Haematologica 2008;93:741-52.
2. Piga A, Fisher R, Harmatz P, St. Pierre TG, Longo F, Fung E, et al. Comparison of LIC obtained from biopsy, BLS and R2-MRI in iron overloaded patients with β-thalassemia, treated with deferasirox (Exjade, ICL670). Blood 2005; 106:755a.
3. Brittenham GM, Farrell DE, Harris JW, Feldman ES, Danish EH, Muir WA, et al. Magnetic-susceptibility measurement of human iron stores. N Engl J Med 1982;307:1671-5.
4. Olivieri NF. The {beta}-Thalassemias. N Engl J Med 1999;341:99-109.
5. Angelucci E, Brittenham GM, McLaren CE, Ripalti M, Baronciani D, Giardini C, et al. Hepatic iron concentration and total body iron stores in thalassemia major. N Engl J Med 2000;343:327-31.

Effective use of imatinib-mesylate in the treatment of relapsed chronic myeloid leukemia after allogeneic transplantation

Despite recent advances in the treatment of chronic myeloid leukemia (CML), allogeneic stem-cell transplantation (SCT) remains the only curative option. The success of SCT is limited because of relapse in 20-30% of patients.[1,2] As the graft-versus-leukemia (GvL) effect contributes to cure, the cessation of immunosuppression[3] and use of donor lymphocyte infusions (DLI)[4] have become established treatment for relapse. DLI is most effective if given for molecular or cytogenetic relapse. However, GvHD is increased if used in the first year post-transplant. Other options include interferon α (IFN) or a second transplant.

Imatinib mesylate (IM) is a potent inhibitor of the BCR-ABL tyrosine kinase and achieves complete cytogenetic responses (CCR) in 87% of previously untreated patients.[5] More recently its role in relapse after SCT has been highlighted.[6,7] We describe the use of imatinib in 14 patients who relapsed post-SCT and were subsequently treated with IM.

All patients with Ph-positive CML in first chronic phase (CP) who relapsed (n=14) following an allogeneic transplant performed between 1987 and 2004 are included. There were 7 males and 7 females. The median age at diagnosis was 31 years (range 15-48) (Table 1). Pre-transplant treatment included either hydroxycarbamide (OHU) and/or IFN. All patients were IM naïve at the time of transplant and were transplanted in CP, 13 using a matched sibling donor and one a matched unrelated donor.

The conditioning regimen was Bu/Cy (9 patients) or Cy/TBI (5 patients). All the transplants were T-cell replete and cyclosporine and methotrexate were used as GvHD prophylaxis. Four patients developed acute GvHD limited to the skin and were treated with corticosteroids. Follow-up included clinical evaluation, blood counts, bone marrow examination including morphology and cytogenetics. From 2002, patients were monitored by qualitative, nested BCR-ABL RT-PCR and if positive, had BCR-ABL transcript levels determined by real-time quantitative PCR (RQ-PCR). Patients were deemed to have had a molecular relapse if greater than a five-fold increase in BCR-ABL transcript levels was observed.

Median time to first relapse was 36 months (range 7-180) (Table 2). Prior to the availability of IM, 4 patients received DLI at incremental doses with only one patient showing any durable response. The other 3 patients proceeded to a reduced intensity-conditioning transplant with short responses before relapsing (Patients 2, 7 and 9 in Table 2). At the time of introduction of IM, 10 patients were in their first relapse and 4 patients were in second relapse. Four patients had a hematologic [3 CP and one accelerated phase (AP)] relapse, 4 had a cytogenetic relapse and the remaining 6 had a molecular relapse. Imatinib was started at a dose of 400 mg daily in all patients except the patient with AP disease who received 600 mg daily.

Table 1. Pre-transplant.

Case#	Age at diagnosis	Rx before BMT	Time to transplant days	EBMT risk score	Conditioning	Type of transplant
1	35	OHU[1]	372	2	Cy / TBI	Sib-Allo
2	48	OHU	402	3	Bu / Cy	Sib-Allo
3	45	OHU	334	2	Bu / Cy	Sib-Allo
4	21	OHU/IFN	207	1	Cy / TBI	Sib-Allo
5	15	OHU	447	0	Bu / Cy	Sib-Allo
6	32	OHU/IFN	502	2	Bu / Cy	Sib-Allo
7	36	OHU	152	1	Bu / Cy	Sib-Allo
8	40	OHU/IFN	170	2	Bu / Cy	Sib-Allo
9	25	OHU	731	2	Cy / TBI	Sib-Allo
10	15	OHU/IFN	503	2	Cy / TBI	MUD
11	30	IFN	847	2	Bu / Cy	Sib-Allo
12	28	OHU	2227	1	Bu / Cy	Sib-Allo
13	25	Bu/Thiogua	948	2	Cy / TBI	Sib-Allo
14	30	OHU / IFN	334	2	Bu / CY	Sib-Allo

[1] *Oxyhydroxyurea.*

Thirteen (93%) patients responded to IM with a median time to response of four months (range 3-15). Of the 4 patients treated in hematologic relapse, 2 achieved a CCR and became nested PCR negative (<1 BCR-ABL transcripts in 105). The other 2 patients had transient responses before developing progressive disease. Of the 10 patients who were treated for cytogenetic or molecular relapses, all achieved a CCR and 9/10 became nested BCR-ABL PCR negative.

