Key words: myeloproliferative syndrome, eosinophilia, lymphoblastic lymphoma, 8p11

Correspondence: Rosangela Invernizzi, M.D., Medicina Interna ed Oncologia Medica, Policlinico S. Matteo, Piazzale Golgi, 27100 Pavia, Italy. E-mail: r.invernizzi@smatteo.pv.it

References

1. Bain BJ. Cytogenetic and molecular genetic aspects of eosinophilic leukaemias. Br J Haematol 2003;122:173-9.
2. Macdonald D, Reiter A, Cross NCP. The 8p11 myeloproliferative syndrome: a distinct clinical entity caused by constitutive activation of FGFR1. Acta Haematol 2002;107:101-7.
3. Abruzzo LV, Jaffe ES, Cotelingam JD, Whang-Peng J, Del Duca V, Medeiros LJ. T-cell lymphoblastic lymphoma with eosinophilia associated with subsequent myeloid malignancy. Am J Surg Pathol 1992;16:236-45.
4. Inhorn RC, Aster JC, Roach SA, Slapak CA, Soiffer R, Tantravahi R, et al. A syndrome of lymphoblastic lymphoma, eosinophilia, and myeloid hyperplasia/malignancy associated with t(8;13)(p11;q11): description of a distinctive clinicopathologic entity. Blood 1995; 85:1881-7.
5. Suzan F, Gulasch G, Terre C, Garcia I, Bastie JN, Maarek O, et al. Long-term complete haematological and molecular remission after allogeneic bone marrow transplantation in a patient with a stem cell myeloproliferative disorder associated with t(8;13) (p12;q12). Br J Haematol 2003; 121:312-4.
6. Xiao S, Nalabolu SR, Aster JC, Ma J, Abruzzo L, Jaffe ES, et al. FGFR1 is fused with a novel zinc-finger gene, ZNF198, in the t(8;13) leukaemia/lymphoma syndrome. Nat Genet 1998;18:84-7.
7. Popovici C, Zhang B, Grégoire MJ, Jonveaux P, Lafage-Pochitaloff M, Birnbaum D, et al. The t(6;8)(q27;p11) translocation in a stem cell myeloproliferative disorder fuses a novel gene, FOP, to fibroblast growth factor receptor 1. Blood 1999:93:1381-9.
8. Guasch G, Mack GJ, Popovici C, Dastugue N, Birnbaum D, Rattner JB, et al. FGFR1 is fused to the centrosome-associated protein CEP110 in the 8p12 stem cell myeloproliferative disorder with t(8;9)(p12;q33). Blood 2000;95:1788-96.
9. Demiroglu A, Steer EJ, Heath C Taylor K, Bentley M, Allen SL, et al. The t(8;22) in chronic myeloid leukemia fuses BCR to FGFR1: transforming activity and specific inhibition of FGFR1 fusion proteins. Blood 2001;98:3778-83.
10. Heath C, Cross NC. Critical role of STAT5 activation in transformation mediated by ZNF198-FGFR1. J Biol Chem 2003;12:(Epub ahead of print).

Chronic Myeloid Leukemias

c-myc expression in cell lines derived from chronic myeloid leukemia

We analyzed proliferation and *c-myc* expression in three chronic myeloid leukemia (CML)-derived cell lines treated with interferon-α, hydroxyurea, busulfan and imatinib. We found that c-Myc levels did not universally correlate with CML cell proliferation and that c-Myc down-regulation correlated to imatinib activity but not to imatinib-induced apoptosis.

haematologica 2004; 89:241-243
(*http://www.haematologica.org/2004/1/241*)

The molecular hallmark of chronic myeloid leukemia (CML) is the Bcr-Abl kinase, generated by the 9;22 translocation.[1] CML has been treated with busulfan, hydroxyurea and interferon-α.[2] Recently, the Bcr-Abl inhibitor imatinib (STI571, Gleevec®) was introduced for CML therapy and has displaced other drugs.[1,3] c-Myc is a transcription factor involved in cell proliferation,[4] and *c-myc* expression has been found to be elevated in CML blast crisis.[5,6] Bcr-Abl and c-Myc co-operate in cell transformation[7] and Bcr-Abl activates *c-myc* transcription.[8,9]

