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Maintenance treatment with azacytidine for patients with high-risk myelodysplastic syndromes (MDS) or acute myeloid leukaemia following MDS in complete remission after induction chemotherapy

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Summary

This prospective phase II study is the first to assess feasibility and efficacy of maintenance 5-azacytidine for older patients with **high-risk MDS, CMML and** MDS-AML syndromes in complete remission (CR) after induction chemotherapy. Sixty patients were enrolled and treated by standard induction chemotherapy. Patients that reached CR started maintenance therapy with subcutaneous azacytidine, 5/28 days until relapse. Promoter-methylation status of ***CDKN2B* (*P15*)**, *e-cadherin* and *hypermethylated in cancer 1* was examined pre-induction, in CR and 6, 12 and 24 months post CR. Twenty-four (40%) patients achieved complete remission after induction chemotherapy and 23 started maintenance treatment with azacytidine. Median CR duration was 13.5 months, >24 months in 17% of the patients, and 18-30.5 months in the four patients with trisomy 8. CR duration **was not associated with** *P15* methylation status or karyotype. Median overall survival was 20 months. Hypermethylation of *E-cadherin* was significantly associated with low CR rate, early relapse, and short OS (P=0.003). 5-azacytidine treatment in a dose of 60 mg/m^2 was well tolerated. Grade III-IV thrombocytopenia and neutropenia occurred after 9.5 and 30% of the cycles, respectively, while hemoglobin levels increased during treatment. 5-azacytidine treatment is safe, feasible and may be of benefit in a subset of patients.

Introduction

Until recently, standard treatment in the Nordic countries for patients with high-risk myelodysplastic syndromes (MDS) or acute myeloid leukaemia (AML) following MDS (MDS-AML) not eligible for stem cell transplantation has been standard induction chemotherapy **or only supportive care including palliative chemotherapy**. Several studies show that around 50% (41-56%) of such patients reach a complete remission (CR). However, CR durations are short with almost no long-term survivors (Hast *et al*, 2003; Ganser *et al*, 2000; Kantarjian *et al*, 2006; Hofmann *et al*, 2007; Wattel *et al*, 1997) and standard consolidation chemotherapy is not associated with prolonged CR (Hast *et al*, 2003; Ganser *et al*, 2000; Hofmann *et al*, 2007; Wattel *et al*, 1997; Kantarjian *et al*, 2006). The dismal prognosis for these patients lead to the search for other treatment regimens, where demethylating therapy is the most promising. In 2002, a randomized study comparing 5-azacytidine (azacytidine) to best supportive care only, reported prolonged time to the composite endpoint leukemic transformation or death in the azacytidine treated group (Silverman *et al*, 2002). **Also decitabine, another hypomethylating agent, had showed to be effective in MDS (Lübbert *et al,* 2001)**. This led us to hypothesize that maintenance treatment with azacytidine for patients in CR after induction chemotherapy might prolong time to relapse and survival. Azacytidine was initially used in high-doses for the treatment of patients with AML but was abandoned in favour for other agents due to severe gastrointestinal side effects and prolonged myelotoxicity (O'Dwyer *&* Maslak, 2008). In lower doses, however, azacytidine was later shown to cause DNA demethylation by irreversible inhibition of DNA methyl transferases (Singal *&* Grinder, 2009; Esteller, 2008; Grønbaek *et al*, 2008). The exact mechanisms of action in vivo are not known but it is clear that it can cause hypomethylation and re-expression of

previously silenced genes as well as induction of apoptosis **and immunomodulation** (Guo *et al*, 2006; Berg *et al*, 2007; Schmeltz *et al*, 2005; Khan *et al*, 2008; **Sánchez-Abarca *et al*, 2010**). Genes previously known to be frequently methylated in MDS include ***CDKN2B* (*P15*)**, *E-cadherin* (*CDH*) and *Hypermethylated in Cancer 1* (*HIC*). Promoter-methylation of these genes has also been reported to be associated with poor prognosis and leukemic transformation of MDS (Aggerholm *et al*, 2006; Tien *et al*, 2001; Christiansen *et al*, 2003). We recently reported a strong association between promoter-methylation of *CDH* or of more than one of these three genes and failure to induction chemotherapy (Grövdal *et al*, 2007). This study was designed to assess the feasibility and efficacy of long-term maintenance treatment with azacytidine in a cohort of elderly patients with high-risk MDS and AML following MDS in CR after conventional induction chemotherapy.

