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Differences in Bone Marrow Cytogenetic Characteristics Between Treated and Untreated Myeloma

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Abstract—*Clonal karyotypic abnormalities in myelomatosis at initial diagnosis have been widely studied, but little data are available on the karyotypic status following treatment. We have studied bone marrow (BM) from 17 cases of untreated myelomatosis at initial diagnosis and from a further 29 cases who had received chemotherapy with regimens containing alkylating agents. At the time of study all previously treated cases had been off treatment for at least 152 days, had a paraprotein level in plateau phase, had a BM with less than 4% blasts, and in 28 of these 29 cases had less than 20% BM plasma cells. Two cases had more than 15% BM ringed sideroblasts; one other case was transfusion dependent.*

Clear differences in cytogenetic characteristics between the two groups were seen. At initial diagnosis clonal karyotypic abnormalities were observed in six of 11 assessible cases. All had hyperdiploid clones (of 49–54 chromosomes) and showed characteristic involvement of chromosomes 3, 5, 7, 9, 11, 15, 19 and 21. Additional structural rearrangements were present in only two of these cases. Following treatment, clonal abnormalities were seen in 10 of 25 assessible cases, of which only two showed hyperdiploidy (one with a hyperdiploid line, one with an additional derived chromosome). The remaining eight showed hypodiploid or pseudodiploid lines, and seven of these showed complex karyotypes with multiple rearrangements particularly affecting chromosomes 1, 2, 3, 6 and 7. After a minimum follow-up of 16 months, only four of these 10 cases (40%) remain alive compared with 12 of 15 (80%) with a normal karyotype after treatment ($P = 0.03$). No correlation was observed between the presence of an abnormal karyotype and the total dose or timing of previous therapy, though cases with an abnormal karyotype tended to have received treatment for longer (790 ± 166 days) than cases with a normal karyotype (486 ± 50 days). It is not clear whether the ploidy difference between the two groups represents a change in the disease state due to treatment or a direct effect of treatment itself.

INTRODUCTION

THE CYTOGENETIC study of multiple myeloma (MM) utilizing banding techniques has been severely limited because malignant plasma cells characteristically have low proliferative activity [1]. Moreover, such cells may be more resistant to G-banding [2]. For these reasons, information on cytogenetic abnormalities in MM is less extensive than for the leukaemias, and our knowledge of how such abnormalities relate to clinical features of the disease is poor.

Approximately 30–65% of cases of MM demonstrate a clonal karyotypic abnormality [3–8]. However, the majority of these studies have been carried out on mixtures of untreated cases at initial diagnosis, treated cases in stable plateau phase, cases currently on treatment and cases with aggressive refractory disease. There is some evidence that cases at initial diagnosis or in remission show a lower frequency of cytogenetic abnormalities than relapsed cases [5, 8]. The pattern of cytogenetic abnormalities in relapsed or refractory disease broadly resembles that at initial diagnosis [5] but very little information is available on the characteristics of cases in stable plateau phase. Such patients are known to be at risk of developing secondary myelodysplastic syndrome (MDS) and acute leukaemia and it has been suggested that this complication is related to previous therapy rather than disease evolution [9]. There is now much evidence that specific involvement of certain chromosomes, especially 5 and 7, is associated with this complication (reviewed in [10]).

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We have studied the bone marrow (BM) cytogenetic findings in 29 previously treated cases of MM in plateau phase, and compared them with 17 serial cases of newly diagnosed MM. We have compared the karyotypic findings in previously treated cases with the type and amount of therapy received.

PATIENTS AND METHODS

Chromosome analyses were carried out on BM aspirates processed by direct and short-term culture techniques without the addition of mitogens. Preparations were fixed with 3:1 methanol and acetic acid according to standard methods and slides stained by Giemsa–Trypsin–Leishman banding. In addition, high resolution banding techniques were carried out using methotrexate synchronization as previously described [11] on cases 3, 4, 8, 11, 16–19, 21, 24, 25, 55, 57 and 64.

