ance to ATRA after multiple therapies, the best treatment is more questionable: the problem is hampered by the lack of randomized studies which are extremely difficult because of the very small numbers involved, but the association of ATRA plus chemotherapy does not seem effective. Thus, the introduction of new active drugs, such as ATO, is warranted although these need to be very carefully evaluated. In our experience, 8/11 patients achieved HCR and all patients in HCR also achieved MCR. Considering the very advanced phase of disease and the heavy pretreatment, ATO seems to retain a substantial efficacy also in this subset of patients. However, the duration of the MCR was always short. In the present series, autologous transplantation performed in 3rd or more advanced MCR did not produce long-lasting disease-free survival, differently from its beneficial effect when performed in 2nd MCR.[3] As a matter of fact, allogeneic BMT seems to be the only effective treatment capable of prolonging remission after ATO treatment in patients with advanced disease.

In conclusion, while the results of ATO as front-line treatment of APL are awaited, our data highlight the efficacy of this drug in advanced APL and the need to use allogeneic transplantation to consolidate the remission.

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Acute Myeloid Leukemia

5'-(3')-nucleotidase mRNA levels in blast cells are a prognostic factor in acute myeloid leukemia patients treated with cytarabine

We analyzed cytosolic 5'-(3')-nucleotidase (dNT-1) mRNA expression by quantitative polymerase chain reaction at diagnosis in leukemic blasts from 114 patients with acute myeloid leukemia (AML) treated with ara-C. Our results show that low dNT-1 mRNA expression in leukemic blasts at diagnosis is correlated with a worse clinical outcome and suggest that this enzyme may have a role in sensitivity to ara-C in AML patients.

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5'-nucleotidases comprise a large and complex group of enzymes differing in substrate specificity and cellular localization.[1] These enzymes dephosphorylate the monophosphate form of nucleoside analogs and, therefore, may affect the pharmacologic activity of ara-C in the treatment of patients with acute myelogenous leukemia (AML). Overexpression of an IMP-selective 5'-nucleotidase (cN-II) has been associated with ara-C resistance in experimental models[2,3] and in the clinical setting.[4,5] However, to date, the role of other 5'-nucleotidases in resistance to ara-C and their influence on clinical outcome in AML is unknown. The objective of this study was to evaluate the clinical significance of dNT-1 nucleotidase, a cytosolic 5'-(3')-nucleotidase (accession number: NP_055410) which possesses higher affinity for the 5'-phosphates of deoxyribonucleosides and the 2'- and 3'-phosphates of either ribonucleosides or deoxyribonucleosides.[6] For this purpose, dNT-1 mRNA expression was determined in leukemic blasts obtained at diagnosis from bone marrow aspirates and peripheral blood of 114 AML patients treated with ara-C-containing regimens. The patients' characteristics are listed in Table 1. Separation of bone marrow blasts, extraction of cellular RNA, cDNA synthesis and real-time polymerase chain reaction (PCR) analysis were performed as previously described[4] using the following primers and probe: for: 5'-GGACACGCAGGT CTTCATCTG; rev: 5'-GCGGTACTTCTCACCCACACA; probe: 5'-FAM-CTCTTCAGGGCTGCCCATGTCTTGATAMRA. The final amount of mRNA (arbitrary quantitative PCR units) was obtained using RelQuant software (Roche, Mannheim, Germany).

We found that lower than median levels of dNT-1 mRNA in AML blasts at diagnosis were independently correlated with shorter disease-free survival (DFS) in univariate (median DFS values: 11 vs. 17 months; p =0.01) (Figure 1) and multivariate analysis (p =0.02; odd ratio: 2.11; 95% CI:1.1-4.2), and with shorter overall survival (OS) only by univariate analysis (median OS values: 14 vs. 20.5 months; p=0.01) (Figure 1). To our knowledge, this is the first time that low levels of expression of dNT-1, as determined by quantitative PCR, have been related to worse clinical outcome in patients with AML.