Materials and methods

Patients

Patients with intermediate-2 or high-risk MDS, chronic myelomonocytic leukaemia (CMML) with >10% blasts or with AML following a documented MDS phase, according to the WHO 2001 classification and the International Prognostic Scoring System (IPSS), were eligible for the protocol (Jaffe *et al*, 2001; Greenberg *et al*, 1997). **Patients should not be eligible for AML-like induction chemotherapy followed by intensive consolidation courses and allogeneic stem cell transplantation**, but should be considered to tolerate at least one cycle of standard induction chemotherapy. The study was approved by ethical committees and medical product agencies of the participating Nordic countries. All patients gave their written

informed consent. Diagnosis as well as CR was verified at a central haematopathology unit (A.P.) according to established Nordic MDS Group routines (Grövdal *et al*, 2007; Jädersten *et al*, 2005). Bone marrow cellularity was assessed on bone marrow biopsies and the percentage of blasts in bone marrow smears was determined by counting 500 cells in representative areas. Cytogenetic analyzes were performed locally only at enrolment, using standard techniques and patients were classified according to the IPSS into good, intermediate, or poor prognostic subgroups (Greenberg *et al*, 1997). The criteria for CR were <5% bone marrow blasts, stable haemoglobin >100 g/L, WBC >1.5 x 10^9 with normal differential count and platelets >100 x 10^9. Persistent dysplastic features were allowed.

Study design

Induction chemotherapy consisted of a DA regimen: daunorubicin 60 mg/m^2 i.v. day 1 and 2 and cytarabine 150 mg/m^2 s.c. or i.v. days 1 to 7. Patients could have a second induction if they did not reach CR on the first one and were judged fit enough by treating physician. No standard consolidation courses were given. **Patients that did not reach CR were given best supportive care with or without low-dose palliative chemotherapy, such as hydroxyurea, according to the choice of the treating physician and were followed only for survival**. Patients achieving CR started maintenance therapy with azacytidine given subcutaneously 5/28 days starting within 28 days from CR. The protocol specifically aimed at administering this treatment on an outpatient basis, and prolonged grade 4 haematological adverse events therefore constituted a basis for dose reduction. Initial azacytidine dose was 75 mg/m^2 but, due to high incidence of grade 4 neutropenia in the first five enrolled patients, the protocol was amended and the starting dose was reduced to 60 mg/m^2. Further reduction of the azacytidine dose was also allowed to avoid severe

cytopenias and hospitalization. Patients continued on azacytidine until relapse or intolerable toxicity. Anti-emetic treatment was given prior to azacytidine. The primary endpoint was duration of CR and secondary endpoints were overall survival and the impact of pre-treatment parameters on prognosis.

Bone marrow sampling, DNA isolation and bisulfite modification

Bone marrow for methylation analyzes was sampled in standardized flasks and medium at enrolment, at CR and 6, 12 and 24 months after CR. Samples were shipped by DHL using a <24 h service to a central laboratory (Lund) for isolation of mononuclear cells (MNC) and CD34+ cells by density gradient technique by Lymphoprep (Axis Shield) and magnetic bead cell sorting (MACS, Miltenyi Biotech) as previously described (Grövdal *et al*, 2007; Nilsson *et al*, 2002). Cells were stored as pellet at -80℃. Genomic DNA was isolated from MNC and CD34+ cells using the QiAmp DNA mini kit (Qiagen) according to manufacturer's guidelines. Bone marrow sampled in CR and during follow up frequently rendered an insufficient CD34+ yield, and methylation analyzes were therefore consequently performed on un-separated MNC, after first confirming a strong correlation between methylation results in CD34+ and MNC on the pre-induction samples (P<0.001) (Grövdal *et al*, 2007). The amount of DNA obtained was measured by spectrophotometer (ND-100, Nano-Drop Technologies). DNA was further modified by sodium bisulfite as previously described (Grövdal *et al*, 2007; Zeschnigk *et al*, 1997).

Polymerase chain reaction and denaturing gradient gel electrophoresis (DGGE)

PCR specific for bisulfite-reacted *P15*, *CDH* and *HIC* promoters was carried out as previously described (Aggerholm *et al*, 2006; Grövdal *et al*, 2007). **Primer**

sequences were: [CCGCC]-GTTAGGAGTTTTTTTTTAGAAGTAATTT (*P15*: F), [GC3]-AAACTAAACTCAACTTCATTACCCTC (*P15*: R), [GC1]-GTTTATTTGGT-TGTAGTTA (*CDH*: F), CTCCAAAAACCCATAACTAAC (*CDH*: R), [GC1]-ATAATT-AGAGTATTAAGGGTTTTTTGTG (*HIC*: F), [CGCCCGCCGC]-CACCCAAAAACT-TAAAATAAACACTACTA (*HIC*: R). Nucleotides in brackets represent GC-clamps; [GC1] = CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCG, [GC3] = CCCGCCGCCCGCCGCTCGCCCGCCGCGCCCCGCGCCCGTCCCGCC-GCCCCCGCCCG. PCR results were examined by electrophoresis in a 2.5% agarose gel. Fifteen to 20 µL of the PCR product was loaded onto a 10% denaturant/6% polyacrylamide -70% denaturant/12% polyacrylamide gradient gel. A fully methylated control (SssI) and an unmethylated control (peripheral blood lymphocytes) were also loaded to each gel. Gels were run at 160 V for 270 minutes in 1 x Tris acetate/EDTA buffer kept at a constant temperature specific for each gene examined as previously described (Aggerholm *et al*, 2006; Grövdal *et al*, 2007). After the electrophoresis the gels were stained in Tris acetate/EDTA buffer containing ethidium bromide (2 mg/mL) and photographed under UV transillumination. Samples were scored as methylated when **bands were** present on the gels below the band corresponding to the unmethylated control (Aggerholm *et al*, 2006; Cremonesi *et al*, 1997).

