Recent studies have identified pathologic mutations in the transcription factor GATA-1 which are implicated in the pathogenesis of both thrombocytopenia and myeloproliferation. Although the precise role of these mutations in the development of TMD and/or AMKL in children with Down's syndrome is not known, they presumably impart a clonal advantage which co-operates with or enhances the fundamental defect provided by increased dosage of a gene on chromosome 21. They are not sufficient for progression to AMKL.[8] Nevertheless, the specificity for the development of immature megakaryoblasts demonstrates that defects in GATA-1 can influence expansion of this lineage. The present study, however, indicates that GATA-1 mutations are not responsible for the increased megakaryocytosis of patients with ET.

Domenica Gandini, Anthony J.R. Allen, Michael J. Nash, David C. Linch, Rosemary E. Gale

Department of Hematology, University College London, UK

Funding: this work was supported by the United Kingdom Medical Research Council.

Key words: GATA-1, essential thrombocythemia, polymorphisms.

Correspondence: Rosemary E. Gale, Department of Hematology, University College London, 98 Chenies Mews, London, WC1E 6HX, UK. Phone: international +44.20.6796232. Fax: international +44.2076796222. E-mail: rosemary.gale@ucl.ac.uk

References

1. Visvader J, Adams JM. Megakaryocytic differentiation induced in 416B myeloid cells by GATA-2 and GATA-3 transgenes or 5-azacytidine is tightly coupled to GATA-1 expression. Blood 1993;82: 1493-501.
2. Vyas P, Ault K, Jackson CW, Orkin SH, Shivdasani RA. Consequences of GATA-1 deficiency in megakaryocytes and platelets. Blood 1999; 93:2867-75.
3. Nichols KE, Crispino JD, Poncz, M, White JG, Orkin SH, Maris JM, et al. Familial dyserythropoietic anaemia and thrombocytopenia due to an inherited mutation in GATA1. Nat Gen 2000;24:266-70.
4. Mehaffey MG, Newton AL, Gandhi MJ, Crossley M, Drachman JG. X-linked thrombocytopenia caused by a novel mutation of GATA-1. Blood 2002;98:2681-8.
5. Freson K, Devriendt K, Matthijs G, Van Hoof A, De Vos R, Thys C, et al. Platelet characteristics in patients with X-linked macrothrombocytopenia because of a novel GATA1 mutation. Blood 2001;98:85-92.
6. Freson K, Matthijs G, Thys C, Marien P, Hoylaerts MF, Vermylen J, et al. Different substitutions at residue D218 of the X-linked transcription factor GATA1 lead to altered clinical severity of macrothrombocytopenia and anemia and are associated with variable skewed X inactivation. Hum Mol Gen 2002;11:147-52.
7. Yu C, Niakan KK, Matsushita M, Stamatoyannopoulos G, Orkin SH, Raskind WH. X-linked thrombocytopenia with thalassemia from a mutation in the amino finger of GATA-1 affecting DNA binding rather than FOG-1 interaction. Blood 2002;100:2040-5.
8. Gurbuxani S, Vyas P, Crispino JD. Recent insights into the mechanisms of myeloid leukemogenesis in Down syndrome. Blood 2004; 103:399-406.
9. Gale RE. Pathogenic markers in essential thrombocythemia. Curr Hematol Rep 2003;2:242-7.
10. Harrison CN, Gale RE Machin SJ, Linch DC. A large proportion of patients with a diagnosis of essential thrombocythemia do not have a clonal disorder and may be at lower risk of thrombotic complications. Blood 1999;93:417-24.
11. Vyas P, McDevitt MA, Cantor AB, Katz SG, Fujiwara Y, Orkin SH. Different sequence requirements for expression in erythroid and megakaryocytic cells within a regulatory element upstream of the GATA-1 gene. Development 1999;126:2799-811.

Acute Myeloid Leukemia

Arsenic trioxide in the treatment of advanced acute promyelocytic leukemia

Eleven patients with advanced APL were treated with ATO (0.15 mg/Kg daily). Eight (73%) achieved molecular CR, but 5 relapsed, 1 died in molecular CR, 1 was lost to follow-up and 1 is still alive in CR after allogeneic transplantation. We suggest that ATO may be effective also in advanced APL, but given the short CR, it seems indicated only in patients eligible for transplant procedures.

haematologica 2004; 89:615-617
(*http://www.haematologica.org/journal/2004/5/615)*

The advent of all-trans-retinoic acid (ATRA) has dramatically improved treatment outcome and survival in patients with acute promyelocytic leukemia (APL).[1-2] However, approximately 30% of patients receiving ATRA-based therapy will eventually relapse.[3] Recent studies have shown that arsenic trioxide (ATO) has a significant antileukemic effect in APL, and may induce complete remission (CR) in more than 80% of patients treated at 1st relapse.[4-8] We report here our experience on ATO treatment for patients with advanced (multiply relapsed or molecularly resistant) APL. From 12/1998 to 12/2000, 11 patients with APL in ≥ 2nd relapse or 1st molecularly resistant disease received ATO as a single agent. Molecular resistance was defined as persistence, in two consecutive marrow samples collected at the end of the AIDA protocol induction and consolidation, of polymerase chain reaction positivity for the PML/RARα hybrid. The main clinical characteristics of the patients and their previous treatments are described in Table 1.

