Table 1. Results of hemostatic tests of the patient with VWD type 3 and her parents.

Hemostatic assays	Father	Propositus	Mother
Closure time (Col/Epi) (sec)	120	>300	192
Closure time (Col/ADP) (sec)	84	>300	144
APTT (sec - ratio)	35-1.06	73.6-2.23	37-1.1
Prothrombin index (%)	91	93	95
Fibrinogen concentration (g/L)	3.2	3.6	2.7
FVIII:C (IU/ml)	0.79	0.01	0.87
VWF:RCo (IU/ml)	0.99	<0.05	0.55
VWF:Ag (IU/ml)	0.90	<0.01	0.47
Platelet count $\times 10^9$/L	165	327	210
Blood group	0	0	0

lyzed with GeneScanv3.7 (Applied Biosystems).

We detected a homozygous 11pb deletion c.2574_2584del (Figure 1) on exon 20 of the *VWF* gene in the proband DNA. To our knowledge, this mutation has never been previously described or referenced to sequence databases. This c.2574_2584del results in a premature stop codon: p.Thr859ValfsX2. This frameshift mutation is clearly deleterious, leading to the truncation of the VWF protein or mRNA damage by nonsense mediated mRNA decay, which would explain the total plasma deficiency of VWF. The non-consanguineous parents of the patient were tested for the mutation by sequencing. We found a heterozygous 11pb deletion in the mother and, surprisingly, an absence of this mutation in the father. To test the hypothesis of uniparental disomy (UPD), 16 polymorphic genetic markers mapped to chromosome 12 were investigated. Every marker indicated a homozygosity of the entire chromosome 12 in the proband, consistent with maternal isodisomy.

To our knowledge, there has been no previous report of either paternal or maternal unidisomy involved in VWD. Interestingly, according to a recent review,[11] UPD of chromosome 12 has never been involved in a case of autosomal recessive disease.

This case highlights the need to perform extensive molecular analysis of the *VWF* gene in VWD type 3 patients in order to improve our knowledge of the molecular mechanisms and pathophysiology of the disease. Indeed, the discovery of a deletion indicates a high-risk factor for the occurrence of anti-VWF antibodies and may affect the therapeutic protocol for the patient. Moreover, identifying an association between UPD of chromosome 12 and VWD is important with regard to genetic counseling to inform parents about the negligible recurrence risk during a new pregnancy compared to the usual 25% risk related to an autosomal recessive transmission of type 3 VWD.

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Minimal residual disease-directed preemptive treatment with azacitidine in patients with NPM1-mutant acute myeloid leukemia and molecular relapse

Therapeutic options are often limited in patients with acute myeloid leukemia (AML) who relapse after inten-

sive chemotherapy or allogeneic hematopoietic stem cell transplantation (HSCT); only a few will achieve long-lasting remissions with salvage chemotherapy or a 2nd HSCT. Furthermore, relapse treatment with intensive chemotherapy is often associated with significant morbidity and mortality. Therefore, the prevention of hematologic relapse (HR) remains the main goal in the care of patients with AML.

Patients who are at an increased risk of HR can be identified by monitoring minimal residual disease (MRD) using leukemia specific molecular markers. Among many aberrations recently identified, mutations of the *NPM1* gene, coding for the nucleophosmin protein, are the most frequent genetic alterations in AML with normal karyotype (NK), which allows a polymerase chain reaction (PCR)-based quantification of MRD.[1] In contrast to other molecular aberrations, *NPM1* mutations appear to be stable during the disease course, because they are considered to be a primary pathogenetic lesion in AML.[2] The detection of increasing *NPM1*-defined MRD is predictive of impending hematologic relapse[3-5] and can thus guide early preemptive interventions.

Generally, the optimal therapeutic approach for the treatment of MRD should be associated with a reliable efficacy and also be well tolerated. The DNA-methylation inhibitor 5-azacitidine (AZA) seems to be a promising drug for this treatment indication. Indeed, it is active in patients with AML and advanced myelodysplastic syndrome (MDS),[6] has a low rate of extramedullary toxicity and can be administered on an outpatient basis.

We report for the first time a notable number of patients (n=10) with NK-AML and NPM1 mutation (NPM1+) who underwent preemptive treatment with AZA to avoid HR (Table 1). All patients were in 1st or 2nd complete remission (CR) after intensive pretreatment with conventional chemotherapy, autologous or allogeneic HSC but displayed molecularly detectable MRD consistent with imminent relapse. One of these patients was discussed as a case report in 2010, however only with a short follow up; this has now been extended.[7]

Table 1. Patient and treatment characteristics.

