

Case Report

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Clonal Karyotype Evolution Involving Ring Chromosome 1 with Myelodysplastic Syndrome Subtype RAEB-t Progressing into Acute Leukemia

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Hans Jochen Kolb^{a,b} Helga Schmetzer^{a,b}^aDepartment of Medicine III, Klinikum Grosshadern, University of Munich and ^bGSF – Research Center for Environment and Health, Munich, Germany**Key Words**

Acute myeloid leukemia · Bone marrow transplantation, allogeneic · Clonal evolution · Cytogenetics · Myelodysplastic syndrome · Ring chromosome 1 · Stem cell transplantation · Donor lymphocyte infusion

Abstract

Karyotypic evolution is a well-known phenomenon in patients with malignant hematological disorders during disease progression. We describe a 50-year-old male patient who had originally presented with pancytopenia in October 1992. The diagnosis of a myelodysplastic syndrome (MDS) FAB subtype RAEB-t was established in April 1993 by histological bone marrow (BM) examination, and therapy with low-dose cytosine arabinoside was initiated. In a phase of partial hematological remission, cytogenetic assessment in August 1993 revealed a ring chromosome 1 in 13 of 21 metaphases beside BM cells with normal karyotypes [46,XY,r(1)(p35q31)/46,XY]. One month later, the patient progressed to an acute myeloid leukemia

(AML), subtype M4 with 40% BM blasts and cytogenetic examination showed clonal evolution by the appearance of additional numerical aberrations in addition to the ring chromosome [46,XY,r(1),+8,-21/45,XY,r(1),+8,-21,-22/46,XY]. Intensive chemotherapy and radiotherapy was applied to induce remission in preparation for allogeneic bone marrow transplantation (BMT) from the patient's HLA-compatible son. After BMT, complete remission was clinically, hematologically and cytogenetically (normal male karyotype) confirmed. A complete hematopoietic chimerism was demonstrated. A relapse in January 1997 was successfully treated using donor lymphocyte infusion and donor peripheral blood stem cells (PB-SC) in combination with GM-CSF as immunostimulating agent in April 1997, and the patient's clinical condition remained stable as of January 2005. This is an interesting case of a patient with AML secondary to MDS. With the ring chromosome 1 we also describe a rare cytogenetic abnormality that predicted the poor prognosis of the patient, but the patient could be cured by adoptive immunotherapy and the application of donor's PB-SC. This case confirms the value of cytogenetic analysis in characterizing the malignant clone in hematological neoplasias, the importance of controlling the quality of an induced remission and of the detection of a progress of the disease.

Thomas Duell and Brigitte Poleck-Dehlin contributed equally to this publication.

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Introduction

Cytogenetic abnormalities are known to occur in bone marrow (BM) cells in about 50% of patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) [1–3]. Clonal karyotype evolution is a well-known phenomenon in these patients, often preceding a clinical progression of the disease. This is why cytogenetic examinations make a valuable tool to estimate the prognosis; usually, patients with certain cytogenetic markers or with a complex karyotype or with a new arising marker in the course of the disease have a worse prognosis than those without a cytogenetic abnormality [1, 4]. Ring chromosomes are genetic abnormalities occurring in leukemic and other neoplastic cells and are often related to a poorer prognosis [5, 6]. Ring chromosomes have been described in patients with MDS [7] but so far no ring chromosome 1.

Despite the advent of new therapeutic agents, the prognosis of an MDS and secondary AML is still very poor [8]. Generally, secondary leukemias are harder to treat than primary malignancies. Bone marrow transplantation (BMT) is regarded as the only curative treatment for patients younger than 60 years [9]. We report an MDS (RAEB-t subtype) patient who had presented with a ring chromosome 1, followed by a clonal evolution in BM cells, who had an early progress which could be treated by BMT and achieved a long-term remission after a relapse by adoptive immunotherapy using donor lymphocyte infusion (DLI) and donor peripheral blood stem cells (PB-SC).