We studied *c-myc* expression in response to the four drugs used to treat CML (hydroxyurea, busulfan, interferon-α and imatinib) on three CML-derived cell lines (KU812, MEG01 and K562). We used drug concentrations slightly above the minimal cytostatic concentrations for the three cell lines: 2000 UI/mL interferon-α, 0.5 mM hydroxyurea, 0.5 mM busulfan and 0.5 μM imatinib. Proliferation (as determined by cell counting and ^{3}H-thymidine incorporation) and *c-myc* expression (analyzed by Northern and Western blots) were monitored during 3 days of treatment. The results are summarized in Table 1. We found that each drug inhibited cell growth. However, growth arrest was reversed when imatinib, interferon-α, and hydroxyurea were removed from the media. Consistent with the different mechanisms of action, we found a differential regulation of *c-myc* in response to the drugs. It was expected that *c-myc* expression would correlate with proliferation, as shown for the KU812 and MEG01 cell lines treated with hydroxyurea and busulfan. Cells with repressed *c-myc* were always non-proliferating, suggesting that CML growth requires c-Myc. However, there was no universal correlation between *c-myc* repression and cessation of proliferation: a) in the three cell lines, interferon-α arrested

Table 1. Effects of interferon-α, hydroxyurea, busulfan and imatinib on *c-myc* expression and cell proliferation of CML-derived cells.

Cell line	*Effect*	*IFNα (2000 UI/mL)*	*Hydroxyurea (0.5 mM)*	*Busulfan (0.5 mM)*	*Imatinib (0.5 μM)*
KU812	Growth arrest	+ reversible	++ reversible	++ irreversible	++ reversible
	c-myc	no change	↓ reversible	↓ irreversible	↓ NT
MEG01	Growth arrest	+ reversible	++ reversible	++ irreversible	++ reversible
	c-myc	no change	↓ reversible	↓ reversible	↓ NT
K562	Growth arrest	+ reversible	+ reversible	++ irreversible	++ reversible
	c-myc	no change	no change	no change	↓ reversible

++ denotes a faster or more profound inhibition of cell growth than + , as assessed by thymidine incorporation and cell counting. The data are summarized from three independent experiments in each case. ↓ denotes c-myc down-regulation, as assessed by Northern blot analysis. "Reversible" refers to the recovery of c-myc mRNA levels after removal of the corresponding drug by cell washing. NT: not tested.

