



# Increased frequency of dicentric chromosomes in therapy-related MDS and AML compared to *de novo* disease is significantly related to previous treatment with alkylating agents and suggests a specific susceptibility to chromosome breakage at the centromere

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Dicentric chromosomes are observed in many malignant diseases including myelodysplasia (MDS) and acute myeloid leukemia (AML) and have often been observed in a subset of these diseases, namely therapy-related MDS (t-MDS) and AML (t-AML). Using fluorescence *in situ* hybridization (FISH) with centromere-specific probes, we investigated the frequency and type of dicentric chromosomes in 180 consecutive patients with t-MDS and t-AML and in 231 consecutive patients with *de novo* MDS and AML, whose karyotypes had been studied previously by conventional G-banding. Twenty-seven out of 180 patients with t-MDS or t-AML presented dicentric chromosomes compared to only seven out of 231 patients with *de novo* disease ( $P = 0.00003$ ). A dic(1q;7p) was observed in 10 cases, a dic(5p;17q) was observed in six cases, whereas various isodicentric chromosomes were observed in six cases. Excluding these six cases with isodicentrics, all 25 patients with dicentric chromosomes had involvement of at least one of the chromosome arms 1q, 5p, or 7p resulting in monosomy for 5q or 7q, and/or trisomy for 1q. Patients with dicentric chromosomes presented significantly more often as t-MDS compared to patients without dicentrics ( $P = 0.046$ ), and the presence of a dicentric chromosome was significantly related to previous therapy with alkylating agents ( $P = 0.026$ ). Thus, only one out of 27 patients with a dicentric chromosome had not previously received an alkylating agent. A specific susceptibility to breakage at the centromere after exposure to alkylating agents is suggested and may explain the frequent loss of whole chromosomes, in particular chromosomes 5 and 7 in t-MDS and t-AML, if the breaks are not followed by rejoining. *Leukemia* (2000) 14, 105–111.

**Keywords:** therapy-related myelodysplasia; therapy-related acute myeloid leukemia; dicentric chromosomes; alkylating agents

## Introduction

Dicentric chromosomes are the result of translocations with a fusion of two chromosome arms and centromeric or pericentromeric sequences from both chromosomes involved. Two general types of dicentric chromosomes are observed: those derived from a fusion of non-homologous chromosome arms and the isodicentrics derived by a fusion of two short arms or two long arms of homologous chromosomes. Because the breakpoints in both participating chromosomes most often occur at, or very close to, the centromere, in a region presumably devoid of genes, these translocations do not directly result in gene rearrangement, as do the reciprocal balanced translocations. Most likely, the genetic consequences of these dicentric chromosomes merely relate to the characteristic loss of the arms reciprocal to those forming the dicentric or, to an apparent duplication of one of the chromosomes or chromosome arms involved.

So far, little is known about why chromosome aberrations arise in malignant diseases, including MDS and AML. How-

ever, in a subset of these disorders, namely t-MDS and t-AML, the etiology is well established as being previous therapy with an alkylating agent, treatment with a DNA topoisomerase II inhibitor or, in a small number of patients, previous radiotherapy.<sup>1–5</sup> As the two general types of cytostatic drugs or irradiation have all been shown experimentally to be mutagenic and to induce chromosome aberrations, there are good reasons to suggest that the leukemias as well as the chromosome aberrations are both a direct result of the previous therapy.

In previous studies of t-MDS and t-AML dicentric chromosomes have often been observed, and specific dicentric chromosomes such as the dic(1;7),<sup>6,7</sup> the dic(5;17),<sup>8</sup> and the dic(7;12)<sup>9</sup> have been discussed in separate reports. However, the occurrence of dicentric chromosomes has not been studied in general in a large series of patients with t-MDS and t-AML.