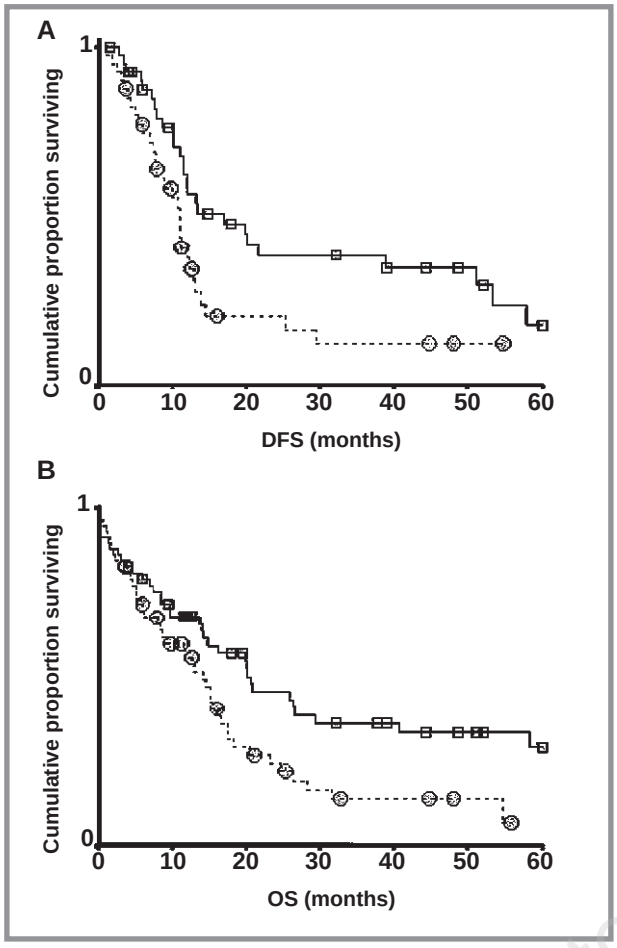

Figure 1. Kaplan-Meier estimate of disease-free survival (A) and overall survival (B) and expression of dNT-1 in the whole population at diagnosis. Dotted line and (○), patients who expressed low levels of dNT-1 mRNA; continuous line and (□), patients who expressed high levels of dNT-1 mRNA.

Table 1. Laboratory characteristics of acute myeloid leukemia patients at diagnosis.

	Mean ± SD	*Median*	
White blood cells (10^9/L)	63.6±69.9	37.8	
Peripheral blood blasts (%)	56.8±31.2	61	Complete response rate to induction therapy: 75%
Bone marrow blasts (%)	73.3±20.2	80	Median DFS at 5 years: 11 months
Hemoglobin (g/L)	88.1±25.9	88	Median OS at 5 years: 12.7 months
Platelets (10^9/L)	67.3±54	59	

Cytogenetic risk (N=96)	*N*	*%*
Low	19	19.8
Intermediate	38	39.6
High	39	40.6

WBC: white blood cells; PB: peripheral blood; BM: bone marrow.

A first explanation of our results is based on the possible accumulation of pyrimidine nucleotides associated with low dNT1 levels, as Mazzon *et al.* recently demonstrated that dNT-1 does not dephosphorylate ara-CMP *in vitro*.[7] In fact, low levels of dNT-1 would lead to higher levels of dCTP[6] and consequently higher resistance to induction treatment. Various cell lines and AML blasts containing high levels of dCTP have been found to be resistant to ara-C.[8] dCTP levels regulate ara-C metabolism at three levels: by feedback inhibition of dCK activity with a consequent decrease in ara-C phosphorylation; by allosteric activation of the catabolic enzyme, cytidine deaminase; and by competing with ara-CTP for incorporation into DNA. Thus, elevated levels of dCTP will favor the survival of blast cells exposed to ara-C.

Another possible explanation of our results could be that dNT-1 has phosphotransferase activity. Although not described for dNT-1, other pyrimidine 5'-nucleotidases such as PN-I and PN-II, have been shown to have phosphotransferase activity.[7,9] In the presence of a suitable nucleoside, these enzymes can catalyze the transfer of the phosphate moiety from a 5'-nucleotide monophosphate donor to the 5'-position of a nucleoside acceptor. PN-I has phosphotransferase activity toward ara-C, phosphorylating this drug to ara-CMP.[10] Moreover, Rampazzo recently concluded that PN-II which has phosphatase and phosphotransferase activity actually belongs to the class of dNTs, showing a substrate specificity remarkably similar to that displayed by dNT-1.[6]

In summary, our study reveals that low dNT-1 mRNA expression levels are an independent prognostic factor for poor outcome in patients with AML. Confirmation of the prognostic value of dNT-1 on larger series, either by real time-PCR or by analysis of protein expression is clearly warranted.

