that renders SCA patients more susceptible to infection. A multicenter prospective study may determine whether such patients should be treated with prophylatic antibiotic therapy throughout their lives to prevent infection.

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Funding: FAPESP, Brazil.

Key words: sickle cell anemia, myeloperoxidase polymorphism, infection.

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Chronic Myeloid Leukemia

Outcome of four patients with chronic myeloid leukemia after imatinib mesylate discontinuation

Imatinib mesylate (IM) therapy is effective in patients with chronic myeloid leukemia (CML). However, whether it should be discontinued in patients who achieve sustained molecular response is debated. We describe 4 patients with undetectable levels of BCR-ABL transcripts in whom IM therapy was discontinued. Two patients relapsed after 7 and 10 months and promptly responded after restarting therapy; 2 patients are off therapy at the last follow-up visit after 14 and 15 months and are still in complete molecular remission.

haematologica 2005; 90:979-981
(*http://www.haematologica.org/journal/2005/7/979.html*)

Imatinib mesylate (IM) therapy leads to a complete cytogenetic response (CCyR) in the majority of patients with chronic myeloid leukemia (CML) in chronic phase. A few patients achieve complete molecular remission, defined by a four log-reduction of BCR-ABL transcripts. The negative quantitative real time polymerase chain reaction (Q-RT-PCR) is confirmed by nested PCR negativity.

Although IM therapy is effective in CML patients, some unanswered questions remain. In particular, it is unclear whether IM can actually cure CML and whether this therapy can be safely stopped in patients with complete cytogenetic and molecular responses.[1] In these patients, it is unknown whether and for how long continued therapy is required to maintain clinical, cytogenetic and molecular responses. Between 2000 and 2004, we treated 88 CML patients with IM: 62 were interferon-α (IFN) pre-treated patients in late chronic phase and 26 were newly- diagnosed patients in early chronic phase. Sixty-three patients (71.5%) achieved a CCyR and in 15 of them (24%) BCR-ABL transcripts became undetectable.

Here we describe the cytogenetic and molecular outcome of 4 patients with CML in whom IM therapy was discontinued after the achievement of a complete molecular response in the bone marrow and peripheral blood. In all cases, IM was discontinued because of the patients' requests and not because of toxic effects. The patients' characteristics are shown in Table 1. All patients were pre-treated with IFN; only patient #2 was in CCyR and was switched to IM because of IFN-intolerance. No patient had a family donor for allotransplant or was a candidate for an unrelated transplant. During IM therapy at 400 mg/day, no patient required dose reduction or discontinuation due to hematologic or non-hematologic toxicity. At the time of IM withdrawal, all patients had been in sustained CCyR for 17 to 30 months and in complete molecular response for 13 to 19 months. All patients showed normal cell morphology on bone marrow examination and none of them had additional cytogenetic abnormalities.

The relative quantification of BCR-ABL transcripts was performed by Q-RT-PCR using 1 μg of total RNA, isolated by an RNeasy mini kit (Qiagen) from 10^7 Ficoll-hypaque separated mononucleated cells, reverse-transcribed as previously described.[2] Relative quantification

Table 1. Clinical characteristics of 4 patients in whom IM therapy was discontinued.

	Gender/ age	Sokal risk	CHR to IFNα	CCyR IFNα	Time from diagnosis to IM therapy (months)	Time to CCyR on IM therapy (months)	Time to Q-PCR negativity on IM therapy (months)
1	M/60	low	yes	NR	137	6	9
2	M/63	low	yes	NR	33	6	9
3	F/51	high	yes	CCyR	33	–	12
4	F/52	int	yes	NR	66	6	12

CHR: complete hematologic response; CCyR: complete cytogenetic response; IM: imatinib; Tx: therapy.

Table 2. Molecular and cytogenetic outcome of the 4 CML patients who discontinued IM therapy after the achievement of sustained molecular response.