The present study was undertaken to analyze the frequency of dicentric chromosomes in our previously reported cases of t-MDS and t-AML compared to the frequency in *de novo* MDS and AML. Furthermore, we report all chromosomes involved in the formation of dicentrics and verify by FISH the results obtained by conventional karyotyping. We focused on dicentric whole-arm translocations, but also included cases presenting translocations with breakpoints close to the centromeres, because such cases in fact could represent dicentric chromosomes, as shown in a recent study.<sup>10</sup> Finally, we have related the presence of dicentric chromosomes in patients with t-MDS and t-AML to the presence of other chromosome aberrations and to the most important clinical parameters, particularly the type of previous therapy, and whether the disease presented as either t-MDS or overt t-AML.

## Materials and methods

One hundred and eighty consecutive cases of t-MDS and t-AML for which the cytogenetic and clinical characteristics have been published previously in detail,<sup>11–17</sup> and a further 231 consecutive cases of *de novo* MDS and AML studied at our laboratory by conventional G-banding chromosome analysis of bone marrow cells, were re-analyzed. The cytogenetic nomenclature of ISCN (1995) was used. In 32 patients an apparently dicentric chromosome had been diagnosed by conventional G-banding and in an additional 25 patients translocations with breakpoints close to the centromeres had been observed.

In order to reassess the presence of dicentric chromosomes in the two groups of patients, two-color FISH was performed on methanol-acetic acid fixed bone marrow cells stored at  $-20^{\circ}\text{C}$  from the time of diagnosis. Only in five patients out of 57 was material not available for FISH. Two of the following centromere-specific probes: CEP 1, 3, 6, 7, 10, 11, 12, 15, 17

**Table 1** Results of conventional G-banding compared with the results of FISH analysis for dicentric chromosomes

	<i>t-MDS and t-AML</i>	<i>MDS and de novo AML</i>
Dicentric chromosomes confirmed by FISH	17/22	3/6
Dicentric chromosomes identified only by FISH	10/15	4/9
Dicentric chromosomes total	27/180 <sup>a</sup>	7/231 <sup>a</sup>
Material unavailable for FISH	4	1

<sup>a</sup> $\chi^2 = 17.56, P = 0.00003$ .

(Vysis, Downers Grove, IL, USA) or the alpha satellite probes 1/5/19, 7, 8, 13/21, 14/22, 16, 20 (Oncor, Gaithersburg, MD, USA) were hybridized to metaphase cells using standard procedures as recommended by the manufacturers. FISH signals were visualized by a Zeiss Axioskop epifluorescence microscope (Oberkochen, Germany), and images were captured by the Quips Smart Capture FISH Imaging Software from Vysis. An average of 15 abnormal mitoses were examined, and only cases with two different and distinct signals from the centromeric region in at least five mitoses were classified as dicentric. Whenever necessary whole chromosome painting probes for chromosomes 1, 5, 7 and 17 (Vysis) were applied to positively identify the dicentric chromosome. In six cases with a dic(5;17) and in one case with a dic(7;12) additional arm-specific painting probes for 5q, 17p, 7p, 7q, 12p, and 12q (AL Technologies, Arlington, VA, USA) were used.

Chromosome derivatives were classified as isodicentric in cases presenting a fusion of homologous whole chromosome arms and a FISH signal larger, most often twice as large as the centromeric signal from the normal chromosome.