When these patients were started on IM, there was no data to indicate whether the molecular remissions achieved would be durable or whether these patients should be maintained indefinitely on therapy. Imatinib was stopped in 7 of the surviving 12 patients. The median duration of treatment for patients who stopped IM was 11 months (range 6-35). No patient stopped the drug because of toxicity. Only 2 of the 7 patients who stopped IM have remained in molecular remission with a median follow-up of 42 months. The other 5 patients all had re-emergence of BCR-ABL transcripts. One patient has stable low levels of transcripts and has not received any further treatment. Four patients were restarted on IM, one has again become PCR negative. The remaining 3 patients received DLI in combination with IM, one remains in molecular remission and 2 have low level stable BCR-ABL transcripts and remain in CCR. Five patients were continued on IM: 4 of these patients remain disease free while one has low level stable BCR-ABL transcripts.

DLI is an effective treatment for patients relapsing after SCT for CML and can restore durable molecular remissions in a high percentage of patients.[4,8,9] However, a significant proportion of patients are unresponsive. Toxicity

Table 2. Post transplant.

Case#	Months post BMT to 1st relapse	DLI pre Imatinib	Type of relapse prior to imatinib (1st/2nd)	Starting dose for imatinib	Best Response (TTR in months)[4]	Imatinib stopped	Imatinib restarted	Current status
1	120	Donor RIP	Hematologic (1)	600 mg	MMR[1] (5)	Yes	Yes	PCR -ve
2	7	x 2	Hematologic (2)	400 mg	MCR[2] (6), + by pcr	No	–	Died AML blast crisis
3	122	No	Molecular (1)	400 mg	MMR (4)	Yes	No	PCR +ve
4	36	x 2	Molecular (2)	400 mg	MMR (3)	Yes	No	PCR -ve
5	36	No	Molecular (1)	400 mg	MMR (6)	Yes	Yes	Post DLI, PCR -ve
6	122	No	Cytogenetic (1)	400 mg	MMR (15)	No	–	PCR -ve
7	36	x 3	Molecular (2)	400 mg	MMR (3)	No	–	PCR -ve
8	24	No	Molecular (1)	400 mg	MMR (4)	Yes	Yes	Post DLI, PCR -ve
9	12	x 2	Hematologic (2)	400 mg	NR[3]	No	–	Died
10	108	No	Molecular (1)	400 mg	MMR (4)	Yes	No	Post DLI, PCR -ve
11	72	No	Cytogenetic (1)	400 mg	MMR (4)	Yes	No	PCR -ve
12	18	Donor pregnant	Cytogenetic (1)	400 mg	MMR (3)	No	–	PCR +ve
13	180	No	Hematologic (1)	400 mg	MMR (6)	No	–	PCR -ve
14	7	No (GVH)	Cytogenetic (1)	400 mg	MMR (3)	No	–	PCR -ve

[1]Major molecular response; [2]major cytogenetic response; [3]No response; [4]time to first response.

from GvHD remains a concern although it can be reduced by using incremental dose regimens.[10] Additionally DLI may not be an option because of unavailability of the original donor, (2/14 patients in our series), or due to GvHD. This report demonstrates the effectiveness of IM in re-establishing leukemia control without serious toxicity and supports the published data.[6,7] However the response in most patients was not durable unless IM was continued, as has been previously shown for patients treated with IM as a primary treatment.[11] Interestingly, the 2 patients who remain in continued molecular remission had both received IFN therapy (patients 4 and 11, Table 2) pre-transplant. This is analogous to reports of occasional patients treated with IFN and subsequent IM who maintained durable molecular remissions when IM is discontinued.[12]

What is the best treatment strategy for a patient who relapses post-BMT from the pre-IM era? We have shown that IM achieves excellent responses without toxicity although it usually needs to be continued for durable responses. DLI has proven to be effective for early relapses albeit with serious adverse effects in some patients. There may be a role for IM in the induction of responses, with a possibility to consolidate with lower doses of DLI. In the post-IM era, most patients who relapse post-transplant will have already been exposed to IM and deemed unresponsive. Determining the mechanism of resistance in these patients is important and mutational analysis of the BCR/ABL oncogene should be considered. The role of newer tyrosine kinase inhibitors such as nilotinib and dasatinib in these patients is unknown; EBMT is exploring this with the use of dasatinib in a recently opened trial.