#	Time from Q-RT-PCR negativity to IM withdrawal (months)	Molecular relapse	Follow up Q-RT-PCR and karyotype
1	13	No	Sustained complete molecular remission at 15+ months after IM discontinuation. Normal karyotype.
2	19	Yes	Complete molecular remission at 6 months after IM discontinuation. Q-RT-PCR= one log increase of BCR-ABL transcripts at 7 months → two log increase at 8 months → IM therapy resumed. Second complete molecular response duration: 4+ months Normal karyotype.
3	17	Yes	Complete molecular remission at 7 months after IM discontinuation. Q-RT-PCR= one log increase of BCR-ABL transcript at 10 months → IM therapy resumed → Second complete molecular response: 2+ months Normal karyotype.
4	14	No	Sustained complete molecular remission at 14+ months after IM discontinuation. Normal karyotype.

of the BCL-ABL fusion transcript was performed with GeneAmp 5700 SDS (Applied Biosystems) in a reaction volume of 25 µL, using 1/10 cDNA volume, SYBR Green PCR Master Mix and the primers already reported.[3] The dissociation curves were analyzed to assess amplification specificity. A standard curve was obtained through serial dilutions (5×10^5 – 5 BCR-ABL copies) of K562 RNA into normal control RNA. BCR-ABL expression levels were calculated by the ΔΔCt method, using ABL as the normalizing gene and K562 as a calibrator sample. Nested RT-PCR assay was used to confirm the negative results of quantitative RT-PCR2. After IM discontinuation, Q-RT-PCR was performed every 3 months on bone marrow and peripheral blood samples.

As shown in Table 2, patients #2 and #3 experienced molecular relapse 7 and 10 months, respectively, after IM discontinuation. The cytogenetic Philadelphia marker was negative and the karyotype was normal. Both patients resumed IM at 400 mg/day and both achieved a second complete molecular response. They are currently receiving IM therapy. Patients #1 and #4 are still in complete molecular response (15+ and 14+ months, respectively) and both are off therapy. Our experience suggests that withdrawal of IM therapy in chronic phase CML patients after achievement of a complete molecular response may result in different molecular outcomes. It is likely that the absence of detectable BCR-ABL transcript by Q-RT-PCR does not equate with cure. To our knowledge, there are 6 other reported cases of IM discontinuation due to intolerance or patients' request: 5 had undetectable levels of BCR-ABL transcript[4,5] and 1 was in sustained cytogenetic response.[6] Overall, in 4 of the literature cases a molecular and cytogenetic relapse occurred rapidly, whereas our 2 cases only had molecular relapse. Moreover, patients who restarted IM had prompt cytogenetic and molecular responses. There is evidence that CML patients have a leukemic population of non-cycling Go *quiescent stem cells* that are not sensitive to IM.[7,8] This subclinical *reservoir* can be a source for disease relapse. On the other hand, the prompt improvement seen after restarting therapy argues against the development of resistance. However, the selection of resistant clones after IM exposure and the emergence of Philadelphia-negative clones with secondary cytogenetic abnormalities are matter of concern, particularly in patients receiving IM for a long time.[5-7] Although the follow-up of our patients is short, the improved quality of life while off therapy and the prompt response to resumed IM therapy suggest that the subset of patients who have sustained complete molecular response may be candidates for intermittent therapy. Future studies should determine the optimal duration of BCR-ABL negativity before IM therapy can be safely discontinued.

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Acute Myeloid Leukemia

Establishment of a human myeloid cell line with trisomy 8 derived from overt leukemia following myelodysplastic syndrome

A human myeloid cell line with trisomy 8 was newly established from overt myelogenous leukemia arising in myelodysplastic syndrome. The cells of this cell line showed immature myelocyte characteristics. The karyotype retained trisomy 8. This cell line could improve understanding of the pathophysiology of myelogenous leukemia with trisomy 8.

haematologica 2005; 90:981-982
(*http://www.haematologica.org/journal/2005/7/981.html*)

We recently cared for an interesting patient with myelodysplastic syndrome (MDS) who demonstrated unique abnormalities in his karyotype: clonal expansion of leukemic cells carrying trisomy 8 replaced the clone carrying trisomy 11, which was the initial chromosome aberration, when the patient became resistant to treatment. We tried to establish the cell line from a peripheral blood sample of this patient. Here we describe the characteristics of this myeloblastic cell line, SKK-1, established from the patient with acute myeloid leukemia (AML) arising from MDS.