## Results

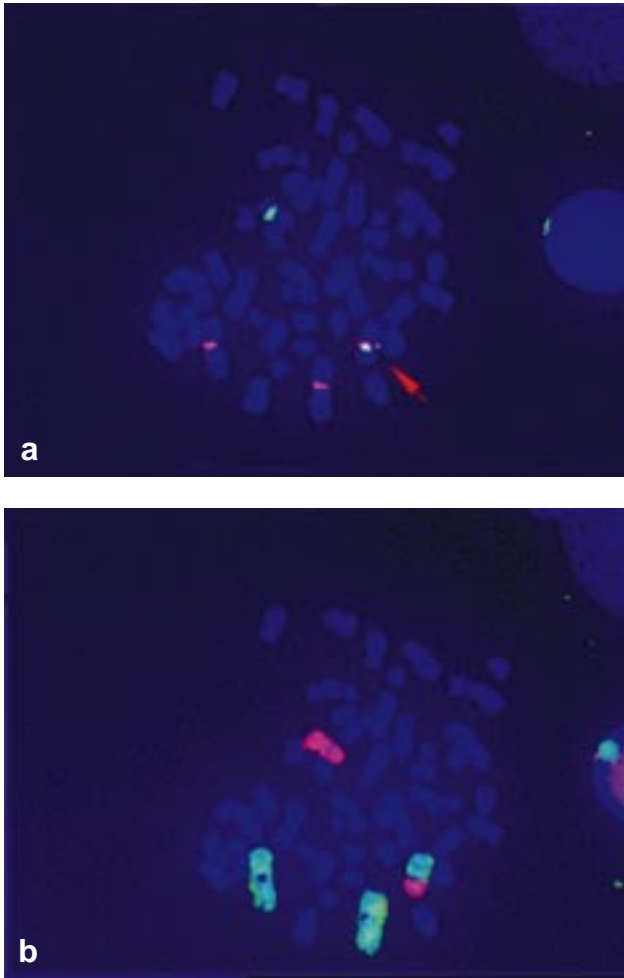
Dicentric chromosomes were identified by FISH analysis in 27 out of 180 patients with t-MDS or t-AML and in seven out of 231 patients with *de novo* MDS or AML (Table 1). This difference is highly significant:  $\chi^2 = 17.56, P = 0.00003$ . In 22 patients with t-MDS or t-AML the presence of a probably dicentric chromosome had previously been reported according to G-banding and in 17 of these patients the results were confirmed by FISH, whereas in five patients a centromeric signal from only one of the two chromosomes was present on the derivative. Similarly, the presence of a dicentric chromosome was verified in three out of six patients with *de novo* MDS or AML. In patients with translocations and breakpoints close to the centromere according to G-banding, 10 out of 15 patients with t-MDS or t-AML and four out of eight patients with *de novo* MDS or AML were verified by FISH as dicentric (Table 1). In four patients with t-MDS or t-AML and in one patient with *de novo* AML, bone marrow material was not available for FISH.

Table 2 shows the specific type of dicentric chromosome observed in 27 patients with t-MDS or t-AML and in seven patients with *de novo* MDS or AML, and the resulting gain or loss of whole, or almost whole chromosome arms. Most often a dic(1;7) (Figure 1), or a dic(5;17) was observed (Figure 2). All 25 patients with dicentrics derived by a fusion of the two arms from non-homologous chromosomes had involvement of at least one of 5p, 7p, or 1q. This resulted in monosomy for 5q or 7q and trisomy 1q, due to the loss of one whole chromosome 5 or 7, or in cases with involvement of 1q, the presence of two apparently normal chromosomes 1 in addition to the 1q participating in the formation of the dicentric. Conversely, in nine patients with isodicentrics all patients presented trisomy for the involved chromosome arm and eight out of nine patients simultaneously showed monosomy for the other arm of the chromosome (Table 2). None of the nine cases with isodicentrics involved chromosomes 5 or 7 and