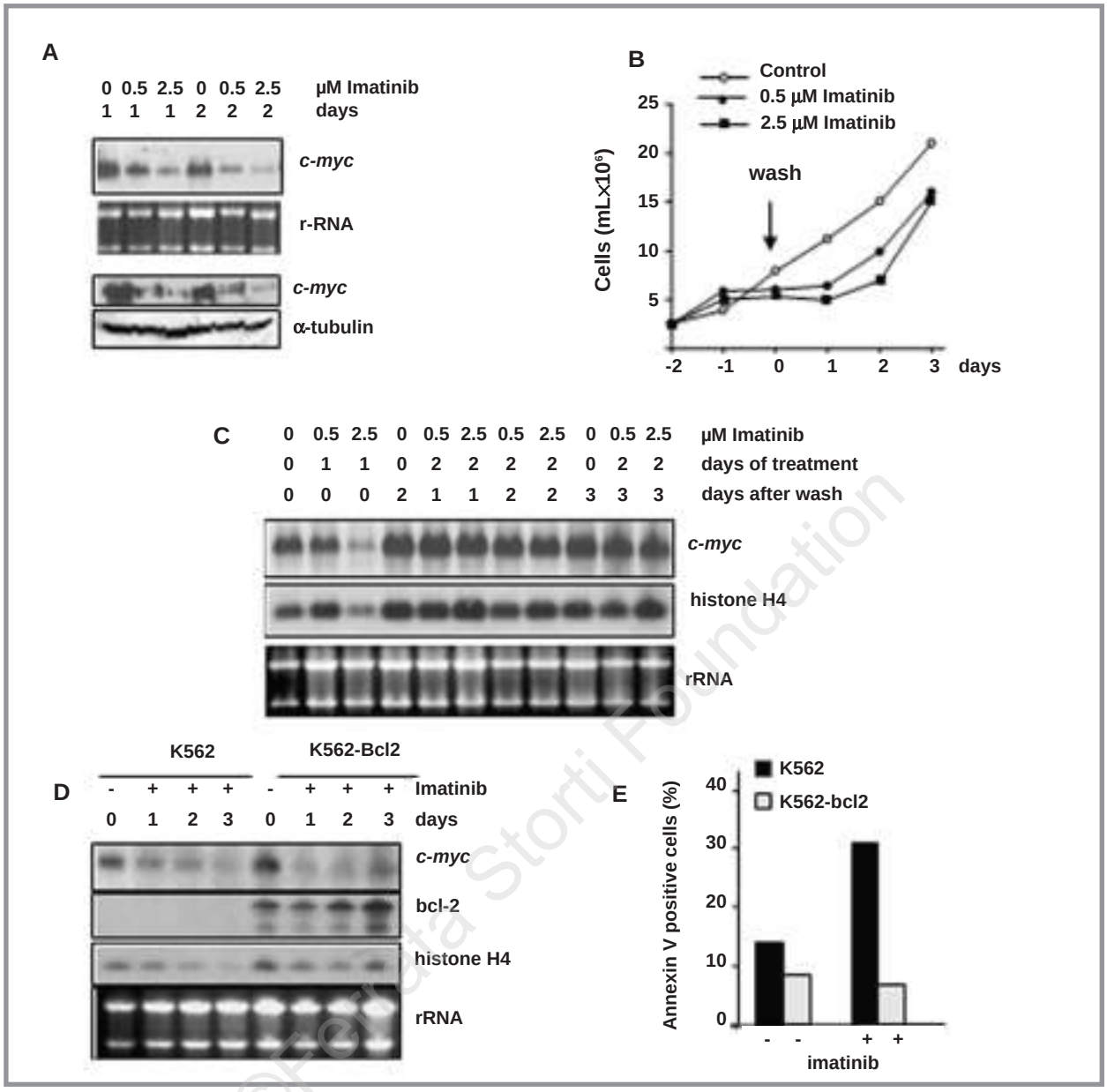

Figure 1. ***c-myc*** **down-regulation mediated by imatinib in K562 correlates with imatinib activity but not with apoptosis. (A) Cells were treated for the indicated periods of time with imatinib and the expression of *c-myc* RNA and protein was determined by Northern blot analysis (upper panel) and Western blot analysis (lower panel). A picture of the filter after transfer, showing the rRNA stained with ethidium bromide and an immunoblot for α-tubulin are shown to assess the loading of RNA and protein, respectively. (B) Cells were treated for 2 days with the indicated imatinib concentrations, washed, and placed in fresh medium. Cell counts were taken for 3 more days after washing. (C) *c-myc* expression in cells treated with 0.5 µM or 2.5 µM imatinib for 1 or 2 days, washed and further incubated in media without imatinib for 1, 2 or 3 days as indicated. The filter was also hybridized to *histone H4* probe to evaluate DNA synthesis (linked to histone expression) in the same samples. (D) *c-myc* expression in K562 and K562-Bcl2 cells treated for 1, 2 or 3 days with 0.5 µM imatinib, as analyzed by Northern blot analysis. The filter was consecutively hybridized to *c-myc*, *bcl-2*, and *histone H4* probes. (E) Apoptosis of K562 and K56-Bcl2 cells in the presence of imatinib. Cells were treated for 2 days with 0.5 µm imatinib and the apoptosis was measured by annexin V binding as determined by flow cytometry.**

growth after 48 h, but *c-myc* expression remained unabated; b) in MEG01 cells, *c-myc* expression increased after removal of busulfan but cells did not resume growth; c) in the K562 line, *c-myc* expression was unchanged in response to interferon-α, hydroxyurea, and busulfan, despite the antiproliferative effect of these drugs.