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Acute Myeloid Leukemia

Differentiating agents + low-dose chemotherapy in the management of old/poor prognosis patients with acute myeloid leukemia or myelodysplastic syndrome

13-cis retinoic acid + $(OH)_2$ vitamin D3 + low-dose 6-thioguanine and cytarabine were tested in 26 patients with acute myeloid leukemia (AML) and in 4 patients with myelodysplastic syndrome (MDS) (median age 72.5), ineligible for standard chemotherapy. The response rate was 50%, with 27% complete remission. The median survival of the whole group and responders was 7.5 (1-47+) and 16.5 months (3.5-47+), respectively.

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Acute myeloid leukemia (AML) and high risk myelodysplastic syndromes (MDS) in patients above the age of 60 usually have an unfavorable outcome.[1-4] In particular, patients unsuitable for standard chemotherapy have a median survival of < 6 months.[3,4] On the basis of previous *in vitro* and *in vivo* observations,[5-7] we treated 26 AML and 4 MDS patients (Table 1), ineligible for intensive chemotherapy because of age, poor clinical conditions or treatment refusal, with a combination of 13-cis retinoic acid (RA) (Roaccutan®, 20-40 mg daily) + $(OH)_2$ vitamin D3 (D3) (Rocaltrol®, 1 μg daily) for 5 weeks, with the addition of 6-thioguanine (6-TG) (Thioguanine®, 40 mg daily) and cytosine arabinoside (ARA-C) (Aracytin®, 8 mg/m^2 × 2/day by subcutaneous injections) during the first 2-3 weeks. After the 5th week of treatment, in the absence of disease progression, the 5-week course was repeated. After the 2nd course the patients were re-assessed. Patients who had obtained at least a partial response continued a maintenance treatment, until disease progression, with RA + D3 + intermittent 6-TG (14-21 days) or ARA-C + 6-mercaptopurine (Purinethol®, 50 mg daily) for 14 days every 5-6 weeks, at the above described dosages.

A complete response (CR) was defined by < 5% bone marrow (BM) blasts with normal cellularity and Hb > 10 g/dL, neutrophils >1.5×10^9/L, and platelets >100×10^9/L. A partial response was defined by the achievement of neutrophil and platelet counts >1×10^9/L and 50×10^9/L, respectively, for at least one month, together with a >50% reduction of BM blasts and/or disappearance of circulating blasts.

Remission duration and survival were calculated by Kaplan-Meier curves.[8] The treatment was reasonably well tolerated: grade 4 neutropenia and thrombocytopenia were observed in 28 patients (94%) during the first two courses of low-dose chemotherapy and 4 patients (13%) aged > 75 died of neutropenia-related infections. Thirteen patients completed the induction treatment as outpatients, while 17 were hospitalized for a median time of 18 days (5-45). Eleven of the 30 patients required intravenous antibiotics and 2 required G-CSF administration for prolonged neutropenia. In responsive patients, the median time to reach neutrophil and platelet values above 0.5 and 20×10^9/L,

Table 1. Patients' clinical features.

	AML	*MDS*
Sex	M 17 F 9	M 3 F 1
Age[1] (years)	72.5 (41-88)	69.5 (61-84)
Age Distribution	<60 2 60-70 8 71-80 13 >80 3	60-70 2 >70 2
Diagnosis[2]	M1 6 M2 10 M4 7 M5a 2 M7 1	RAEB 2 4
Secondary Disease	Post MDS 7 Post chronic MPD 2 Therapy related 1 All 10/26	 Therapy related 1 All 1/4
Disease status	Diagnosis 21 1st relapse 2 2nd relapse 1 3rd relapse 2	Diagnosis 4
Karyotype	Normal 7 Abnormal 4 N.E. 15	Normal 2 Abnormal 2
WBC[1]×10^9/L	3.8 (1.5-93)	2.5 (0.9-3.8)
WBC > 20×10^9/L	4/26	
IPSS[3]	N.E.	Intermediate[2] (2 patients with intermediate 2 score) High 2

[1]: median value and (range); [2]: diagnosis according to FAB and WHO classification; [3]: International Prognostic Scoring System.