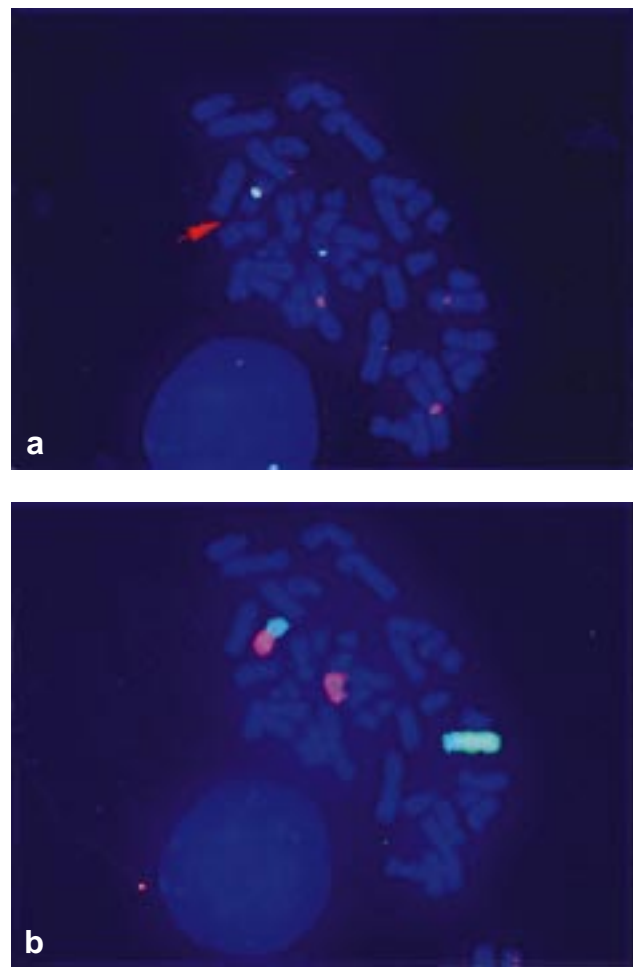
**Table 2** Number of patients and types of dicentric chromosomes observed in 180 therapy-related and in 231 *de novo* cases of MDS and AML

<i>Dicentric chromosome</i>	<i>t-MDS or t-AML</i>	<i>de novo MDS or AML</i>	<i>Loss of</i>	<i>Gain of</i>
dic(1;6)(p11;q11)	1	—	— <sup>a</sup>	1q
dic(1;8)(q32;q22)	—	1	8p	1q
dic(1;12)(p11;p11)	1	—	12p	1q
dic(1;15)(p11;p11)	—	1	15p	1q
dic(1;7)(p11;q11)	10	2	7q	1q
dic(5;7)(q11;q11)	1	—	5q, 7q	—
dic(5;17)(q11;p11)	4	—	5q, 17p	—
— (q12 or q13;p11)	1	—	5q, 17p	—
— (q11;p12)	1	—	5q, 17p	—
dic(7;12)(q?;p?)	1	—	7q, 12p	—
dic(7;13)(p11;p11)	1	—	7p, 13p	—
Total	21	4		
<i>Isodicentrics</i>				
idic(1)(p11)	—	1	1p	1q
idic(8)(p11)	1	—	8p	8q
idic(13)(p11)	1	—	13p	13q
idic(14)(p11)	—	1	— <sup>a</sup>	14q
idic(17)(p11)	1	1	17p	17q
idic(21)(p11)	3	—	21p	21q
Total	6	3		

<sup>a</sup>Supernumerary dicentric chromosomes not accompanied by loss.



**Figure 1** A case of dic(1;7) examined by FISH. (a) Centromere specific signals for chromosome 1 (red) and chromosome 7 (green). The dic(1;7) contains two closely related centromeric signals (arrow). (b) The same metaphase spread with whole chromosome painting probes for chromosome 1 (green) and chromosome 7 (red).



**Figure 2** A case of dic(5;17) examined by FISH. (a) Centromere specific signals for chromosomes 1 and 5 (red) and chromosome 17 (green). The dic(5;17) contains two closely related centromeric signals (arrow). (b) The same metaphase spread with whole chromosome painting probes for chromosome 5 (green) and chromosome 17 (red).

only one involved 1q. In 10 patients with t-MDS and t-AML for whom dicentrics were identified only by FISH, and in five patients in whom a suspected dicentric could not be confirmed by FISH, the previous karyotype was corrected according to our new findings as shown in Table 3.