Imatinib was the only drug that down-regulated *c-myc* in K562 cells, and did so in a time- and dose-dependent manner (Figure 1A). When imatinib was removed, cells resumed normal growth rates after 72 h (Figure 1B), whereas *c-myc* expression reached control levels after 24 h (Figure 1C) when Bcr-Abl kinase activity was recovered. Moreover, imatinib repressed *c-myc* in K562-Bcl2 transfectants[10] (Figure 1D) despite their partial resistance to imatinib-mediated proliferative arrest and total resistance to imatinib-induced apoptosis (Figure 1E).

Thus, *c-myc* repression correlated with imatinib-mediated inhibition of Bcr-Abl (confirming that Bcr-Abl acti-

vates *c-myc* expression) but not with imatinib's pro-apoptotic effects. In summary, *c-myc* expression is not linked to CML cell proliferation as the growth of cells can be arrested in the presence of high *c-myc* expression, indicating that c-Myc is not sufficient to trigger cell proliferation. However, *c-myc* expression could serve as a molecular marker of imatinib activity.

M. Teresa Gómez-Casares, Jose P. Vaqué,° Angelina Lemes,* Teresa Molero,* M. Dolores Delgado,° Javier León°*

**Servicio de Hematología, Hospital Dr. Negrín, Las Palmas, Spain; °Grupo de Biología Molecular del Cáncer, Departamento de Biología Molecular and Unidad de Biomedicina-CSIC, Universidad de Cantabria, Santander, Spain*

Acknowledgments: we thank Silvia de la Iglesia, María Perera, Teresa Ramírez, Pilar Gutierrez, Pilar Frade, and Guillermo Santana for technical assistance, Elizabeth Buchdunger (Novartis) for providing the imatinib, M. Jesús Alsar (Roche) for providing the interferon α, and Carlos Richard for critical reading of the manuscript.

Funding: this study was supported by grants SAF2002-04193 from Spanish Ministry of Science and Technology to J.L., and FIS01-1129 from Spanish Ministry of Health to M.D.D.

Key words: c-myc, CML, imatinib, interferon-α, hydroxyurea.

Correspondence: Javier León, Departamento de Biología Molecular, Facultad de Medicina, Universidad de Cantabria, 39011 Santander, Spain. Phone: international +34.9.42201952; Fax: international +34.9.42201945. E-mail: leonj@unican.es

References

1. Goldman JM, Melo JV. Chronic myeloid leukemia-advances in biology and new approaches to treatment. N Engl J Med 2003; 349:1451-64.
2. Silver RT, Woolf SH, Hehlmann R, Appelbaum FR, Anderson J, Bennett C, et al. An evidence-based analysis of the effect of busulfan, hydroxyurea, interferon, and allogeneic bone marrow transplantation in treating the chronic phase of chronic myeloid leukemia: developed for the American Society of Hematology. Blood 1999;94:1517-36.
3. O'Dwyer ME, Mauro MJ, Druker BJ. Recent advancements in the treatment of chronic myelogenous leukemia. Annu Rev Med 2002;53:369-81
4. Lutz W, Leon J, Eilers M. Contributions of Myc to tumorigenesis. Biochim Biophys Acta 2002;1602:61-71.
5. Handa H, Hegde UP, Kotelnikov VM, Mundle SD, Dong LM, Burke P, et al. Bcl-2 and c-myc expression, cell cycle kinetics and apoptosis during the progression of chronic myelogenous leukemia from diagnosis to blastic phase. Leuk Res 1997;21:479-89.
6. Jennings BA, Mills KI. c-myc locus amplification and the acquisition of trisomy 8 in the evolution of chronic myeloid leukaemia. Leuk Res 1998;22:899-903.
7. Sawyers CL, Callahan W, Witte ON. Dominant negative MYC blocks transformation by ABL oncogenes. Cell 1992;70:901-10.
8. Stewart MJ, Litz-Jackson S, Burgess GS, Williamson EA, Leibowitz DS, Boswell HS. Role for E2F1 in p210 BCR-ABL downstream regulation of c-myc transcription initiation. Studies in murine myeloid cells. Leukemia 1995;9:1499-507.
9. Xie S, Lin H, Sun T, Arlinghaus RB. Jak2 is involved in c-Myc induction by Bcr-Abl. Oncogene 2002;21:7137-46
10. Lerga A, Richard C, Delgado MD, Canelles M, Frade P, Cuadrado MA, et al. Apoptosis and mitotic arrest are two independent effects of the protein phosphatases inhibitor okadaic acid in K562 leukemia cells. Biochem Biophys Res Commun 1999;260:256-64.