	Median (range)
Age in years	49 (33-68)
	N. (%)
Sex	
Male	5 (50)
Female	5 (50)
AML subtype according to the French–American–British (FAB) classification	
FAB M1	1
FAB M2	4
FAB M4	1
FAB M5	2
Unclassified	2
Karyotype	
normal	10 (100)
Molecular markers	
NPM1 +/FLT3 ITD+	3 (30)
NPM1 +/FLT3 ITD-	7 (70)
Remission status prior to molecular relapse	
1st CR	7 (70)
2nd CR	3 (30)
Pretreatment	
Conventional chemotherapy	5 (50)
(Induction/consolidation) autologous HSCT	2 (20)
Allogeneic HSCT	3 (30)
Time from last therapy to MRD	
Persistent MRD after last therapy	3 (30)
MRD recurrence after ≤6 months	3 (30)
≥6 months	4 (40)

According to our institutional MRD guidelines, screening was routinely performed during the disease course by using sequential real-time PCR specific for NPM1 mutants A, B, and D in bone marrow and peripheral blood samples as described in recent studies.[4,7] In cases of molecular relapse or persistent MRD defined as an increase or a persistence of NPM1/ABL ratio of more than 1% in the bone marrow after the last therapy, preemptive treatment with AZA was initiated at a dose of 75 mg/m²/day s.c. on days 1-7 every 28 days. Two patients received dose-modified therapy (100 mg/day, days 1-5). Molecular response was defined as a minimum of 1-log MRD reduction compared to the baseline MRD value before treatment initiation.

Patients started treatment of MRD with AZA at a median NPM1/ABL ratio of 194% (range 3-7129%) in the bone marrow. The median time from last therapy to molecular relapse was seven months. At this time point all patients were still in CR with a median neutrophil and platelet count of 3.1×10^9/L and 137×10^9/L, respectively. A median of 5 cycles were given (range 2-12 cycles) and were usually well tolerated. The most frequent side effect was myelosuppression with reversible neutropenia and thrombocytopenia grades 3/4 in 80% and 40% of patients, respectively. Although similar results were reported in the AZA001 study,[6] these patients had a manifest active disease, whereas in our study they were treated for MRD, being otherwise not impaired by the imminent disease recurrence. Therefore, patients undergoing AZA treatment for MRD should be monitored closely to avoid a reduction in their quality of life due to cytopenia associated complications. Nevertheless, in future trials the dose of AZA might be further reduced possibly without loosing its efficiency, as recently described by Lima *et al.*[8]

After a median follow-up time of 10 months (range 2-

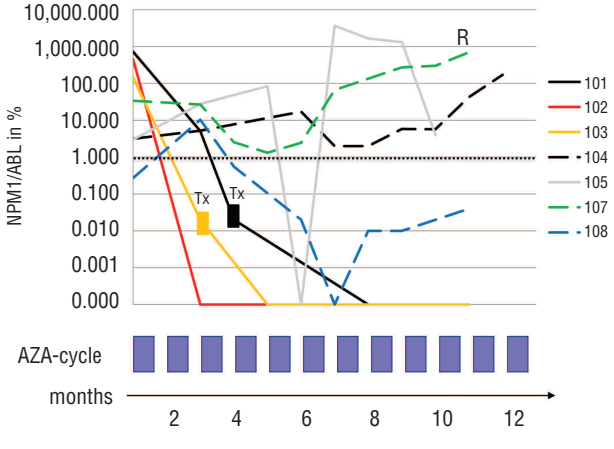

Figure 1. MRD course of 7 patients with molecular response to single agent azacitidine. Tx: date of allogeneic HSCT; R: hematologic relapse. MRD monitoring in bone marrow samples is represented by the continuous lines; dashed lines reflect MRD monitoring from peripheral blood samples.

12 months) from initiation of AZA treatment only 3 patients developed a hematologic relapse. A molecular response with an at least 1-log decrease in the MRD level was observed in 7 of the 10 (70%) patients. Five of them responded within 3 cycles, another 2 patients responded after cycle 4 and 5. However, some patients had only a temporary response consistent with the opinion that AZA treatment might only have delayed the time to hematologic relapse in some patients (Figure 1). A stable course of MRD was documented in one patient and early progressive increase of the NPM1+ clone despite AZA treatment was observed in 2 patients.

Interestingly, the 2 patients with the highest NPM1+ values (>1000%) before initiation of AZA treatment ultimately relapsed after 4 and 6 cycles, respectively. This might reflect the existence of a threshold above which a patient is not likely to obtain long-term benefit from preemptive AZA application.

Among the 7 responders there were 3 patients suffering from molecular relapse after previous allogeneic SCT. One of these showed complete clearance of NPM1+ after 4 cycles. We, therefore, speculate that AZA, besides its direct effects on leukemic cells, might also influence the donor immune system and reconstitute the graft-*versus*-leukemia (GvL) effect as suggested recently.[9]

Taken together, the results of the present analysis are promising, especially when considering the published data describing the natural disease course in NPM1+ AML, with disease progression to full clinical relapse within a median of eight weeks after detection of MRD.[4,5,10] In contrast to these data, we found 7 of 10 patients to be still in complete hematologic remission after a median follow-up time of ten months, suggesting a potential efficacy of AZA in NPM1-defined treatment of MRD. Especially elderly patients and those with a history of serious treatment-related toxicities may benefit from this well tolerated treatment approach. Furthermore, for patients with no available matched donor at the time of molecular relapse, this strategy might be preferable to bridge to subsequent allogeneic HSCT.

Further investigations are necessary to define the patient population who could benefit most from this treatment approach. Since our study included only a relatively small number of patients, prospective clinical trials addressing the impact of MRD-directed therapy with AZA in NPM1 mutant AML are warranted.

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