ATO, kindly provided by PolaRx Biopharmaceuticals Inc., was administered at a dose of 0.15 mg/kg daily until the achievement of hematologic complete remission (HCR) and for a cumulative maximum duration of 60 days. HCR and molecular remission (MCR) were defined as reported elsewhere.[9] Patients who achieved HCR, were planned to receive an additional course of ATO as consolidation therapy, with the same dosage for a cumulative period of 25 days.

Eight patients (73%) achieved HCR after induction treatment with ATO. Three patients died of cerebral hemorrhage, on day 7, 15 and 25: all of them developed an APL differentiation syndrome, characterized by high leukocyte count and respiratory distress (Table 2). The median treatment duration in patients who achieved CR was 37.5 days (range 28-50) and the median cumulative dose was 300 mg (range 108-564). All but one of the patients in HCR received one cycle of consolidation with ATO. Among the 8 patients in HCR, 6 achieved MCR after the first cycle of ATO and the remaining 2 after consolidation. As to follow-up, 1 patient was lost to follow-up after 2 months while in MCR, 1 patient did not receive any other treatment and relapsed after 3 months, 2 patients received one further cycle of ATRA + idarubicin and both relapsed after 3 and 4 months. The remaining 4 patients underwent transplant procedures: two received an autologous bone marrow transplantation (BMT) and both relapsed, after 13 and 22 months, while 2 received

Table 1. Clinical characteristics of the patients.

Patient N.	Age	Sex	PML/RARα isoform	Treatment at diagnosis	HCR/MCR duration (months)	Salvage treatments	Response to salvage treatments	Disease status at ATO treatment	Months from diagnosis to ATO
1	39	F	BCR1	AIDA	5/0	APLres	Res	Molecular resistance	15
2	51	M	BCR3	AIDA	4/0	APLres	Res	Molecular resistance	8
3	20	M	BCR1	AIDA	7/6	a) APLres b) IEV c) ATRA+IFN d) AAT	a) Res b) Res c) Res d) Res	1st Resistant Rel	29
4	41	F	BCR3	AIDA	8/5	APLres	Res	1st Resistant Rel	18
5	37	M	BCR3	AIDA	16/14	APLres→ABMT	CR (8 mo)	2nd Relapse	33
6	62	M	BCR3	AIDA	12/6	APLres→ABMT	CR (7 mo)	2nd Relapse	25
7	5	M	BCR3	AIDA	9/6	APLres→BMT	CR (15 mo)	2nd Relapse	35
8	34	F	BCR3	AIDA	8/1a)	APLres b) HdHU	a) CR (1 mo) b) Res	2nd Resistant Rel	18
9	38	F	BCR1	AIDA	6/5	a) APLres b) AAT→PBSCT c) ATRA+IFN	a) Res b) CR (2 mo) c) Res	2nd Resistant Rel	32
10	53	M	BCR3	AIDA	11 / 8	a) APLres b) DNR	a) CR (8 mo) b) Res	2nd Resistant Rel	29
11	34	F	BCR3	AIDA	8 / 3	a) APLres→ABMT b) ATRA c) AAT	a) CR (6 mo) b) Res c) Res	2nd Resistant Rel	34

HCR: hematologic complete remission; MCR: molecular complete remission; AIDA: idarubicin + ATRA; APLres: mitoxantrone + aracytin + ATRA; AAT: amsacrine + aracytin + thioguanine; HdHU: high dose hydroxyurea; ABMT: autologous bone marrow transplantation; BMT: allogeneic bone marrow transplantation; PBSCT: peripheral blood stem cell transplantation.

Table 2. Patients outcome after ATO treatment.

Patient N.	WBC > 10×10^9/L during ATO	ATO treatment (days) Induction	Consolidation	Outcome	PML/RARα post-induction	CR duration (months)	Post-remission treatment	Present status
1	134	7	NP	Early[3] death	–	–	–	Dead
2	15.3	25	NP	Early[2] death	–	–	Dead	
3	–	41	25	CR	Neg.	3	–	Dead
4	–	30	25	CR	Pos.	+24	BMT	CR
5	–	39	22	CR	Neg.	4	IDA+ATRA	Dead
6	62.5	15	NP	Early[1] death	–	–	–	Dead
7	135	36	25	CR	Neg.	22	PBSCT	Dead
8	43.9	30	25	CR	Pos.	20	BMT	Dead
9	–	28	NP	CR	Neg.	13	ABMT	Dead
10	–	47	10	CR	Neg.	?	?	Lost to FU
11	10.4	50	25	CR	Neg.	3	IDA+ATRA	Dead

[1]Due to cerebral hemorrhage on day 15; [2]Due to cerebral hemorrhage on day 25; [3]Due to cerebral hemorrhage on day 7; NP: not performed; CR: complete remission; BMT: allogeneic bone marrow transplantation; ABMT: autologous bone marrow transplantation; PBSCT: peripheral blood stem cell transplantation.

an allogeneic BMT (1 died in MCR from second neoplasia after 20 months, and 1 is still alive in MCR after 24 months).