In patients with t-MDS and t-AML the dicentric chromosomes were apparently true whole-arm translocations with fusion of the centromeres in all but three cases. In two out of six cases with a dic(5;17) the centromeres were separated by chromosomal material from 5q in one case and from 17p in another case, and the breakpoints were reassessed as dic(5;17)(q12 or q13;p11) and dic(5;17)(q11;p12), respectively. In the third case with a dic(7;12) the centromeric signals were separated by a small region of non-centromeric chromosomal material, by arm-specific painting probes demonstrated to derive from 12p as well as 7q (Figure 3).

In patients with t-MDS and t-AML an interesting and significant difference was observed between the two largest groups of dicentrics, the dic(1;7) and the dic(5;17), with regard to the presence of additional cytogenetic abnormalities (Table 4). Thus, a dic(1;7) was observed in eight out of 10 patients alone or together with one single (two patients) or two (one patient) other cytogenetic abnormalities, which was trisomy 8

in two cases. Conversely, all six patients with a dic(5;17) presented at least three other cytogenetic abnormalities ( $P = 0.007$ , Fisher's exact test, two-sided).

As far as presentation of the disease as t-MDS or as overt t-AML is concerned, 23 out of 27 patients with dicentric chromosomes presented as t-MDS, vs 97 out of 153 patients without dicentrics. This difference is significant ( $\chi^2 = 3.94$ ,  $P = 0.046$ ). The result is not unexpected, since 17 out of 27 patients with a dicentric chromosome showed monosomy for 5q or 7q, an abnormality previously shown to be significantly related to t-MDS.<sup>15,16,19</sup> Patient age, latent period from primary tumor to development of t-MDS or t-AML, and response to intensive antileukemic chemotherapy, did not show any significant difference between patients with dicentric chromosomes and patients without dicentrics.

Finally, and most important, the presence of a dicentric chromosome was related to the general type of therapy for the primary tumor (Table 5). Twenty-six out of 27 patients with dicentric chromosomes had previously been treated with alkylating agents either alone (13 patients), combined with radiotherapy (five patients), or a topoisomerase II inhibitor (six patients), or combined with both (two patients). Only a single patient had previously received therapy not including alkylat-

**Table 3** Revision of the previously reported karyotype in 10 patients with and five patients without dicentric chromosomes and t-MDS or t-AML based on the results of FISH analysis

Case No.	Presentation of the disease	Revised karyotype
32 <sup>a</sup>	MDS	44,XY,dic(5;17)(q11;p11),-6,-7,-8,+2mar[19]
89 <sup>a</sup>	MDS	45,XY,dic(5;17)(q11;p12),add(6)(p23),+add(6)(p23),del(7)(q22),dic(15;16)(p11;q11)[11]/45,idem,idelic(8)(p11)[5]
114 <sup>a</sup>	MDS	46,XY,+1,dic(1;7)(p11;q11)[16]/46,XY[12]
117 <sup>a</sup>	MDS	46,XX,+1,dic(1;7)(p11;q11)[19]/46,XX[6]
124 <sup>a</sup>	MDS	43,XX,dic(5;17)(q12 or q13;p11),-7,-9,-22,add(21)(p11),+mar1[17]/43,XX,dic(5;17)(q11;p11),-6,-19,-21,+mar2[9]
165 <sup>a</sup>	AML	46,XX,+1,dic(1;7)(p11;q11)[27]
166 <sup>a</sup>	MDS	44,XX,dic(5;7)(q11;q11),-15,-20+mar[3]/46,XX[21]
167 <sup>a</sup>	MDS	45,XY,dic(7;13)(p11;p11)[3]/46,XY[24]
168 <sup>a</sup>	MDS	43,X,-X,+1,dic(1;12)(p11;p11),t(3;21)(q26;q22),del(4)(p14p16),-6,-8,del(10)(p11),-13,-14,-14,del(20)(p12),add(22)(q12),+mar1,+mar2,+mar3[14]/46,XX[13]
173 <sup>a</sup>	MDS	44,XX,dic(5;17)(q11;p11),-7[6]/43,idem,del(3)(p21),-4,add(8)(p21),-12,-21,+mar1,+mar2[8]/43,idem,del(3)(p21),-4,add(8)(p21),add(11)(q23),-12,-21,+mar1,+mar2[3]/46,XX[9]
63 <sup>b</sup>	MDS	45,XX,der(5)t(5;21)(q11;q11),-21[19]/44,XX,-5,-21[3]
81 <sup>b</sup>	AML	46,XY,idelic(13)(p11),der(17)t(11;17)(q11;p13)[34]
152 <sup>b</sup>	MDS	44,X,-Y,inv(3)(q21q26),-5,-7,-9,add(9)(p13),der(13;21)(q10;q10),+der(13;21)(q10;q10),der(15)t(13;15)(q11;p11),-21,-22,+4mar[21]
154 <sup>b</sup>	MDS	47,XX,del(5)(q11.2q33),add(7)(q11.2),add(12)(p11),-18,-18,del(20)(q12),+mar[9]/46,idem,t(10;12)(q11;p11),-20[8]/46,idem,-8,del(12)(p11.2),+12,-add(12)[7]
160 <sup>b</sup>	AML	46,XX,46,XX,-5,der(12)t(3;12)(p11;p11),+mar[6]/46,XX[23]

