Nevertheless the molecular and hematologic characterization of more patients with e6a2 transcript will be needed to verify the real correlation of this transcript with more aggressive disease. This knowledge is important in order to improve the choice of treatment and thus the clinical outcome of single patients.

*Simona Colla,* Gabriella Sammarelli,* Simone Voltolini,* Monica Crugnola,* Paola Sebastio,° Nicola Giuliani**

**Chair of Hematology and BMT Unit, University of Parma; °Laboratory of Molecular Genetics and Diagnostic Biotechnology, Hospital of Parma, Italy*

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*Correspondence: Simona Colla, Chair of Hematology and BMT Unit, University of Parma, via Gramsci 14, 43100 Parma, Italy. E-mail: ematopr@unipr.it*

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Chronic Myeloproliferative Disorders

## No mutations in the GATA-1 gene detected in patients with acquired essential thrombocythemia

**Mutations in the GATA-1 gene have been identified in patients with familial macrothrombocytopenia and Down's syndrome patients with a transient myeloproliferative disorder and/or acute megakaryoblastic leukemia. We screened this gene in 46 patients with essential thrombocythemia and identified only a common single nucleotide polymorphism that is unlikely to be of pathological significance.**

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Although GATA-1 was originally described as an erythroid-specific nuclear binding factor, it is now known to play a critical role in megakaryocytic proliferation and differentiation. Over-expression of GATA-1 in a mouse pluripotent myeloid cell line 416B induces megakaryocytic differentiation[1] and megakaryocyte-specific loss of GATA-1 expression in a knock-out mouse leads to thrombocytopenia with hyperproliferation of morphologically retarded megakaryocytes.[2] Furthermore, two different types of mutation in GATA-1 have recently been shown to have pathologic consequences for the megakaryocytic lineage. Firstly, five different inherited point mutations identified in exon 4, which codes for the N-terminal zinc finger, lead to disrupted binding to either its essential co-factor FOG-1 or DNA and cause macrothrombocytopenia with variable degrees of dyserythropoiesis and anemia in affected individuals.[3-7] Secondly, acquired mutations have been detected in exon 2 in most patients with the transient myeloproliferative disorder (TMD) and/or acute megakaryoblastic leukemia (AMKL) associated with Down's syndrome. The mutations include deletions, insertions, splice mutations, nonsense and missense mutations, and cause loss of the full length GATA-1 protein but not of a shorter length variant lacking the N-terminal activation domain which can still bind DNA and interact with FOG-1 but has reduced transactivation potential.[8]

The etiology of the sustained thrombocytosis and megakaryocytic hyperproliferation characteristic of the acquired myeloproliferative disorder essential thrombocythemia (ET) is not known. No specific cytogenetic abnormalities or molecular markers have been associated with the disorder and its diagnosis remains one of exclusion. There is, however, evidence that it is biologically heterogeneous, with patients having either polyclonal or clonal myelopoiesis as demonstrated by X-chromosome inactivation patterns (XCIPs) in informative females, and normal or elevated PRV-1 expression.[9] We would hypothesize that, especially in patients with polyclonal myelopoiesis, mutations in genes implicated in megakarycytopoiesis which cause subtle differences in function may contribute to increased megakaryocytic production. We therefore looked for mutations in the GATA-1 gene in DNA from neutrophils of 46 female ET patients. The DNA had been previously used to investigate clonality in these patients, whose demographic and clinical details have been previously published.[10]

Polymerase chian reaction (PCR) analysis was performed using Optimase Polymerase™ (Transgenomic Limited, Crewe, UK) with 7 different primer sets covering the GATA-1 coding region (exons 2-6), the hematopoietic-specific promoter (IE, exon 1) and a 317 bp *cis*-acting regulatory element, HS1, which is associated with a hematopoietic-specific DNAse I hypersensitive site and is required in full for megakaryocytic- but not erythroid-specific expression[11] (Table 1). PCR products were analyzed using denaturing high performance liquid chromatography (DHPLC) on a Transgenomic WAVE®, DNA fragment analysis system at optimal temperatures for each fragment as determined by WAVEMAKER® software (Table 1). Patterns from patients' sam-