Clinical features of all cases are given in Tables 1 and 2. Forty-five of the 46 cases studied had a serum paraprotein at initial diagnosis. The remaining case had Bence-Jones proteinuria only. At diagnosis, all cases had advanced (stage III) disease [12]. All untreated cases had at least 50% BM plasma cells; 28 of the 29 treated cases had less than 20% BM plasma cells. The remaining case (No. 29 in Table 1) had 28% plasma cells, and required further treatment approximately 6 weeks after study. All previously treated cases had less than 4% BM blasts, had completed treatment with alkylating agents (and in some cases radiotherapy) at least 152 days previously, and had a serum and/or urinary paraprotein either undetectable or in plateau phase. Two treated cases (Nos 11 and 24) had more than 15% BM ringed sideroblasts at the time of study (though not at initial diagnosis); one other case was transfusion dependent (No. 17).

For previously treated cases, the following parameters were recorded:

1. Total dose (per square metre) of melphalan and cyclophosphamide. All cases had received melphalan, and 13 of the 29 had received cyclophosphamide as well.
2. Duration of chemotherapy, in days.
3. Number of discrete coures of chemotherapy.
4. Days since last treatment was given.

RESULTS

Karyotypes were successfully obtained from 11 of 17 untreated and 25 of 29 previously treated cases. Details of these are given in Table 1 and 2. Abnormal clones other than constitutional abnormalities were observed in six of 11 (55%) untreated and 10 of 25 (40%) treated cases. Marked differences were observed in the nature of karyotypic abnormalities between the two groups. A clonal increase in chromosome number particularly involving chromosomes 3, 5, 7, 9, 11, 15, 19 and 21 is the dominant feature in untreated cases; additional structural rearrangements are present in only two such cases. In contrast, clonal chromosome loss and multiple structural rearrangements affecting chromosomes 1, 2, 3, 6 and 7 are the dominant feature in the treated group.

Only two cases in the treated group show a gain in chromosome number (case 1 with 46,XX/50–54,XX and case 24 with an additional derived

Table 1. Clinical and cytogenetic data on 17 untreated cases of MM. Survival is in months after initial study

No.	Age/sex	Survival	Number of cells Examined	Abnormal	Karyotype
51	62F	1029+	—	—	No metaphases
52	68M	517	5	4	46,XY/55,XY,+2,+3,+7,+11,+19,+20,+1p−,+2mar
53	75M	313	10	2	46,XY/49–50,XY
54	62M	940+	—	—	No metaphases
55	76M	938+	26	13	46,XY/47,XYY (constitutional)
56	72M	493	28	8	46,XY/53,XY,+3,+5,+9,+11,+15,+19,+21
57	72F	812+	20	0	46,XX
58	66F	212	20	0	46,XX
59	71F	762+	7	2	46,XX 50,XX
60	60F	747+	—	—	No metaphases
62	58F	283	—	—	No metaphases
63	70M	36	—	—	No metaphases
64	56M	181	60	10	46,XY/53–55,XY,+3,+5,+7,+8/9,+11,+14,+15, +18,+19,+20,+21 (variable)
65	78F	568+	24	0	46,XX
66	84M	489	20	0	46,XY
67	74M	558+	15	6	46,XY/52,XY,+3,+6,+9,+11,+19,+21,−13,t(7;?), t(15;?),+mar
68	70F	533+	—	—	No metaphases

Table 2. Clinical and cytogenetic data on 29 previously treated cases of MM in stable plateau phase