An APL differentiation syndrome developed in 3 patients and was managed with dexamethasone (10 mg every 12 hours for 4 days). All these 3 patients died from cerebral hemorrhage, apparently not related to concomitant APL differentiation syndrome. Other extra-hematologic toxicity included Q-T prolongation in 2/11 patients (18%), which did not however require ATO discontinuation, and severe peripheral neuropathy in 2/11 (18%) cases. Hyperleukocytosis (WBC > 10×10^9/L) during the induction treatment with ATO was observed in 6/11 patients (54.5%), with a median peak WBC count of 57×10^9/L. Cytotoxic drugs were added in only one patient.

APL patients in first relapse after ATRA-based protocols, need a salvage scheme able to re-induce molecular remission. Provided that at least 6 months have elapsed since the last ATRA administration, such a remission can be obtained by using ATRA again in combination chemotherapy or, alternatively, agents that have proven to be extremely active and non-cross resistant with ATRA, such as ATO. For those patients in very advanced phase with an acquired resist-

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.

Ida Carmosino , Roberto Latagliata,* Giuseppe Avvisati,° Massimo Breccia,* Erica Finolezzi,* Francesco Lo Coco,* Maria Concetta Petti#*

**Dipartimento di Biotecnologie Umane e Ematologia, Università "La Sapienza"; °Ematologia, Università Campus Bio-Medico; #Ematologia, Istituto Regina Elena, IFO, Rome, Italy;*

Key words: arsenic trioxide, acute promyelocytic leukemia.

Correspondence: Roberto Latagliata, MD, Dipartimento di Biotecnologie Umane e Ematologia , Università "La Sapienza", via Benevento 6, 00161 Rome, Italy. Fax: international +39.06. 44241984. E-mail: rob.lati@libero.it

References

1. Fenaux P, Chomienne C, Degos L. Acute promyelocytic leukemia: biology and treatment. Semin Oncol 1997;24:92-102.
2. Lo Coco F, Nervi C, Avvisati G, Mandelli F. Acute promyelocytic leukemia: a curable disease. Leukemia 1998;12:1866-80.
3. Meloni G, Diverio D, Vignetti M, Avvisati G, Capria S, Petti MC, et al. Autologous bone marrow transplantation for acute promyelocytic leukemia in second remission: prognostic relevance of pretransplant minimal residual disease assessment by reverse - transcription polymerase chain reaction of the PML/RARα fusion gene. Blood 1997;90:1321-5.
4. Soignet SL, Maslak P, Wang ZG, Jhanwar S, Calleja E, Dardashti LJ, et al. Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. N Engl J Med 1998;339: 1341-8.
5. Ohnishi K, Yoshida H, Shigeno K, Nakamura S, Fujisawa S, Naito K, et al. Arsenic trioxide therapy for relapsed or refractory Japanese patients with acute promyelocytic leukemia: need for careful eletrocardiogram monitoring. Leukemia 2002;16:617-22.
6. Soignet SL, Frankel SR, Douer D, Tallman MS, Kantarjian H, Calleja E, et al. United States multicenter study of arsenic trioxide in relapsed acute promyelocytic leukemia. J Clin Oncol 2001; 19: 3852-60.
7. Lu DP, Qiu JY, Jiang B, Wang Q, Liu KY, Liu YR, Chen SS. Tetraarsenic tetra-sulfide for the treatment of acute promyelocytic leukemia: a pilot report. Blood 2002;99:3136-43.
8. Leoni F, Gianfaldoni G, Annunziata M, Fanci R, Ciolli S, Nozzoli C, et al. Arsenic trioxide therapy for relapsed acute promyelocytic leukemia: a bridge to transplantation. Haematologica 2002; 87:485-9.
9. Mandelli F, Diverio D, Avvisati G, Luciano A, Barbui T, Bernasconi C, et al. Molecular remission in PML/RAR α-positive acute promyelocytic leukemia by combined all-trans retinoic acid and idarubicin (AIDA) therapy. Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto and Associazione Italiana di Ematologia ed Oncologia Pediatrica Cooperative Groups. Blood 1997;90:1014-21.

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.

haematologica 2004; 89:617-619
(*http://www.haematologica.org/journal/2004/5/617*)

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.