<sup>a</sup>Cases reassessed by FISH to have a dicentric chromosome.

<sup>b</sup>Cases disproved by FISH to have a dicentric chromosome.

ing agents, as she had had radiotherapy only. On the other hand, 37 out of 153 patients without dicentrics had received therapy not including an alkylating agent. Thus, the occurrence of dicentric chromosomes in general was significantly related to previous therapy with alkylating agents ( $\chi^2 = 4.94$ ,  $P = 0.026$ ).

## Discussion

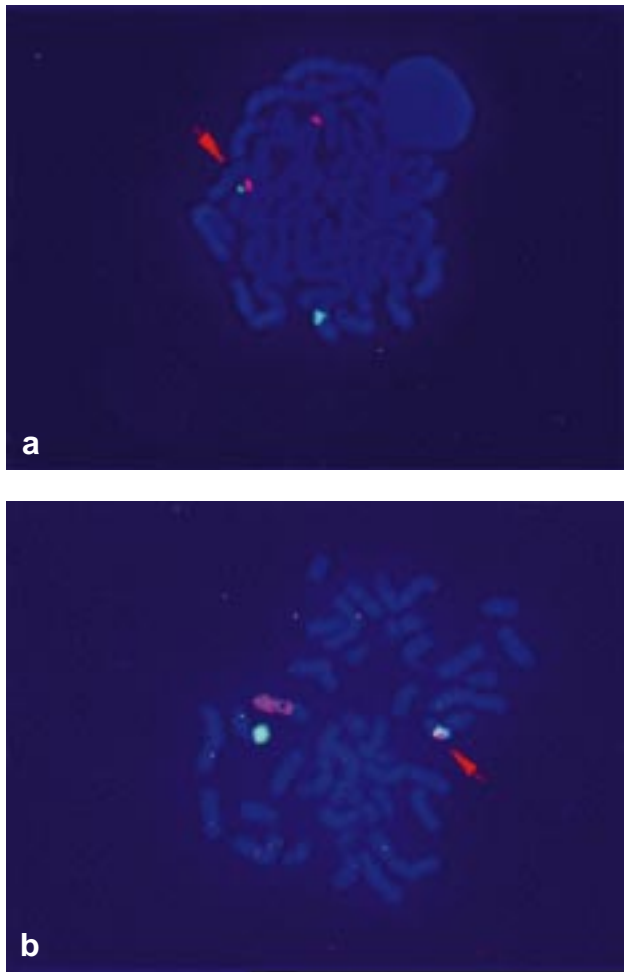
The present study demonstrates that dicentric chromosomes in general are significantly more common in therapy-related leukemia as compared to *de novo* MDS and AML, and are easily overlooked or misinterpreted without FISH analysis. In patients with a dicentric chromosome diagnosed by G-banding this could be confirmed in only 71% by FISH, whereas 58% of patients with translocation breakpoints close to, but not at the centromere according to G-banding were shown by FISH in fact to represent dicentric chromosomes. This clearly demonstrates the superiority of the FISH technique for identification of this type of chromosome abnormality. However, as the centromere-specific FISH probes also cover the alpha satellite sequences flanking the centromeres, it can not be excluded that some breakpoints are not located in the centromere itself, but in the flanking repetitive sequences.