Pyrosequencing

In a fraction of samples, the DGGE results for *P15* (N=29) and *CDH* (N=19) were compared in a blinded way to methylation analysis by pyrosequencing according to a previously described method with a congruence of 80% and 84% respectively (Tost *et al*, 2007; Geli *et al*, 2008). In this article, DGGE results are used when referring to methylation status data.

Statistics

Median haemoglobin levels during azacytidine maintenance were compared using Wilcoxon signed-rank test. Survival analyzes were performed by the Kaplan-Meyer method and compared using the Log Rank test. The size of the material did not allow for an extended multivariate analyzes to be performed. All statistical calculations were carried out using SPSS 15.0 software for windows (SPSS Inc, Chicago, IL, USA).

Results

Patients

Sixty patients (median age 68 years, range 54-83) were enrolled between February 2004 and June 2006, with the last follow up per 1'st of August, 2008, 24 months after the last CR was reported. Median follow up time was 20.0 months (4.5-52.3). Clinical CR data and methylation status before treatment has previously been reported (Grövdal *et al*, 2007). Of 24 patients (40%) who achieved CR, one underwent allogeneic stem cell transplantation and was taken off study, and 23 patients (median age 70 years, range 62-76) started maintenance therapy with azacytidine. Ten patients had MDS (RAEB-1 (1) or RAEB-2 (9)), 10 patients had AML, and 3 CMML-2 with >10% blasts. All AML patients had AML with multilineage dysplasia following a myelodysplastic syndrome according to WHO 2001. The median blast count in this group was 36% (range 20-98). RAEB patients fulfilled the criteria for IPSS intermediate-2 or high risk, and the CMML patients had myelodysplastic features with marrow blasts >10%. Twelve patients had a favourable prognostic karyotype (normal or isolated - Y, del(5q) or del(20q)) according to the IPSS, 6 had a poor prognostic

karyotype (≥3 aberrations or chromosome 7 abnormalities) and 5 had an intermediate karyotype (not fulfilling the criteria for good or poor) (Table I).

Feasibility and safety of azacytidine maintenance treatment

In case of grade 3 or 4 cytopenia or severe adverse event after azacytidine, the subsequent course was delayed with one week. However, according to protocol, a maintained interval between azacytidine courses was prioritized to dose and consequently, the protocol was amended when 3 of the first 5 patients treated with azacytidine 75 mg/m^2 developed grade 3 (n=1) or 4 (n=2) neutropenia. The new starting dose was 60 mg azacytidine per m^2 and further dose reductions were allowed to avoid severe cytopenias and hospitalization. The median dose of all administered azacytidine cycles was 56.3 mg/m^2/day. The administered mean dose for each patient ranged between 30.0 and 63.3 mg/m^2. Median time between azacytidine courses was 29 days (20-53). The most frequent adverse event was grade 3 or 4 neutropenia which was reported at any time point in 43.5% and 30.5% patients respectively (Table II). However, only 22% (grade 3) and 8% (grade 4) of the total number of given courses were associated with neutropenia of this magnitude. Thrombocytopenia grade 3 occurred in 43.5% of the patients and after 9.5% of the courses. No grade 4 thrombocytopenia was reported. The majority of observed cytopenias preceded a relapse. Interestingly, haemoglobin levels rose during the first courses in 16/23 patients. Median haemoglobin level before azacytidine cycle 1 was 112 g/L (87-135) compared with 131 g/L (78-151) before cycle 4 (P=0.02) (Figure 1). Local rash at the injection site was common and was reported for 8 patients (35%). All adverse events are shown in Table 2. All but one patient stopped azacytidine because of relapse. This patient had a thrombosis in the optic artery and lost vision in this eye.

CR duration

Median CR duration for the 23 azacytidine treated patients was 13.5 months (2-49+). Four patients (17%), without any obvious unifying characteristics, had a CR lasting for more than 24 months and two patients were still in CR at the last follow up. The study was not powered for subgroup analysis, however, no obvious differences in CR duration or survival were observed according to age or pre-induction diagnosis or cytogenetic subgroup (Figure 2). Pre-treatment platelet count below median was the only factor associated with time to relapse (P=0.04). The actual maintenance dose given (above or below the median of 56.3 mg/m^2) did not affect time to relapse. Four out of 5 patients with a karyotype including trisomy 8 reached CR. Interestingly, all 4 had CR durations well above the median for the whole maintenance group (18-30.5 months).