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Citation: Hayat A, McCann SR, Langabeer S, Irvine S, Frances McMullin M, and Conneally E. Effective use of imatinib-mesylate in the treatment of relapsed CML after allogeneic transplantation. Haematologica 2009; 94:296-298. doi: 10.3324/haematol.13353

References

1. Gratwohl A, Hermans J, Niederwieser D, Frassoni F, Arcese W, Gahrton G, et al. Bone marrow transplantation for chronic myeloid leukemia: long-term results. Chronic Leukemia Working Party of the European Group for Bone Marrow Transplantation. Bone Marrow Transplant 1993; 12:509-16.
2. Horowitz MM, Rowlings PA, Passweg JR. Allogeneic bone marrow transplantation for CML: a report from the International Bone Marrow Transplant Registry. Bone Marrow Transplant 1996;17(Suppl 3):S5-6.
3. Collins RH Jr, Rogers ZR, Bennett M, Kumar V, Nikein A, Fay JW. Hematologic relapse of chronic myelogenous leukemia following allogeneic bone marrow transplantation: apparent graft-versus-leukemia effect following abrupt discontinuation of immunosuppression. Bone Marrow Transplant 1992;10:391-5.
4. Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, Arcese W, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. European Group for Blood and Marrow Transplantation Working Party Chronic Leukemia. Blood 1995;86:2041-50.
5. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. IRIS Investigators. N Engl J Med 2003;348:994-1004.
6. Olavarria E, Ottmann OG, Deininger M, Clark RE, Bandini G, Byrne J, et al. Response to imatinib in patients who relapse after allogeneic stem cell transplantation for chronic myeloid leukemia. Chronic Leukaemia Working Party of the European Group of Bone and Marrow Transplantation (EBMT). Leukemia 2003;17:1707-12.
7. Hess G, Bunjes D, Siegert W, Schwerdtfeger R, Ledderose

G, Wassmann B, et al. Sustained complete molecular remissions after treatment with imatinib-mesylate in patients with failure after allogeneic stem cell transplantation for chronic myelogenous leukemia: results of a prospective phase II open-label multicenter study. J Clin Oncol 2005;23:7583-93.
8. Dazzi F, Szydlo RM, Craddock C, Cross NC, Kaeda J, Chase A, et al. Comparison of single-dose and escalating-dose regimens of donor lymphocyte infusion for relapse after allografting for chronic myeloid leukemia. Blood 2000;95:67-71.
9. Kaeda J, O'Shea D, Szydlo RM, Olavarria E, Dazzi F, Marin D, et al. Serial measurement of BCR-ABL transcripts in the peripheral blood after allogeneic stem cell transplantation for chronic myeloid leukemia: an attempt to define patients who may not require further therapy. Blood 2006;107:4171-6.
10. Mackinnon S, Papadopoulos EB, Carabasi MH, Reich L, Collins NH, Boulad F, et al. Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: separation of graft-versus-leukemia responses from graft-versus-host disease. Blood 1995;86:1261-8.
11. Cortes J, O'Brien S, Kantarjian H. Discontinuation of imatinib therapy after achieving a molecular response. Blood 2004;104:2204-5.
12. Rousselot P, Huguet F, Rea D, Legros L, Cayuela JM, Maarek O, et al. Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. Blood 2007;109:58-60.

Late relapse of acute myeloid leukemia with mutated *NPM1* after eight years: evidence of *NPM1* mutation stability

Late relapse (>5 years) of acute myeloid leukemia (AML) is rare.[1] Detecting at the time of late relapse the same genetic alteration as at diagnosis strongly suggests it may play a critical role in leukemogenesis. We previously reported in this journal that in AML with mutated nucleophosmin (NPM1), NPM1 mutation is very stable at relapse.[2] However, this conclusion was based upon molecular analysis of AML patients in whom the interval between diagnosis and relapse was short (median one year).[2] Here, we describe for the first time the clinico-pathological and molecular features of a patient with *NPM1*-mutated, *FLT3*-ITD positive AML who, after eight years, relapsed with *NPM1*-mutated, *FLT3-ITD* negative AML. These findings provide the most compelling clinical evidence to date that *NPM1* mutation is stable, strongly suggesting it is a founder genetic lesion and further supporting the view that AML with mutated NPM13 is a separate entity with distinct biological and clinical features.[4,5]