No.	Age/sex	Therapy*	Survival	Number of cells Examined	Abnormal	Karyotype
1	55F	VCBM	1030+	16	3	46,XX/50–54,XX
2	70M	M	1009+	11	0	46,XY
3	69F	MCEV	968+	14	0	46,XX
4	73M	VMR	959+	36	0	46,XY
5	59M	MEV	945+	38	0	46,XY
6	64M	CM	938+	40	0	46,XY
7	70F	CMR	938+	52	0	46,XX
8	81M	M	505	18	(−Y) 5	45,X/46,XY
					inv(1) 5	46,XY,inv(1)(p36q21)
					t(1;10) 4	46,XY,t(1;10)(p36;p13)
9	79F	M	903+	—	—	No metaphases
10	70F	M	868+	—	—	No metaphases
11	69M	MV	868+	22	0	46,XY
12	52F	ABCM	183	41	0	46,XX
13	66M	ABCM	751+	—	—	No metaphases
14	58M	ABCMVR	756	14	(−20) 3	45,XY,−20/46,XY
					(7q−) 2	46,XY,del(7)(q36)
15	64M	MEVR	750+	31	0	46,XY
16	57M	MVP	744+	57	0	46,XY
17	79F	MR	555	23	4	41–44,XX,del(1)(q32qter),t(7;11)(p14;p14), 6p+/46,XX†
18	72M	M	728+	17	(−Y) 5	45,X
					(−21) 5	45,XY,−21/46,XY
19	61F	M	715+	20	0	46,XX
20	74M	ABCM	488	20	0	46,XY
21	67M	CBM	183	12	7	46,XY,/46,XY,−6,−8,+i(6p), +der(8)t(3;8)(q12;p23)
22	63M	CM	700+	9	0	46,XY
23	63F	CVYR	50	—	—	No metaphases
24	56M	MCL	28	16	9	46,XY/47,XY,−6,+i(6p), t(2;3)(p11;p11),+der(3)t(2;3)(p11;p11)
25	55F	ABCM	658+	15	8	46,XX/46,XX,tdic(1;7),2p−,17p−
27	73F	M	249	23	0	46,XX
28	72M	MC	428	28	19	46,XY but non-specific loss
29	56M	M	531+	8	complex 5	44,XY,−13,−16,−21,−22,+i(1q),
					47–48,XY 3	t(1;2)(q12;q25),t(1;4) (q11;p16),19q+,+mar/ 47–48,XY
30	49M	ABCM	463+	29	0	46,XY

*A = Adriamycin®, B = BCNU, C = cyclophosphamide, E = epipodophyllotoxin, L = chlorambucil, M = melphalan, P = etoposide, R = radiotherapy, V = vinca alkaloid (vincristine and/or vinblastine), Y = cytosine arabinoside.

†Hypervariable.

chromosome). The remainder show hypodiploid or pseudodiploid lines. Case 25 demonstrates a rearrangement involving chromosome 7; this case has gone on to develop a refractory anaemia with severe trilineage cytopenia and BM morphological features of MDS. Case 17 also shows a chromosome 7 lesion, and has since died of MDS with severe trilineage cytopenia. Of the two cases with an excess of ring sideroblasts at the time of study (though not at initial diagnosis), case 11 (normal karyotype) remains well with a normal blood count 27 months later, whilst case 24 (complex abnormal karyotype) died of bronchopneumonia, though with a normal blood count, 5 weeks after study. Case 19 (normal karyotype) has developed secondary polycythaemia rubra vera; it is of interest that further BM karyotypes on this case remain normal. Cases 21 and 24 demonstrate a novel chromosomal abnormality, iso (6p), the subject of a separate report [13]. Case 28 was relapsing at the time of study, and required further therapy 6 weeks later. It is of interest that his karyotype reveals a mixture of hypodiploid and hyperdiploid lines.

The Kaplan–Meier survival curves of the treated cases with normal and abnormal karyotypes are shown in Fig. 1. A total of nine treated cases have died. With a minimum follow-up of 16 months, the overall survival is significantly better for patients with a normal karyotype ($P = 0.005$, log-rank test). The treatment parameters of treated cases with

normal and abnormal karyotypes are summarized in Table 3. No significant differences are seen (Mann–Whitney test), although those cases with an abnormal karyotype tend to have received treatment for longer than those with a normal karyotype.

DISCUSSION

Despite difficulties in obtaining satisfactory banded preparations in MM, approximately 30–65% of cases demonstrate an abnormal karyotype. Abnormalities tend to be complex, with a predominance of clonal hyperdiploidy particularly affecting chromosomes 3, 5, 7, 9, 11, 15, 19 and 21 [5–8]. Structural changes, often multiple, are also common and include anomalies of 14q in 30% of cases [5], rearrangements affecting chromosome 1 in 49% of cases [8], and anomalies of 11q and 17p. Clonal hypodiploidy is less frequent, occurring in only 11% of cases studied by Gould *et al.* [8].

