S1:S71-S3.
5. Cortes J, O'Brien S, Kantarjian H. Discontinuation of imatinib therapy after achieving a molecular response. Blood 2004; 104:2204-5.
6. Ghanima W, Kahrs J, Dahl TGIII, Tjonnfjord G. Sustained cytogenetic response after discontinuation of imatinib mesylate in a patient with chronic myeloid leukemia. Eur J Haematol 2004;72:441-3.
7. Bumm T, Müller C, Al-Ali HK, Krohn K, Shepherd P, Schmidt E, et al. Emergence of clonal cytogenetic abnormalities in Ph-cells in some CML patients in cytogenetic remission to imatinib but restoration of polyclonal hematopoiesis in the majority. Blood 2003;101:1941-9.
8. Graham SM, Jorgensen HG, Allan E, Pearson C, Alcorn MJ, Richmond L, et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood 2002;99:319-25.

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.

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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

cell line establishment. Abnormalities of 12p are relatively common in adverse risk AML.[8] These abnormalities might have some effects on disease progression. Finally, since SKK-1 cells probably contain undetected genetic alterations which could influence the progression of MDS to AML, analyzing this cell line may reveal alterations and their role in leukemogenesis.

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Key words: trisomy 8, myeloid cell line, SKK-1, leukemogenesis AML, MDS,

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References

1. Mitelman F. Catalog of Chromosome Aberrations in Cancer. New York: Wiley-Liss. 1992.
2. Rowley AD, Golomb HM. The Fourth International Workshop on Chromosomes in Leukemia: a prospective study of acute nonlymphocytic leukemia. Cancer Genet Cytogenet 1984;11:249-360.
3. Fenaux P, Preudhomme C, Lai JL, Morel P, Beuscart R, Bauters F. Cytogenetics and their prognostic value in de novo acute myeloid leukaemia: a report on 283 cases. Br J Haematol 1989; 72:61-7.
4. Heim S, Mitelman F. Secondary chromosome aberrations in the acute leukemias. Cancer Genet Cytogenet 1986;22:331-8.
5. Yunis JJ, Brunning RD, Howe RB, Lobell M. High-resolution chromosomes as an independent prognostic indicator in adult acute nonlymphocytic leukemia. N Engl J Med 1984;311:812-8.
6. Paulsson K, Sall T, Fioretos T, Mitelman F, Johansson B. The incidence of trisomy 8 as a sole chromosomal aberration in myeloid malignancies varies in relation to gender, age, prior iatrogenic genotoxic exposure, and morphology. Cancer Genet Cytogenet 2001;130:160-5.
7. Jacobs RH, Cornbleet MA, Ardiman JW, Larson RA, Le-Beau MM, Rowley JD. Prognostic implications of morphology and karyotype in primary myelodysplastic syndromes. Blood 1986;67:1765-72.
8. Greer JP, Baer MR, Kinney MC. Acute myeloid leukemia in adults. In: Greer JP, Foerster J, Lukens JN, Rodgers GM, Paraskevas F, Glader B, editors. Wintrobe's Clinical Hematology. 11th edition. Philadelphia: Lippincott Williams & Wilkins; 2004. p. 2097-142.
9. Diaz MO, Le-Beau MM, Harden A, Rowley JD. Trisomy 8 in human hematologic neoplasia and the c-myc and c-mos oncogenes. Leuk Res 1985;9:1437-42.
10. Matsuo Y, MacLeod RA, Uphoff CC, Drexler HG, Nishizaki C, Katayama Y, et al. Two acute monocytic leukemia (AML-M5a) cell lines (MOLM-13 and MOLM-14) with interclonal phenotypic heterogeneity showing MLL-AF9 fusion resulting from an occult chromosome insertion, ins(11;9)(q23;p22p23). Leukemia 1997;11:1469-77.

Acute Myeloid Leukemia

The CYP1A1*2a allele is an independent prognostic factor for acute myeloid leukemia

Polymorphisms in carcinogen- and drug-metabolizing enzymes may increase the risk of acute myeloid leukemia (AML) and may influence prognosis. We report that the polymorphic variant of the cytochrome P450 CYP1A1*2A, present in 11.3% of patients, is an independent unfavorable prognostic factor for failure-free and overall survival in patients with AML.

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CYP1A1 belongs to the cytochrome P450 family and is a phase I detoxification enzyme involved in the bioactivation of several chemical carcinogens, including cytotoxic drugs. Cytochrome P450 enzymes transfer electrons onto toxicants to create highly reactive intermediates which are usually coupled to glutathione or other groups, producing water-soluble compounds, but can also interact with DNA, resulting in the formation of DNA adducts.[1] The CYP1A1 polymorphic variants *2A and *2B have increased enzymatic activity and/or inducibility, while the biological significance of the *4 allele is still unknown.[1] We have shown that the CYP1A1*2B and *4 alleles may increase the risk of acute myeloid leukemia (AML), particularly when combined with the glutathione-S transferase (GST) T1 null genotype.[2] On the other hand, polymorphisms of xenobiotic metabolizing enzymes may influence the prognosis of AML and Hodgkin's lymphoma, as previously shown by our group for deletions of GSTM1 and/or T1 genes and for the GSTP1 Ile105Val polymorphism.[3-5] We were interested in the prognostic role of polymorphisms of CYP1A1*2A, *2B and *4 and in possible interactions with established prognostic factors, including age, white blood cell counts (WBC), karyotype and the fms-like tyrosine kinase 3 (FLT3) internal tandem duplications (ITD).[6]

Our analysis included 97 patients (48 females, 49 males, median age 58 years, range 16–70), diagnosed with AML between April 1997 and April 2004. Eleven patients had had a previous cancer: 8 of them had received previous radio/chemotherapy. Patients were treated according to protocols of the GIMEMA group using standard chemotherapy regimens (EORTC-GIMEMA AML 12 and 13, GIMEMA-AIDA 0493, *www.gimema.org*). Complete remission, partial remission and resistance to induction treatment were assessed by bone marrow evaluation on day +28 in 90 patients according to standard criteria. Death due to treatment toxicity precluded evaluation in the other 7 patients.

CYP1A1 polymorphisms T6235C, A4889G and C4887A were characterized by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach.[7] The presence of the T6235C alone identifies the *2A allele, while the combination of T6235C and A4889G identifies the *2B allele. C4887A corresponds to the *4 allele.[2,7] The frequencies of CYP1A1 alleles *2A, *2B and *4 were 11.3% (11/97), 3.1% (3/97) and 22.7% (22/97), respectively. Patients with alleles CYP1A1*2A and *2B were all heterozygous, while 1 of 22 patients with allele *4 was homozygous. The