A secondary objective of this study was to evaluate whether promoter methylation of selected genes could predict response to azacytidine. Eight of 9 patients with promoter methylation of any of the analyzed genes became unmethylated in CR. *P15* methylation status prior to induction chemotherapy did not correspond to CR duration (P=0.82). Only 2 of 15 patients with pre-treatment *CDH* methylation reached CR and they both had very short CR durations (2.5 and 6 months, respectively). One patient developed *P15* methylation in the bone marrow sampled at 12 months after CR and relapsed shortly after, at 15.5 months. Figure 3 shows methylation status and relapse information for all patients.

Overall survival

Median overall survival (OS) for the 23 patients who received maintenance treatment was 20.0 months (4-52+) (Figure 4). Two year survival was 37.5% and 13.9%, in

patients achieving CR and not achieving CR, respectively. Median OS for the whole cohort (N=60) was 8.2 months (0.1-52.3+). *CDH* was methylated in 15 of 41 evaluable patients pre-treatment. Median OS for the 15 patients with pre-treatment *CDH* methylation was 4.0 months (0.1-15.7), compared to 9.3 months (0.5-43.5+) for patients without *CDH* methylation (P=0.003). *P15* methylation status did not affect survival (0.83). In addition a diagnosis of MDS-AML vs. RAEB or CMML, CD34+ expression and platelet counts below median were associated with shorter survival (P=0.04, 0.018 and 0.006 respectively) (Figure 4). There was no significant association between IPSS cytogenetic subgroup and survival.

Discussion

Prolonged therapy with azacytidine was recently shown to significantly improve survival and time to AML transformation in Intermediate-2 and high-risk MDS patients, according to a large randomized phase III trial, data that recently led to the approval of the drug by EMEA and that are likely to influence the European guidelines for treatment of this patient category (Fenaux *et al*, 2009). However, when the present study was designed in 2002 primary therapy for high-risk and transformed MDS in most parts of Northern Europe was moderate intensity induction chemotherapy **or supportive care only**. There is overwhelming evidence that disease recurs in the vast majority of patients achieving a complete remission unless allogeneic stem cell transplantation is performed. Most patients relapse within the first year, and conventional consolidation chemotherapy does not seem to prolong CR duration (Hast *el al*, 2003; Ganser *et al*, 2000; Hofmann *et al*, 2007; Wattel *et al*, 1997; Kantarjian *et al*, 2006). If there is a potential benefit from autologous stem cell

transplantation, this is restricted to younger patients (De Witte *et al*, 1997; Wattel *et al*, 1999).

This trial is the first to evaluate safety and feasibility of maintenance treatment with azacytidine for patients with high-risk MDS and MDS-AML with CR after induction chemotherapy. The hypothesis was that azacytidine maintenance could prolong time to relapse and that the results might constitute a basis for a prospective randomized phase III trial.

Azacytidine treatment was well tolerated at a starting dose of 60 mg /m^2 5/28 days, while higher doses, administered within 28 days from complete remission, induced high degree of grade 3 or 4 neutropenia. Also at the lower starting dose, the most common adverse event was grade 3 neutropenia and/or thrombocytopenia. Interestingly, azacytidine treatment rarely induced anaemia and in fact more than two thirds of the patients experienced an improvement in haemoglobin levels during the first months of treatment (Fig 1). As thrombocytopenia and neutropenia was common, this may not just reflect bone marrow recovery after induction chemotherapy but a positive direct effect of azacytidine on erythropoiesis. Other side effects were mild and manageable and only one patient stopped treatment due to side effects.

CR duration of 13.5 months and an overall survival of 20.0 months is not clearly different from previous studies on induction chemotherapy for patients with high-risk MDS or MDS-AML; however the majority of these studies included slightly younger patients (Hast *et al*, 2002; Ganser *et al*, 2000; Kantarjian *et al*, 2006; Hofmann *et al*, 2007; Wattel *et al*, 1997). Studies on older patients with AML by the SWOG and the HOVON groups, including both de novo cases and secondary AML have shown

overall survivals of 9 and 10 months respectively (Anderson *et al*, 2002; van der Holt *et al*, 2007). Clearly however, azacytidine did not seem to prevent relapses in the majority of the cases.

The question that remains to be investigated is whether relapse was delayed in a subset of patients. This present study was not powered to analyze subgroups. However, we conclude that there were no obvious differences in CR duration or overall survival with regard to age, diagnosis or cytogenetic subgroup, i.e. parameters that in other studies have appeared as prognostic markers for outcome (Ganser *et al*, 2000; Wattel *et al*, 1997; Kantarjian *et al*, 2006). One interesting subgroup was patients with a karyotype including trisomy 8. Four out of 5 patients with trisomy 8 reached CR and all 4 had CR durations well above the median for the whole maintenance group. **This is interesting, since a better response to azacytidince also previously has been reported among patients with trisomy 8** (Raj *&* Mufti, 2006). **Our findings might support a positive effect of azacytidine maintenance in this group but could also just reflect a more robust response to induction chemotherapy. There are previous reports in differences in the immune response between MDS patients with trisomy 8 and other MDS (Meers *et* al, 2007; Kawabata *et al,* 2006) and also on immunomodulatory effects of azacytidine (Sánchez-Abarca *et* al, 2010; Liu *et al*, 2009; Laurenzana *et al*, 2009). Whether immunomodulation, by demethylation or not, may explain the better overall outcome in this group remains to be studied.** As previously reported by us, methylation of *CDH* or of multiple genes was associated with a poor response to induction chemotherapy (P=0.008) (Grövdal *et al*, 2007). Hence, very few patients with any other gene than *P15* methylated actually reached CR and, accordingly, most of these patients never started azacytidine maintenance. In the