Disorders of Hemostasis

Recombinant factor VIIa for the management of severe hemorrhages in patients with hematologic malignancies

Seven patients with hematologic malignancies were treated with recombinant activated factor VII (rFVIIa) for severe bleeding episodes complicating diagnostic procedures or high-dose chemotherapy associated or not with stem cell transplantation. All patients were thrombocytopenic and refractory to standard support. After administration of rFVIIa, 2 complete responses, 3 partial responses and 2 failures were documented.

haematologica 2004; 89:243-245
(*http://www.haematologica.org/2004_2/243.htm*)

Severe bleeding can be a fatal complication of intensive treatment for acute leukemia, and is thus associated with reduced survival. Administration of platelet concentrates is the most common treatment but substantial limitations frequently make this approach unsatisfactory. Recently, recombinant activated factor VII (rFVIIa, Novoseven) has been successfully used for the management of bleeding in patients with hemophilia A and B with inhibitors, congenital or acquired platelet disorders, severe thrombocytopenia associated with hematologic malignancies, or bleeding complications after bone marrow transplantation.[1-6] The mechanism by which rFVIIa can stop bleeding in patients with thrombocytopenia and the doses needed are currently being investigated.[7] From March 2001 to December 2002, seven patients with hematologic malignancies were treated with rFVIIa for severe bleeding episodes that were refractory to standard anti-hemorrhagic therapies. The clinical characteristics, the type and probable cause of the hemorrhage and the planned treatment are reported in Table 1. Two patients affected by acute myeloid leukemia (AML) received rFVIIa during or before induction therapy. Five patients received rFVIIa during the course of allogeneic stem cell transplantation. The initial indications for rFVIIa were a post-liver biopsy hemorrhage and uterine bleeding in the two AML patients; subsequently, gastrointestinal bleeding in the context of severe acute graft-versus-host disease (GVHD) in 3 cases, gastrointestinal bleeding and hemorrhagic cystitis in 1 were treated during the course of allogeneic stem cell transplantation. The type of bleeding was evaluated through a score proposed by Nevo *et al.*[8] Hemorrhages were diffuse in all cases, except in 1 patient in whom bleeding followed a liver biopsy, and were objectively assessed by instrumental procedures. All patients were thrombocytopenic at the time of rFVIIa infusion and had proved refractory to standard anti-hemorrhagic measures, including intravesicular administration of prostaglandin in the patients with hemorrhagic cystitis. No patient had evidence of disseminated intravascular coagulation or a history of a prior bleeding diathesis. Informed consent for the experimental use of rFVIIa was obtained from all the patients or the minor's legal guardian.

The planned administration of rFVIIa was 100 μg/kg (or 40 μg/kg in the case of the presence or a history of thrombosis) every 6 hours, for a total of 6 doses (Table 2). Platelet transfusions were continued during rFVIIa administration to provide a substrate useful for the action of the drug.[9] Treatment efficacy was evaluated 96 hours after the last dose of rFVIIa and was based on daily clinical records and on the number of red blood cell units required to maintain the hemoglobin lev-