Dewald *et al.* [5] found that of 16 cases of aggressive refractory MM, 63% had karyotypic abnormalities, the pattern of which was broadly similar to that of untreated cases. Gould *et al.* [8] found no apparent correlation between specific chromosomal abnormalities and disease state, and no difference in chromosome number between untreated patients and those who had received prior chemotherapy. However, an association between hypodiploidy and resistance to therapy has been suggested by flow cytometric studies [1, 8].

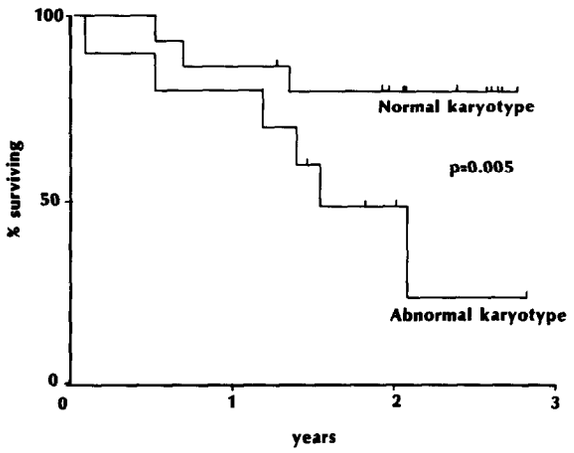

Fig. 1. Kaplan–Meier survival curves for treated cases with a normal (n = 15) *and abnormal* (n = 10) *karyotype.*

The present findings in newly diagnosed MM are in agreement with published series — a predominance of clones showing extensive chromosomal gain with non-random involvement of particular chromosomes in 55% of those cases in whom metaphases were obtained (Table 1). In contrast, the pattern of abnormalities is quite different in our patients with disease in stable plateau phase. Ten of the 25 cases from whom a karyotype was successfully obtained demonstrated a clonal karyotypic abnormality, and nine of these 10 have hypodiploid or pseudodiploid clones (Table 2).

In stable disease in plateau phase, where the tumour load is lower than at diagnosis, we have demonstrated that karyotypic abnormalities are both less frequent and also of a different pattern to those observed at diagnosis. The cell lineage(s) responsible for these abnormalities is uncertain, and we cannot exclude the possibility that BM cells other than the malignant plasma cells account for the abnormalities observed. It is interesting to speculate that at least some of the observed abnormal karyotypes in previously treated cases may represent clonal abnormalities in BM myeloid cells. Case 25 demonstrated a tdic(1;7) 7 months before developing a haematologically obvious secondary myelodysplastic syndrome. This clinical evolution suggests that myeloid BM cells may account for the tdic(1;7) lesion in this patient.

No significant difference in treatment parameters was noted between cases with normal and abnormal karyotypes, though cases with an abnormal karyotype tended to have received treatment for longer periods than those with a normal karyotype (Table 3). The overall survival of treated cases with an abnormal karyotype is significantly worse than cases with a normal karyotype (see Fig. 1; $P = 0.005$ by the log-rank test). It is not clear whether those cases with abnormal karyotypes rep-

Table 3. Treatment parameters for treated cases according to karyotype

	Karyotype		
	Normal	Abnormal	*P*
Total number of cases	15	10	
Total melphalan dose*	322 (39)	336 (75)	NS
Total cyclophosphamide dose*	2224 (846)	4949 (2283)	NS
Total duration of CT (days)	486 (50)	790 (166)	NS
No. of discrete courses of CT	1.3 (0.13)	2.0 (0.54)	NS
Days from last treatment to study	1225 (229)	457 (143)	NS

*Figures in mg/m^2. All 25 cases received treatment with melphalan, though only 13 received cyclophosphamide.
Figures are mean values, with standard error of mean in parentheses. Statistical comparison is by the Mann–Whitney test. CT = chemotherapy; NS means $P > 0.05$.

resent a subgroup with more aggressive or advanced disease, requiring longer periods of therapy to achieve plateau phase, or whether the longer period of therapy is causally related to the presence of an abnormal karyotype. Further studies are needed to define the significance of cytogenetic change in previously treated myeloma.

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