whole study population, *CDH* methylation was related to significantly shorter survival (P=0.003). The only two patients with *CDH* methylation subjected to azacytidine maintenance relapsed early, after 2.5 and 6 months. This strengthens our previous observation that *CDH* methylation is a marker for poor overall prognosis and poor response to induction chemotherapy. Interestingly, methylation status of *P15* was not correlated to CR duration or survival, which contradicts previous reports that *P15* methylation predicts a worse outcome (Aggerholm *et al*, 2006; Christiansen *et al*, 2003). Whether this reflects a selection bias, i.e. *P15* methylation will not add further to the risk profile in a cohort with a high proportion of other high-risk features and confirmed AML transformation, or if it may indicate that azacytidine maintenance actually counteracted the negative effect of *P15* methylation remains to be investigated. At this stage we can only conclude that methylation analysis of any of the three selected genes cannot be used to select patients for maintenance treatment with azacytidine.

Dose reduction of azacytidine was allowed to maintain dose interval and to avoid potentially hospitalizing adverse events in this elderly patient group. Hence, insufficient azacytidine doses might have influenced the overall results. **However, patients treated with doses above or below the median dose showed no differences with regard to any of the measured outcomes.**

In the majority of patients with pre-treatment methylation of any of the three genes, achievement of CR was associated with a disappearance of methylation. Together with the finding of a development of *P15* methylation preceding relapse in one patient this supports a previous report proposing promoter methylation as a marker for residual disease (van der Holt *et al*, 2007). It may be of future interest to clarify if this

is due to demethylation of cells belonging to the clone, or if the methylated clone is reduced below detection level by cytostatic treatment.

This study is the first to evaluate azacytidine as maintenance treatment after successful induction chemotherapy in high-risk and transformed MDS. We show that treatment is very well tolerated, with manageable neutropenia and thrombocytopenia, almost no inhibiting effect on erythropoiesis, and few other side effects. Although no overall positive effect on CR duration, the main efficacy criterion, was observed, certain subgroups of patients, such as those with trisomy 8, may be subject for further investigation. The strong negative effect of hypermethylation on outcome of chemotherapy is a finding that needs to be addressed in high-risk MDS, in particular in patients planned for allogeneic SCT.

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Legends to figures

Figure 1. Peripheral blood counts during first 6 courses of azacytidine. Samples were taken immediately prior to the start of azacytidine. (A),(C),(E) Each line represents one patient during 6 cycles or until relapse. (B),(D),(E) Box plots. Boxes show median values and 25-75 percentile, whiskers represent max and min values, circles represent outliners (values between 1.5 and 3 box lengths from either end of the box) and asterisks represent extremes (values more than 3 box lengths from either end of the box). (A),(B) Haemoglobin (g/L) (C),(D) Platelets (10^9/L) (E),(F) Neutrophils (10^9/L)

Figure 2. CR duration. (A) All 23 patients on azacytidine maintenance (B) MDS-AML vs. RAEB and CMML (C) Cytogenetics IPSS Good vs. Intermediate and Poor (D) Platelet counts above median vs. below median at study start (E) **P15 (CDKN2B)** methylated vs. unmethylated at study start (F) Patients given doses of azacytidine above vs. below the mean dose

Figure 3. Methylation status. Methylation status for each of the 23 patients receiving azacytidine maintenance at the time points when bone marrow was sampled (study start, CR and 6, 12 and 24 months after CR) Patients with karyotype including trisomy 8 are marked with a asterisk.

Figure 4. Overall survival. (A) Overall survival for all 60 patients enrolled (B) MDS-AML vs. RAEB and CMML (C) Bone marrow CD34+ above vs. below the median level (D) Platelets at study start above vs. below the median (E) **P15 (CDKN2B)** methylated vs. unmethylated at study start (F) CDH methylated vs. unmethylated at study start

Table I. Pre-induction characteristics of 23 patients receiving maintenance therapy with azacytidine

Values at start of study	N=23 (%)	Mean (SD)
Sex Male Female	 12 (52) 11 (48)	
Age		70 (62-76)[1]
Diagnosis WHO MDS MDS-AML CMML	 10 (43.5) 10 (43.5) 3 (13)	
Cytogenetic risk group IPSS[2] Good Intermediate Poor	 12 (52) 5 (22) 6 (26)	
Haemoglobin (g/L)		97 (10.1)
WBC (10^9/L)		6.5 (14.4)
Platelets (10^9/L)		121 (96.9)
S-LDH (µkat/L)		5.8 (5.5)
Bone marrow cellularity (%)		75 (21.4)
Bone marrow blasts (%)		26 (24.2)
Methylated *P15* (***CDKN2B***)	9/19 (47.4)	
Methylated *CDH*	2/16 (12.5)	
Methylated *HIC*	2/16 (12.5)	