In t-MDS and t-AML the presence of dicentric chromosomes was significantly related to previous therapy with alkylating

agents, indicating a specific susceptibility to chromosome breakage at the centromeres following alkylation. This observation may be of more general importance in the understanding of the cellular events leading to monosomy, particularly of chromosomes 5 and 7 in t-MDS and t-AML. Finally, the results may lead to a discussion of why only chromosome 1 or its long arm is duplicated, if 1q participates in the formation of a dicentric.

For many years the alkylating agents have been recognized as the most important etiological factor for t-MDS and t-AML, characterized by monosomy for chromosomes 5 or 7 or deletions of various parts of the long arms of the two chromosomes.<sup>11-24</sup> More recently, the DNA topoisomerase II inhibitors have also been shown to be leukemogenic, in most instances administered in combination with platinum derivatives or an alkylating agent.<sup>25-28</sup> These leukemias primarily present balanced translocations to chromosome bands 11q23 or 21q22<sup>29</sup> with rearrangement of the *MLL* and the *AML1* genes, but also less frequently other balanced rearrangements such as the inv(16)(p13q22), and the t(15;17)(q22;q11) known from *de novo* MDS and AML.<sup>30,31</sup>

The whole-arm translocations leading to dicentric chromosomes as observed in t-MDS and t-AML in the present study differ generally from the balanced chromosome aberrations. Thus, the dicentric chromosomes are significantly related to previous therapy with alkylating agents, whereas the balanced translocations are related to therapy with DNA topoisomerase



arm is duplicated. Thus, in the future as has been done in the past, we suggest that the dicentric chromosomes, including 5p and 7p and leading to monosomy 5q and 7q, should be classified together with interstitial deletions of the long arms and the complete loss of the two chromosomes.

Previously, it has been demonstrated experimentally that breakage with loss of the telomeric region of a chromosome results in cellular arrest followed by a loss of the whole chromosome at one of the following cell divisions.<sup>32</sup> As alkylating agents induce double strand breaks of DNA, this could lead to loss of the telomeric sequences and subsequently loss of the whole chromosome by non-disjunction or by anaphase lagging. The present study demonstrates that another non-coding part of the chromosome, the centromere, may be particularly sensitive to breakage following alkylation, because breakage is a prerequisite of the development of a dicentric chromosome. If breaks occur at the centromere without homologous religation, only chromosome arms undergoing rearrangement and fusion with other chromosome fragments, for instance by the formation of a dicentric chromosome, remain as functional and consistent elements. Isolated chromosome arms, on the contrary, are eliminated, as are chromosomes devoid of the telomere. Centromeric breakage could represent an important mechanism for the development of monosomy 5 and monosomy 7 in t-MDS and t-AML as well as for the development of hypodiploidy in malignancy in general.

The results of the present study support the existence of one genetic pathway in t-MDS and t-AML characterized by deletions of various parts of the long arms of chromosomes 5 or 7 or loss of the whole chromosome 7 as the primary cytogenetic event.<sup>16</sup> In this model deletions or loss of the short arm of chromosome 17 or loss of a whole chromosome 17 were secondary cytogenetic events. A dic(5p;17q) has previously been closely related to mutations of the p53 gene located at 17p13, and to a complex karyotype,<sup>8</sup> and our results support the latter association.