Case Report

A 50-year-old male patient was admitted to our facility in April 1993 for a medical workup after outpatient blood examinations had shown persistent asymptomatic pancytopenia since October 1992. The PB revealed pancytopenias with $28 \times 10^9/l$ leukocytes, 11 g/dl hemoglobin, $68 \times 10^9/l$ thrombocytes and 1% blast cells. BM examination revealed a blast count of 29 with 60% of those cells being peroxidase positive and 50% of them being esterase positive. Ringed sideroblasts or Auer rods were not detectable. According to the French-American-British (FAB) classification (table 1) [10], the disease was rated as an MDS subtype RAEB-t with transition into an AML. From May to July 1993, the patient received low-dose cytosine arabinoside chemotherapy. In August 1993, the patient was found to have gone into partial remission with a BM blast count of 10%. Cytogenetic analysis revealed a ring chromosome 1 in 13 of 21 metaphases indicating the persistence of clonal, leukemic cells: (46,XY,r(1)(p35q31)[13]/46,XY[8]) (fig. 1). Only 1 month later, the disease progressed to an AML, subtype M4 (myelomonocytic leukemia) with a BM blast count of 39% and monocytoid-dif-

ferentiated blasts (CD14+, CD33+, CD13+, HLA-Dr+) detectable by flow cytometry. Cytogenetically, disease progression was also indicated by clonal evolution, which consisted of additional numeric aberrations of chromosomes 2, 21 and 22 (45,XY,r(1)(p35q31),+8,-21,-22[7]/r(1)(p35q31),+8,-21[10]/46,XY[4]).

The origin of the ring chromosome could be identified by G banding. The rings were homogeneous in structure. In figure 1, a small fragment of chromosome 1 can be seen demonstrating that not the whole genetic material was included in the ring, but a fragment (q24–43) was deleted. These small q-arm fragments could be seen in the preparation of August but not in that of September 2003 and might have been lost during the preparation.

The patient received intensive chemotherapy according to the sHAM protocol (hexamethylenamine, Adriamycin, melphalan, methotrexate) and total body irradiation to prepare for allogeneic transplant in December 1993, using his son's HLA-compatible BM. A complete chimerism was established with cells of his son characterized by a phosphoglucomutase 1 (PGM1) type a3a1 isoenzyme type (the patient's PGM1 type was a1a1) [11]. The a3a1 donor PGM1 type was demonstrable at 14 different time points between April 1994 and September 1998 in several cellular fractions (granulocytes, platelets, erythrocytes or lymphocytes) obtained from PB or BM of the patient (data not shown). In January 1997 a relapse of the patient's AML was noted on BM examination with a BM blast cell count of 18% and a PB blast cell count of 2%. The presence of donor cells in the PB was demonstrable by the PGM1 donor isoenzyme type a3a1. Therefore, the patient could be treated with a DLI-based therapy in combination with LD-AraC and PB-SC without immunosuppressive therapy and immunostimulation with GM-CSF in April 1997 using his son's lymphocytes. After DLI therapy he developed an acute graft versus host reaction of the skin (grade 3) and the intestine; however, he achieved a second remission which has been stable as of January 2005.

The clinical course of the patient was complicated by several transplantation/therapy-associated diseases like EBV-associated pleural polyserositis, a cataract, glaucoma, and stem cell transplantation (SCT)-associated keratoconjunctivitis. Moreover, the patient suffered from herpes infection, diabetes type 2 and osteoporosis.

Material and Methods

Morphological Examinations

Cytological and cytochemical examinations of the BM cells were performed by staining the cell smears with Pappenheim solution; peroxidase (POX; rendering yellow stain to granulopoietic cells except myeloblasts) and esterase reactions (macrophages and monocytes strongly react positively) were performed according to standard procedures [10, 12].

Immunophenotyping

Flow-cytometric analysis was employed to determine lineage and maturation of the surface markers. Mononuclear BM cells were incubated with a panel of monoclonal antibodies conjugated with fluorescent dyes [13]. Subsequent analysis was done to determine the proportions of cells and the blast phenotype, according to the manufacturer's instruction (Cytoron Absolute, Ortho Diagnostic Systems).

Table 1. Patient's course of the disease

Date	Diagnosis	Clinical stage	Therapy	BM blasts %	Cytogenetics
October 1992	pancytopenia				
April 1993	MDS (RAEB-t)	diagnosis	LD-AraC May to September 1993	29	not available
August 1993		MDS-PR		10	46,XY,r(1)(p35q31)[13]/46,XY[8]
September 1993	sAML-M4	AML diagnosis		39	45,XY,r(1)(p35q31),+8,-21,-22/46XY,r(1)(p35q31),+8,-21[10]/46,XY[4]
			sHAM/Endoxan/TBI September to December 1993		
November 1993	sAML-M4	AML pers	allogeneic SCT December 1993	2	
April 1994	sAML	AML-CR		<5	46,XY[19-32] (5 different analyses)
September 1996	cataract and glaucoma		surgery	<5	
December 1996	sAML	AML-CR			
January 1997	sAML	AML relapse after SCT	LD-AraC January to February 1997	18	
	aGvH		GM-CSF, DLI, prednisolone, cyclosporine February 1997		
May to October 1997	osteoporosis	AML-CR	Aredia	<5	46,XY[23-28] (2 different analyses between May and October 1997)
	aGvH				
June 1999	EBV-associated polyserositis, pleural and pericardial effusion		Cidofovir June 1999 to May 2000		
August 1999	diabetes (subtype 2)		Euglucon August 1999		
May 2000	herpes zoster				
			retinal detachment October 2003		
May 2004	SCT-associated keratoconjunctivitis				
January 2005		AML-CR			