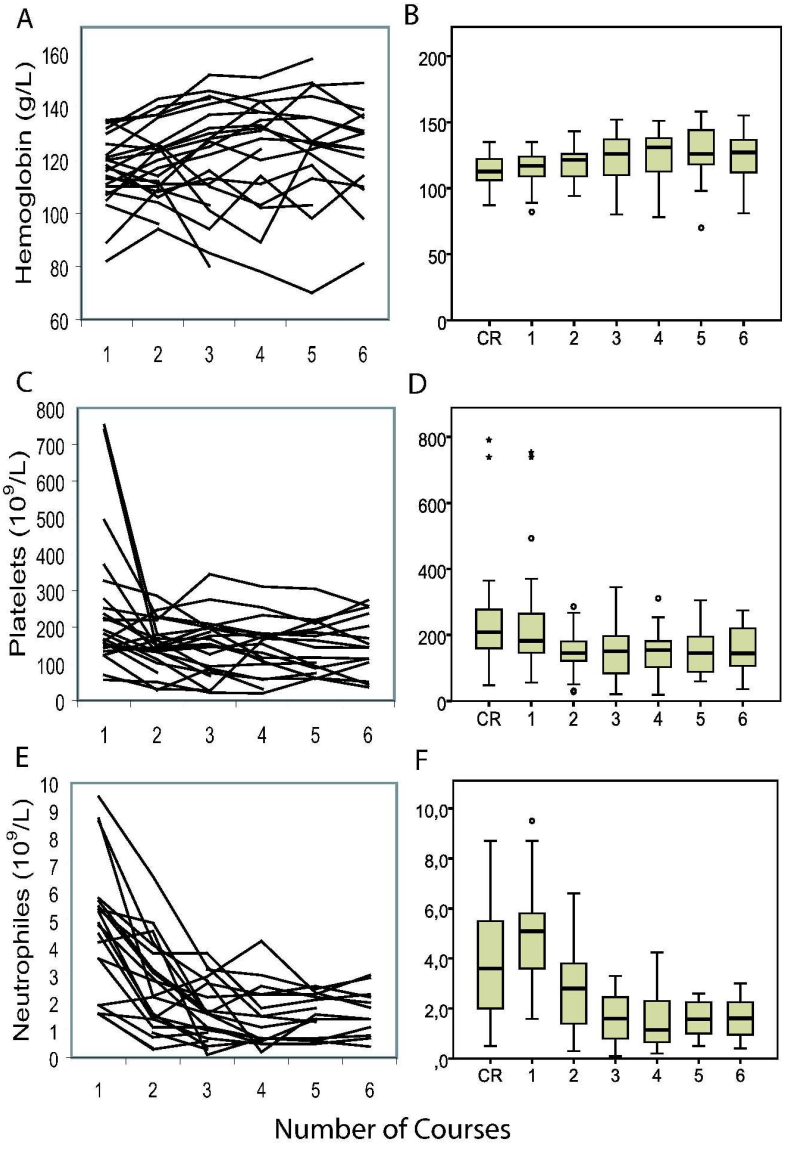
[1]median and range [2]International Prognostic Scoring System (Good: Normal, del(5)q, del(20)q Poor: Complex (≥3 anormalities) or chromosome 7 anomalities Intermediate: Not fulfilling criteria for good or poor

Table II. Adverse events

	Patients N=23 (%)	Courses N=281 (%)
Rash at injection site	8 (35)	
Myelosuppression[1]		
Thrombocytopenia		
Grade 0-1	9 (39)	223 (79.5)
Grade 2	4 (17.5)	31 (11)
Grade 3	10 (43.5)	27 (9.5)
Grade 4	0 (0)	0 (0)
Neutropenia		
Grade 0-1	3 (13)	124 (44.5)
Grade 2	3 (13)	72 (25.5)
Grade 3	10 (43.5)	61 (22)
Grade 4	7 (30.5)	23 (8)
Infectious disease	5 (22)	
Fatigue	3 (13)	
Muscle pain	3 (13)	
Nausea	2 (9)	
Thrombosis in optic artery	1 (4)	