As demonstrated, the presence of a dicentric chromosome in most cases resulted in loss of the arms reciprocal to those forming the dicentric, except for cases including 1q. Such cases were always associated with the presence of two apparently normal chromosomes 1, resulting in trisomy for 1q. A primary duplication of a whole chromosome 1 could precede and perhaps predispose to subsequent formation of a dicentric chromosome containing 1q could precede and perhaps predispose to a subsequent duplication of the whole chromosome. Trisomy for chromosome 1, however, is an uncommon cytogenetic finding in t-MDS and t-AML,<sup>14-24</sup> and the presence of dicentrics apparently does not result in duplication of other chromosomes or parts of chromosomes, except for 1q and the involved arms of the isodicentric chromosomes.

An interesting new model for the concurrent development of a dicentric chromosome or another unbalanced translocation containing a supernumerary 1q, has recently been proposed in advanced multiple myeloma.<sup>33</sup> In this disease decondensation of pericentromeric heterochromatin was observed and suggested to predispose to chromosome recombination at this region. A simultaneous formation of triradial configurations due to duplication of 1q was seen in some cases. This mechanism by which a translocation and a whole arm duplication of 1q develop simultaneously, should also be considered in t-MDS and t-AML with dicentric chromosomes containing 1q. If in a myeloma cell 16p is the partner and if in a myeloid cell 7p is the partner, the two types of cell could

**Figure 3** The case of dic(7;12) examined by FISH. (a) Centromere specific signals for chromosome 7 (green) and chromosome 12 (red). The centromeric signals on the dicentric chromosome are clearly separated (arrow). (b) Another metaphase spread with arm specific probes for 7q (red) and 12p (green). The fragment between the two centromeres contains chromosomal material from 12p as well as 7q (arrow).

**Table 4** Number of additional cytogenetic abnormalities in patients with t-MDS and t-AML and dicentric chromosomes

Number of aberrations	0	1-2	3	≥4
dic(1;7)	5	3	—	2
dic(5;17)	—	—	1	5
others	2	4	2	3
Total	7	7	3	10

dic(1;7) vs dic(5;17),  $P = 0.007$ .  
dic(5;17) vs others,  $P = 0.043$ .  
Fisher's exact test (two sided).

II inhibitors. Furthermore, the breakpoints of the dicentric chromosomes possibly do not directly result in gene rearrangement as the recurrent balanced aberrations, as they occur at, or very close to the non-coding centromeric region of the chromosome. Finally, the dicentrics are always unbalanced, because the reciprocal arms of the two chromosomes are lost, or if the long arm of chromosome 1 is involved, this

**Table 5** Type of previous therapy in t-MDS and t-AML with and without dicentric chromosomes

	Alkyl ± RT	Alkyl + ATTOP ± RT	ATTOP ± RT	RT only	Others	Total
Dicentric chromosome present	18	8	—	1	—	27
No dicentric chromosome present	69	47	11	21	5	153
Total	87	55	11	22	5	180

RT, radiotherapy; Alkyl, alkylating agents; ATTOP, topoisomerase II inhibitors.  
26/142 vs 1/38,  $\chi^2 = 4.94$ ,  $P = 0.026$ .

perhaps achieve a specific proliferative advantage, due to a combination of monosomy for one chromosome arm and trisomy for 1q. As in the study of advanced stage myeloma, demethylation of the centromeric region should be considered as the primary injury also in t-MDS and t-AML. Our results indicating that alkylation of the centromeric region could be the eliciting event in t-MDS and t-AML, could also be the mechanism in advanced stage myeloma, because almost all these patients have previously received alkylating agents, being the cytostatic drug most extensively used in this disease.

More basic research, is required to confirm our conclusions, and may lead to a better understanding of the complicated mechanisms by which chromosome aberrations develop in malignant diseases.

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