Our patient, a 69-year old male, was diagnosed as having refractory anemia with excess of blast (RAEB) in March 1991. Karyotypic study at that time revealed 47,XY,+11 (Table 1). In July 1991 the disease transformed to RAEB in transformation, with no further karyotypic changes. He was treated with combination chemotherapy but had only a poor response. In August the disease progressed to AML and became chemotherapy resistant. The patient died of leukemia in October 1991. Cytogenetic study at this point showed that the most of examined cells had trisomy 8 (Table 1).

Cells for establishing the cell line were obtained from the peripheral blood of the patient in October 1991. Mononuclear cells were separated on a Ficoll-Hypaque gradient and cultured in RPMI-1640 medium supplemented with 20% fetal calf serum (FCS) and recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) at 37°C in a humid condition with 5% CO_2. The stable subclone, designated SKK-1 was established and its clonality was confirmed by surface marker analysis and fluorescent *in situ* hybridization (FISH) analysis. The SKK-1 has been maintained with a doubling time of 32 hours in RPMI-1640 medium supplemented with 10% FCS and 10ng/mL rhGM-CSF. SKK-1 cells proliferate as free-floating cells in suspension. The nuclei are large, round, often lobulated and a few nucleoli are found per cell. The cytoplasm contains a few granules and some cells have vacuoles in the cytoplasm. The cells are negative for myeloperoxidase. They are positive for CD13, CD33, CD71, CD117 and HLA-DR cell surface markers. CD34, CD41, CD42 and EBNA are negative. The cytogenetic data of SKK-1 cells are shown in Table 1.

Table 1. Karyotypic analyses of the original leukemia and the SKK-1 cells.

	Karyotype	*Metaphases*
RAEB cells	47,XY,+11	20/20
RAEB-t cells (July 1991)	47,XY,+11	20/20
Leukemia cells (October 1991)	47,XY,+8,15q? 47,XY,+8,15q?,i(17q) 47,XY,+8,4p+,15q? 47,XY,+11,15q?	30/45 1/45 13/45 1/45
SKK-1 cells	47,XY,add(4)(p16),+8,add(12)(p13)	10/10

The karyotype of these cells is 47,XY,add(4)(p16), +8,add(12)(p13) (10 examined cells) (Table 1). FISH analysis showed that 93.8% of SKK-1 cells carry trisomy 8. This novel cell line, designated SKK-1, derived from the peripheral blood of a patient with the overt myeloblastic leukemia arising from MDS, appears to have properties of primitive myeloblasts, as judged from the morphologic and phenotypic studies. The patient showed an interesting finding in a series of cytogenetic studies (Table 1).

Although trisomy 8 is a very common cytogenetic abnormality found in myeloid disorders such as MDS and AML,[1-6] its precise role in leukemogenesis remains unknown. Trisomy 8 is occasionally noted as an evolutionary change, found together in the same patient added to the initial abnormal karyotype in these diseases.[2,7] Moreover, trisomy 8 confers an intermediate prognosis in MDS and an intermediate to poor risk in AML.[8] These data support the possibility that trisomy 8 correlates with progression in MDS and AML.

In our case, the clone with trisomy 8 had a growth advantage over the clone with trisomy 11, and MDS progressed to AML. We thus considered that analysis of the leukemic cells with trisomy 8 in this patient is a promising way to understand the role of trisomy 8 in leukemogenesis, and tried to establish the cell line from this patient. Molecular analysis of the SKK-1 cell line documented no rearrangement of genes on chromosome 8, such as MTG8 or c-myc, confirming, as previously suggested, that unknown mechanisms are important.[9] There are some other myeloid cell lines with trisomy 8, such as MOLM-13 and MOLM-14.[10] These cell lines could also be excellent models for analyzing the role of trisomy 8 in progression of AML, however their use as a tool to study the progression from RAEB to AML may be limited. SKK-1 cells have additional chromosome abnormalities, add(12p) and add(4p), which appeared in the process of