 44/358

Figure S9D

CI at 1.5 Y.

Toxic death p(Gray)= 0.12

Non-Down   Toxic death .05, SE=.01 Events/N 24/543
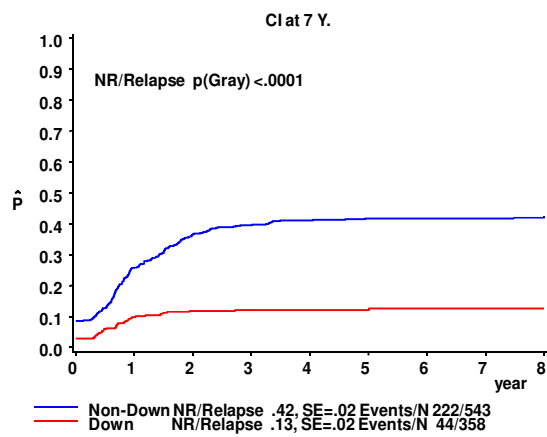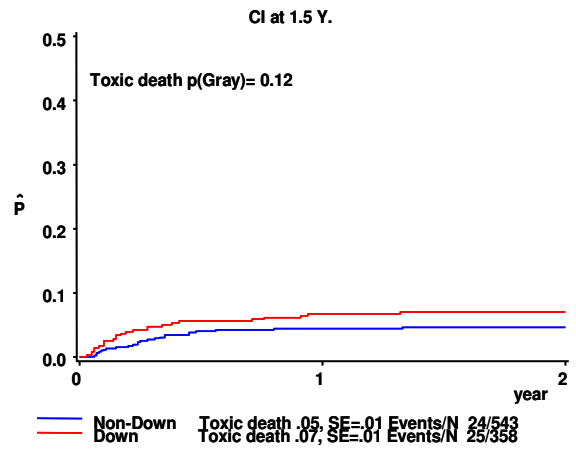Down   Toxic death .07, SE=.01 Events/N 25/358

Figure S9: Survival curves for non- DS AML patients (n=543) compared to ML-DS patients (n=358)
   A. Event-free survival curves
   B. Overall survival curves
   C. Cumulative incidence of relapse
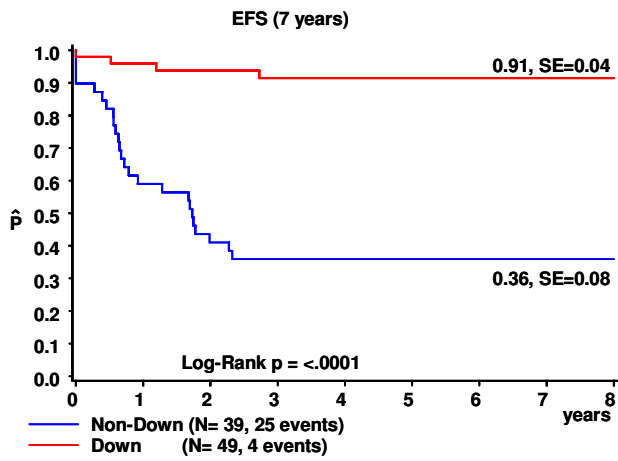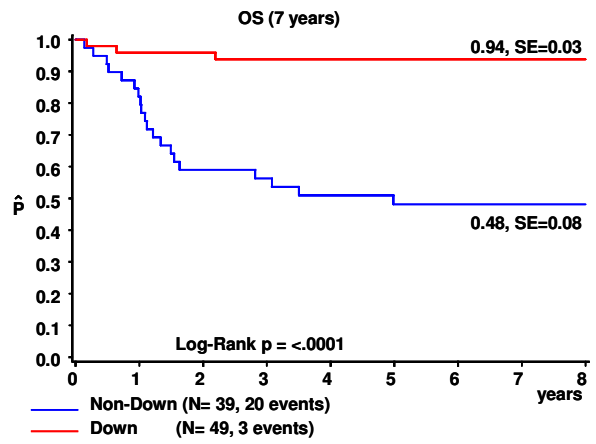   D. Cumulative incidence of toxic death

Figure S10A

EFS (7 years)

0.91, SE=0.04

0.36, SE=0.08

Log-Rank p = <.0001

Non-Down (N= 39, 25 events)
Down   (N= 49, 4 events)

Figure S10B

OS (7 years)

0.94, SE=0.03

0.48, SE=0.08

Log-Rank p = <.0001

Non-Down (N= 39, 20 events)
Down   (N= 49, 3 events)

Figure S10C

CI at 7 Y.

NR/Relapse p(Gray) <.0001

Non-Down .62, SE=.08 Events/N 24/39
Down   .06, SE=.04 Events/N   3/49

Figure S10D

CI at 1.5 Y.

Toxic death (Gray)= 0.88

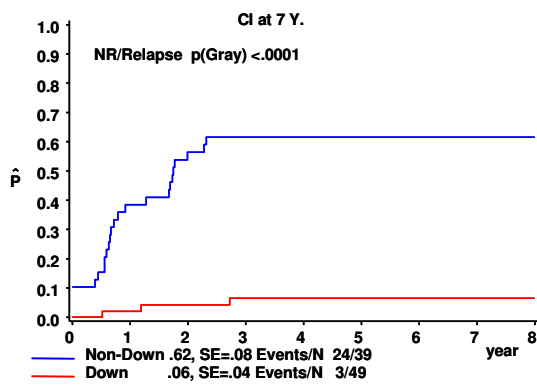Non-Down .03, SE=.03 Events/N 1/39
Down   .02, SE=.02 Events/N 1/49