In May 2000, a 19-year old woman was diagnosed with acute myelo-monocytic leukemia (*Online Supplementary Figures 1A*) at the Institute of Hematology, "La Sapienza" University, Rome. The leukemic cells' immunophenotype in cell suspension was: $CD33^+$ (95%), myeloperoxidase-positive (55%), $CD13^+$ (23%), $CD14^+$ (25%), $CD15^+$ (46%), $CD117^+$ (1%), $HLA\text{-}DR^+$ (1%), $CD34^+$(0%). Results of immunohistochemistry are shown in *the Online Supplementary Figures 1B and C*. Immunohistochemical staining of bone marrow trephine revealed aberrant nucleophosmin expression in leukemic cell cytoplasm[6] (Figure 1A), predicting *NPM1* mutation. *NPM1* gene sequencing showed a 4 base pair (TCTG) insertion after nucleotide 1018 corresponding to mutation A3. Molecular studies also revealed an internal tandem duplication of the FLT3 gene (FLT3-ITD) (Figure 1B). The number of NPM1 mutant copies assessed by quantitative PCR at diagnosis is shown in Figure 1C. Cytogenetic analysis showed a der(13;14) constitutional Robertsonian recombination associated with trisomy 4 in six metaphases and der(13;14) as the sole finding in five (*data not shown*).

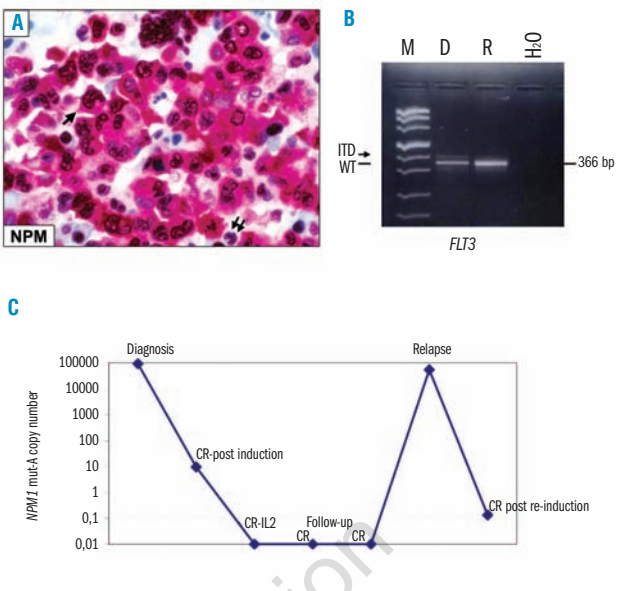

Figure 1. (A) **NPM subcellular expression at diagnosis. Acute myeloid leukemia cells show aberrant cytoplasmic positivity for nucleophosmin (NPM) (arrow) whilst residual hemopoietic cells exhibit a nucleus-restricted positivity for NPM (double arrows). APAAP technique; × 800; hematoxylin counterstaining. (B) *FLT3* gene status at diagnosis and relapse. The arrow shows the presence of a faint extra-band corresponding to the *FLT3-ITD* expressed at low level at diagnosis but not at relapse. The black line indicates the 366bp product corresponding to the amplification of FLT3 wild-type. M: marker; D: diagnosis; R: relapse; H2O=negative control, water line. C) Retrospective quantification of *NPM1* mut-A by real time RT-PCR. Variation in *NPM1* mut-A transcript level at diagnosis, complete remission (CR) and relapse is shown. The highest transcript copies number was detected at diagnosis and relapse, whereas the transcript copies number decreased following induction and became undetectable during follow-up. After re-induction therapy a reduction of NPM1 mut-A copies number was detected again. IL2 indicates interleukin-2.**

The patient was treated under the Gruppo Italiano Malattie EMatologiche dell'Adulto (GIMEMA)/EORTC AML12 protocol (*Online Supplementary Appendix*). Complete hematologic remission, first documented in June 2000, continued until May 2008 when the patient relapsed. Immunohistochemical and molecular studies confirmed nucleophosmin was dislocated into leukemic cell cytoplasm (C23/nucleolin was nucleus-restricted) (Figure 2 A, B) and NPM1 mutation A was present (Figure 2C). FLT3 gene analysis revealed no ITD or D835 mutations (Figure 1B). Cytogenetic investigation again found a 46,XX, der(13;14) (q10;q10), +4 karyotype in 4/9 metaphases (Figure 2D). Rescue therapy was immediately started with ARA-C, idarubicin and mylotarg (*Online Supplementary Appendix*). At present (September 2008), the patient is in complete hematological remission.

Our results raise interesting questions about the significance and stability of NPM1 and FLT3 mutations in