The patient's disease with diagnosis, treatment measures and outcome is demonstrated as related to time. The course of the disease can be seen between October 1992, the diagnosis of pancytopenia, and January 2005, after treatment of AML with BMT, of AML relapse with DLI and of concomitant diseases. The patient is still in remission of AML. PR = Partial remission; CR = complete remission; sAML = secondary AML; aGvH = acute graft versus host disease; TBI = total body irradiation; pers = persisting disease.

Cytogenetics

Cytogenetic techniques were facilitated by incubation of un-separated BM cells for 48 h at 37°C. Metaphase chromosome analysis and Giemsa banding of chromosomes were executed according to standard staining procedures [14].

Discussion

MDS are disorders characterized by hematopoietic abnormalities involving the clonal hematopoietic progenitor cells resulting in uncontrolled proliferation, differentiation or maturation of hematopoietic cells [1, 3, 4]. The

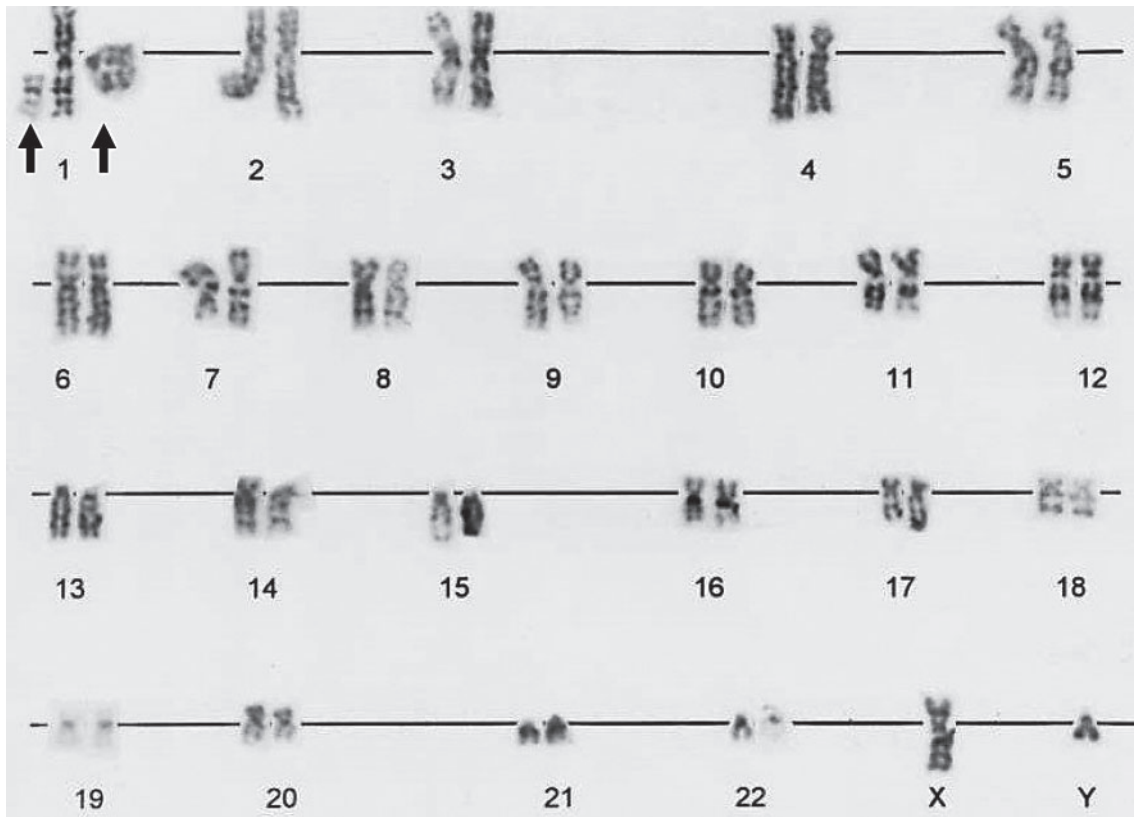
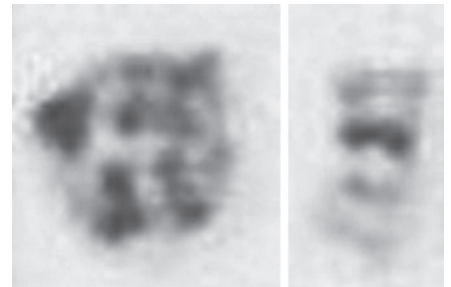