**Table 1.**

| *PCR fragments* | | *Primers* | *Temperatures (°C)* *PCR (annealing)* | *WAVE® analysis* |
|---|---|---|---|---|
| HS1 | F | 5'-gcctgacggagaagacgcgcg-3' | 66.0 | 54.0, 60.0, 64.0 |
| | R | 5'-tggcacaccataaatggtgtgc-3' | | |
| Exon 1(IE) | Fa | 5'-ctatcccactcctcgaggaat-3' | 64.0 | 61.4, 64.0, 65.0 |
| | Ra | 5'-atttgtgcggggccggtgtg-3' | | |
| | Fb | 5'-gacgcacatacacaggagtc-3' | 64.0 | 58.5, 64.0 |
| | Rb | 5'-ggagcctggagcaagcataag-3' | | |
| Exon 2 | F | 5'-gaaggatttctgtgtctgaggac-3' | 64.0 | 63.0, 64.0, 66.0 |
| | R | 5'-tggcaaccccaacagcactca-3' | | |
| Exon 3 | F | 5'-ggaacttggccaccatgttgg-3' | 64.0 | 60.7, 61.7, 62.7 |
| | R | 5'-gaagagggagctaggctcagc-3' | | |
| Exon 4 | F | 5'-tgggaggggtggcccaaagta-3' | 64.0 | 63.0, 64.0 |
| | R | 5'-cctgtgtataaggtgaagcaggt-3' | | |
| Exon 5 | F | 5'-ggcatcaccctgtaaacaaagcc-3' | 63.0 | 62.4, 64.2 |
| | R | 5'-ggcagtggcatgaagacag-3' | | |
| Exon 6 | F | 5'-gtgtccctggttgacacagag-3' | 64.0 | 61.9, 63.5 |
| | R | 5'-gtccttctcctcttgtagcc-3' | | |

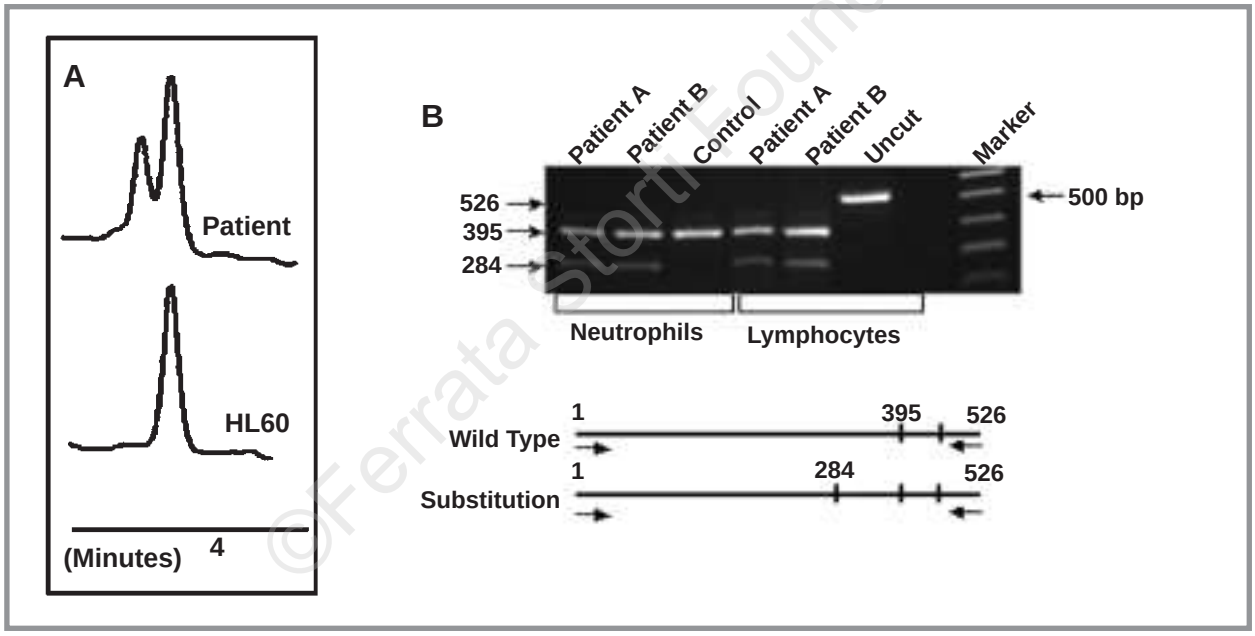


**Figure 1. Detection of a substitution in the promoter region (5' end of exon 1) of GATA-1 in 2 ET patients. (A) WAVE® pattern at 64.0°C for PCR products from a control (HL60) and an ET patient. (B) D*de*I digestion of the same PCR fragment.**

ples were compared with those from DNA of the HL60 myeloid cell line. Positive controls were artificially created by shot-gun cloning of a PCR product from HL60 DNA for each fragment and selection of clones with abnormal WAVE® patterns. Substitutions were determined by direct sequencing of PCR products.

No abnormal DHPLC patterns were detected in any sample using fragments covering either the five coding exons (2–6) or the regulatory element HS1. A heterozygous A to G substitution at position -4628 (Accession number NT_011568) was detected in the 3' region of the promoter (exon 1) in 2 of 46 patients (4%) (Figure 1A). The nucleotide change creates a new cleavage site for the restriction enzyme D*de*I (Figure 1B) and digestion of PCR products demonstrated that the substitution was present in DNA from neutrophils and $CD3^+$ cells of both patients, indicating that it was germline and may be a polymorphism (Figure 1B). DNA from 98 hematologically normal controls was screened by PCR and D*de*I digestion, 12 were heterozygous for the substitution (12%). These results are consistent with the presence of a common single nucleotide polymorphism and the similar frequency in hematologically normal individuals means that it is unlikely to be associated with pathologically altered GATA-1 function.

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*

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*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*

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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.**

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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