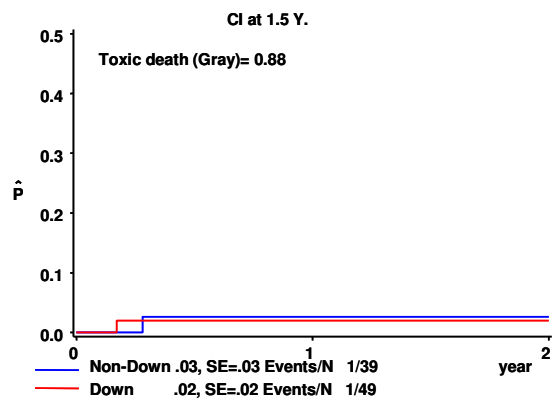**Figure S10: Survival curves for trisomy 8 patients; non-DS AML (n=39) vs. ML-DS (n=49)**
  A. Event-free survival curves
  B. Overall survival curves
  C. Cumulative incidence of relapse
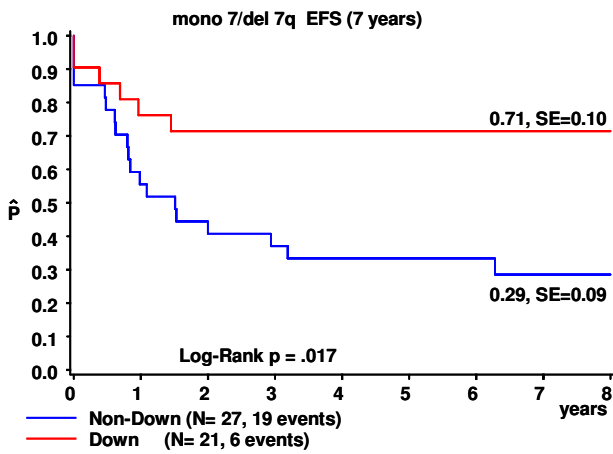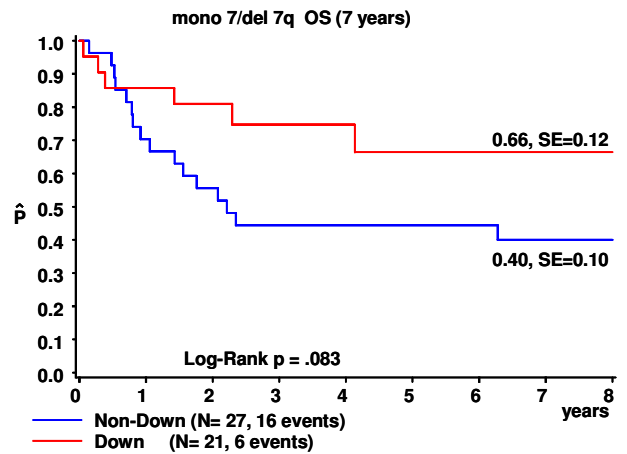 D. Cumulative incidence of toxic death

Figure S11A

mono 7/del 7q EFS (7 years)

0.71, SE=0.10

0.29, SE=0.09

Log-Rank p = .017

Non-Down (N= 27, 19 events)
Down   (N= 21, 6 events)

Figure S11B

mono 7/del 7q OS (7 years)

0.66, SE=0.12

0.40, SE=0.10

Log-Rank p = .083

Non-Down (N= 27, 16 events)
Down   (N= 21, 6 events)

Figure S11C

mono 7/del 7q CI at 7 Y.

NR/Relapse p(Gray)=0.006

Non-Down .52, SE=.10 Events/N 14/27
Down   .14, SE=.08 Events/N 3/21

Figure S11D

mono 7/del 7q CI at 1.5 Y.

Toxic death p(Gray)= 0.41

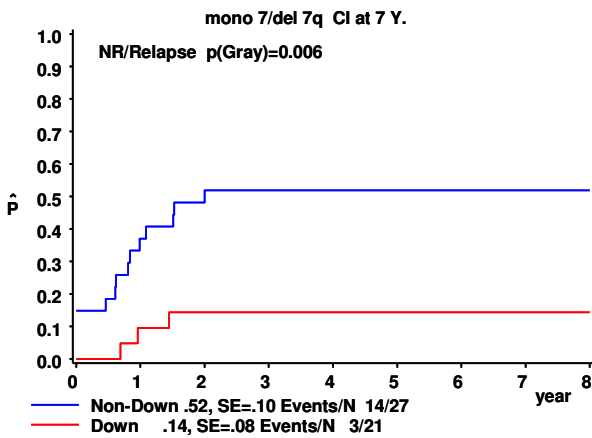Non-Down .07, SE=.05 Events/N 2/27
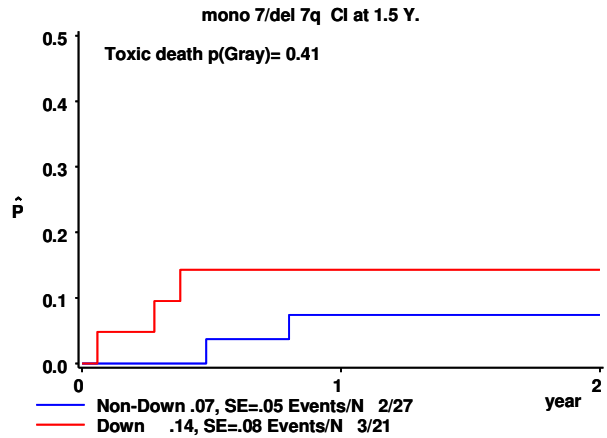Down   .14, SE=.08 Events/N 3/21

Figure S11: Survival curves for patients with monosomy 7/ del 7q; non-DS AML (n=27) vs. ML-DS (n=21)
   A.   Event-free survival curves
   B.   Overall survival curves
   C.   Cumulative incidence of relapse
   D.   Cumulative incidence of toxic death