Fig. 1. Karyotype analysis was performed by Giesma banding of metaphases obtained from uncultured BM cells in August 1993. In 13 of 21 metaphases a ring chromosome 1 could be detected. The rings resulting from a break and fusion at q24 were homogeneous in structure. The remaining genetic material (q24–43) could be seen in some preparations.



only potentially curative treatment for these patients is allogeneic hematopoietic SCT [15, 16]. Relapses after transplantation, however, occur and are due to a reemerging of the malignant cell clones, regularly detectable by an increase of blast cells in PB or BM, reduction of blast phenotypes and/or clonal markers [4, 17–19]. Isoenzyme studies are good means for follow-up analyses of chimerism after SCT. The detection of donor cells in a relapsed patient allows a DLI therapy without preliminary immunosuppression [11, 20]. Relapses after SCT occur and can be successfully treated by DLI-based therapy as described by others and us [21–23], although the details of DLI-based treatment of a relapse post-SCT in MDS (e.g. with purified donor T cells or pooled lymphocytes prior che-

motherapy) remain to be established [21, 24]. In our case a stable remission could be established by DLI therapy pointing to the crucial and impressive therapeutic efficacy of donor lymphocytes in eradicating tumor burden and establishing a stable remission as already shown in a small group of patients treated in a DLI-based pilot study [23].

The clinical course of the patient was complicated by several SCT and/or graft-versus-host disease-associated side effects; ocular abnormalities like cataracts, keratoconjunctivitis or glaucoma due to microvascular lesions are observed especially in patients conditioned with total body irradiation and cyclosporin A and are more often seen in patients with chronic graft versus host disease [25–27]. Loss of bone mineral density resulting in osteo-

porosis as well as the development of diabetes mellitus type 2 could be a late effect of SCT [28–31]. EBV or herpes virus infections are still complications in patients after SCT and are often responsible for morbidity and mortality after SCT [32, 33]. Possibly these infections could be responsible for pleural or pericardial effusions as observed in our patient [34]. Effective prophylactic or preemptive treatment strategies and infection controls after transplantation could help to avoid transplant-related problems [33, 35].

Independent of FAB types cytogenetics contributes important diagnostic and prognostic data in AML and MDS which give clues for therapeutic decisions [17, 36, 37]. Ring chromosomes are rare cytogenetic findings in MDS. Usually they appear in cases with a complex karyotype and are associated with a poor prognosis [6, 7, 38]. Moreover it was shown that clones containing ring chromosomes might be more resistant to chemotherapy than other cytogenetically abnormal clones [7]. It could be demonstrated that the emergence of ring chromosomes correlates well with worsening pancytopenia or transfusion requirements for the patient [38]. In univariate analyses Sole et al. [39] could demonstrate that aberrations at the q-arm of chromosome 1 were associated with an unfavorable prognosis for MDS patients, making patients presenting with such abnormalities good candidates for a more aggressive, curative therapeutic regimen like BMT. Detailed information about the effect of deletions of parts of chromosome 1 on the outcome of MDS patients is still missing.

We describe a case of MDS and secondary AML in a relatively young man with a favorable clinical course, although the cytogenetic findings of a ring chromosome and a complex aberrant karyotype would normally indicate a rather poor prognosis [1, 2, 4, 36, 37]. Also, there was rapid progression to a secondary acute leukemia, which is often harder to treat than the primary leukemia and has a very short survival time. Despite concomitant SCT-related problems, allogeneic BMT and the DLI treatment for a relapse proved to be a successful therapy, with the patient being in a stable condition for more than 7 years after the initial diagnosis.

To our knowledge ring chromosomes of chromosome 1 were not described in MDS. The clinical course of the patient demonstrates that this ring chromosome 1 contributed to the further clinical course of the disease: the clonal evolution and the fast progression, which could only be cured by BMT with an allogeneic transplant. The relevance of a ring chromosome has not been established; more data need to be collected for establishing the role and impact of a ring chromosome in a patient with MDS and AML.

This case illustrates the already well-established importance of performing cytogenetic examinations additionally to histomorphological techniques in patients with MDS and AML [4, 16, 17, 40].

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