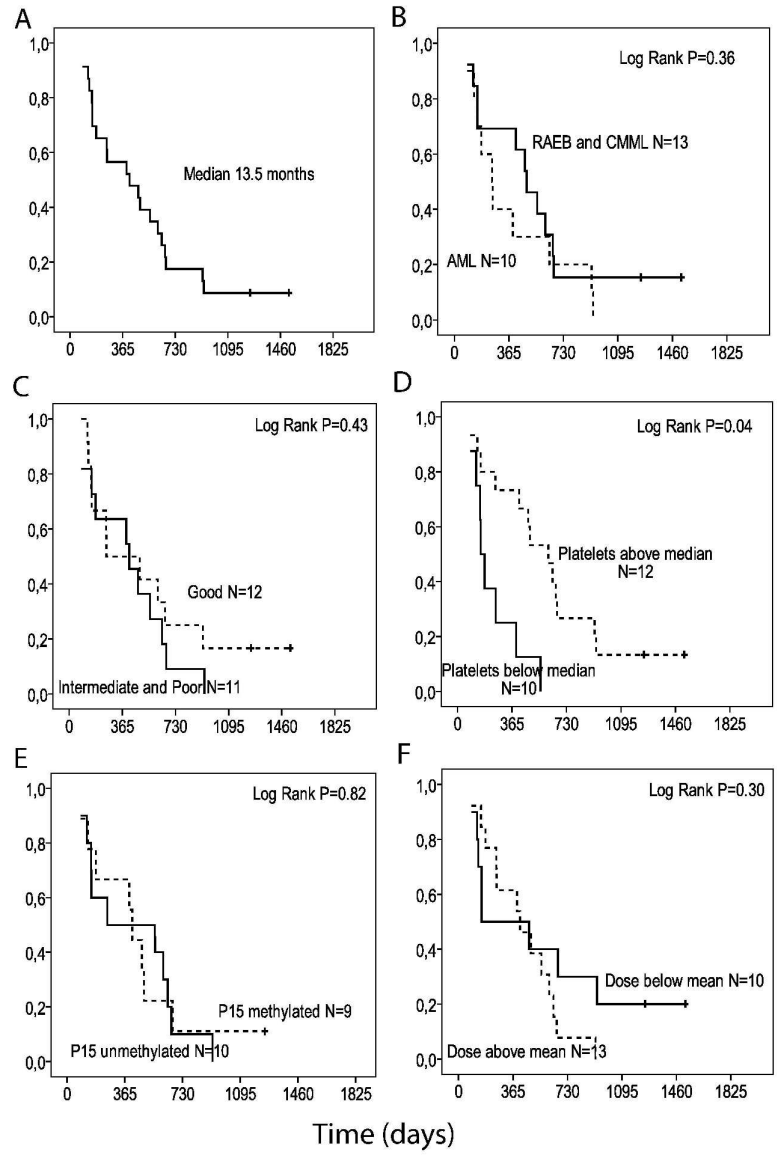
[1]According to the NCI common toxicity criteria 2.0

Figure 1



Peripheral blood counts during first 6 courses of azacytidine. Samples were taken immediately prior to the start of azacytidine. (A),(C),(E) Each line represents one patient during 6 cycles or until relapse. (B),(D),(E) Box plots. Boxes show median values and 25-75 percentile, whiskers represent max and min values, circles represent outliners (values between 1.5 and 3 box lengths from either end of the box) and asterisks represent extremes (values more than 3 box lengths from either end of the box). (A),(B) Haemoglobin (g/L) (C),(D) Platelets (109/L) (E),(F) Neutrophils (109/L)
113x170mm (600 x 600 DPI)

Figure 2



CR duration. (A) All 23 patients on azacytidine maintenance (B) MDS-AML vs. RAEB and CMML (C) Cytogenetics IPSS Good vs. Intermediate and Poor (D) Platelet counts above median vs. below median at study start (E) P15 (CDKN2B) methylated vs. unmethylated at study start (F) Patients given doses of azacytidine above vs. below the mean dose
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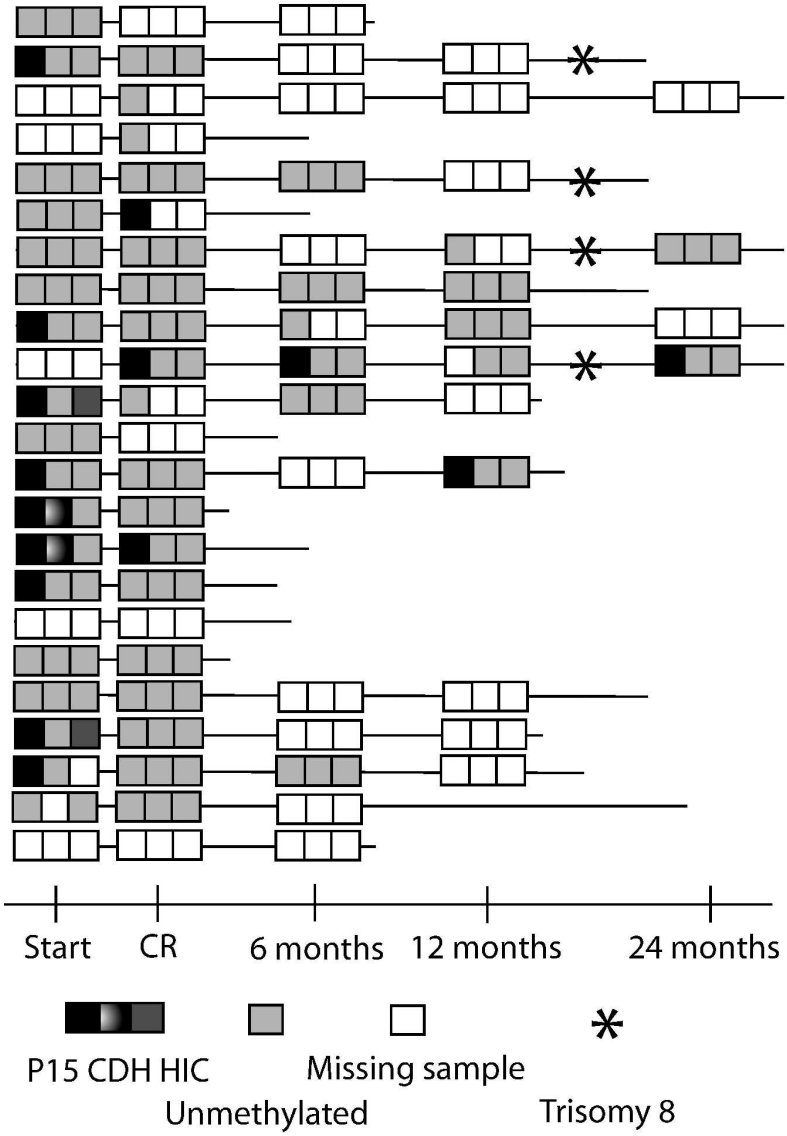
Figure 3



Methylation status. Methylation status for each of the 23 patients receiving azacytidine maintenance at the time points when bone marrow was sampled (study start, CR and 6, 12 and 24 months after CR) Patients with karyotype including trisomy 8 are marked with a asterisk.
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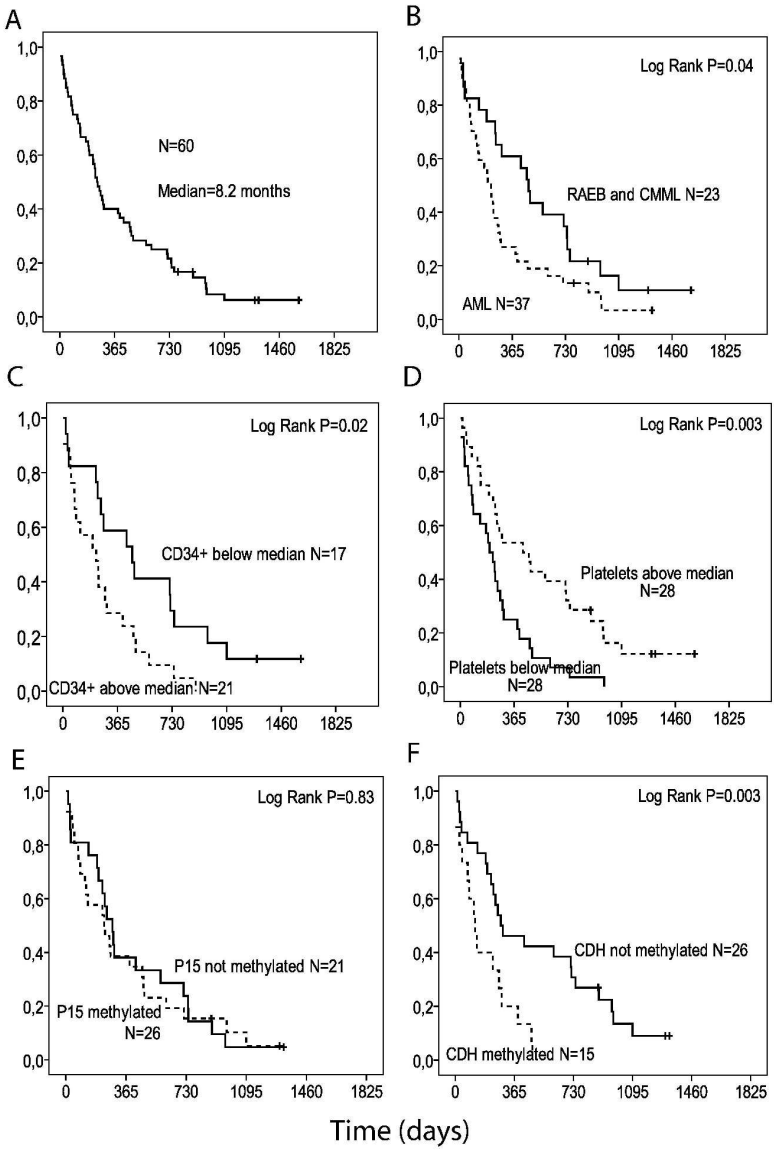
Figure 4



Overall survival. (A) Overall survival for all 60 patients enrolled (B) MDS-AML vs. RAEB and CMML (C) Bone marrow CD34+ above vs. below the median level (D) Platelets at study start above vs. below the median (E) P15 (CDKN2B) methylated vs. unmethylated at study start (F) CDH methylated vs. unmethylated at study start
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