

Autografting

Genetic risk identifies multiple myeloma patients who do not benefit from autologous stem cell transplantation

H Chang^{1,2}, XY Qi^{1,2}, S Samiee³, Q-L Yi⁴, C Chen³, S Trudel³, J Mikhael³, D Reece³ and AK Stewart³

¹Department of Laboratory Hematology, Princess Margaret Hospital/University Health Network, University of Toronto, Toronto, Ontario, Canada; ²Department of Laboratory Medicine and Pathobiology, Princess Margaret Hospital/University Health Network, University of Toronto, Toronto, Ontario, Canada; ³Department of Medical Oncology, Princess Margaret Hospital/University Health Network, University of Toronto, Toronto, Ontario, Canada; and ⁴Department of Biostatistics, Princess Margaret Hospital/University Health Network, University of Toronto, Toronto, Ontario, Canada

Summary:

Genetic aberrations have emerged as major prognostic factors for patients with multiple myeloma (MM). We evaluated 126 MM patients for t(4;14) or t(11;14), 13q or p53 deletions and correlated the number of genetic aberrations with patient's clinical outcome following undergoing autologous stem cell transplantation. We demonstrate the significance of genetic-based risk classification that clearly segregate patients into low (no genetic abnormalities or only t(11;14)), intermediate (any one of the genetic abnormalities other than t(11;14)) and high-risk groups (any two or more of the genetic abnormalities other than t(11;14)). High-risk patients do not benefit from stem cell transplant and should be offered alternative therapies.

Bone Marrow Transplantation (2005) **36**, 793–796.
doi:10.1038/sj.bmt.1705131; published online 22 August 2005

Keywords: multiple myeloma; IgH translocations; 13q deletions; p53 deletions; fluorescence *in situ* hybridization; autologous stem cell transplantation

Multiple myeloma (MM) is a clonal plasma cell malignancy characterized by recurrent genetic changes that include chromosome 13q14 deletions and translocations involving the immunoglobulin heavy chain switch region (IgH) with various partner genes, most commonly t(11;14) and t(4;14).^{1,2} We and others have found that t(4;14) but not t(11;14) detected by cytoplasmic fluorescence *in situ* hybridization (cIg-FISH), is an adverse risk factor for MM patients receiving high-dose therapy and autologous stem cell transplantation (ASCT).^{3,4} In addition, we found that p53 deletions are an independent adverse prognostic factor

in MM patients undergoing ASCT.⁵ We now extend our analysis by studying MM patients evaluated by cIg-FISH for each of these four genetic abnormalities and propose a new genetic-based risk stratification model for MM patients treated with high-dose chemotherapy and ASCT. Specifically, we suggest that genetically high-risk patients do not benefit from high dose melphalan and should be offered alternative therapies.

Patients and methods

A total of 126 MM patients who received melphalan-based high-dose chemotherapy and ASCT were studied. The major clinical and biological features are summarized in Table 1. After institutional ethics review board approval, bone marrow aspirates were obtained, mononuclear cells enriched for by Ficoll separation and cytopspin slides prepared and stored at -70°C . To improve FISH scoring specificity, we combined interphase FISH with immunofluorescent detection of the cytoplasmic light chain as previously described.⁶ The FISH probes for detection of 13q deletions, t(11;14), t(4;14) and p53 deletions were previously described by us.⁶ A total of 100 cells were scored for each abnormality and the percentages of cells with abnormal patterns recorded.

Progression-free survival (PFS) and overall survival (OS) were calculated from the transplant date by the Kaplan–Meier method. Survival curves between risk groups were compared by log–rank tests. Multivariate analysis of PFS and PS was performed using the COX proportional hazards regression model. *P*-values <0.05 were considered significant.

Results

Chromosome 13q deletions were detected by cIg-FISH in 39 of 104 (38%) patients, t(11;14) in 16 of 122 (13%) patients, t(4;14) in 15 of 123 (12%) patients and p53 deletions in 10 of 105 (10%) patients. No genetic abnormalities were detected in 43 patients (41%). In all, 34 patients (33%) had one genetic abnormality, 14 patients (13%) had two abnormalities and one (1%) had three

Correspondence: Dr H Chang, Department of Laboratory Hematology, Princess Margaret Hospital/University Health Network, 610 University Avenue, 4-320, Toronto, Ontario, Canada M5G 2M9;

E-mail: hong.chang@uhn.on.ca

Received 25 February 2005; accepted 8 June 2005; published online 22 August 2005

genetic abnormalities. The coexistence of 13q deletions and t(4;14) was found in eight cases (8%), 13q deletion and p53 deletion in five (5%), t(4;14) and p53 deletion in one (1%).

The PFS and OS for patients with each genetic aberration are outlined in Table 2. Patients with either 13q deletions, or t(4;14), or p53 deletions had significantly shorter PFS and OS. In contrast, t(11;14) did not confer a poor prognosis. p53 deletion was the most powerful adverse factor for PFS and OS, followed by t(4;14), and then 13q deletions (Figure 1). Based on 104 MM cases that had informative FISH results for all four genetic markers, we propose a genetic-based risk stratification model with three distinct prognostic categories: low-risk patients with none of the genetic abnormalities tested or only t(11;14) (55 cases); intermediate risk with any one of the genetic abnormalities other than t(11;14) (34 cases); high-risk patients with two or three genetic abnormalities other than t(11;14) (15 cases). Their median OS was 18 months for the high-risk group, 46 months for the intermediate risk group and was not reached for the low-risk group ($P < 0.0001$). The median duration of PFS was 10, 20 and 32.1 months, respectively ($P = 0.0009$) (Figure 2a and b). The relative risk (RR) for the different combination of any two risk factors was 6.0 (2.4–15.1, 95% CI) for OS and 3.7 (1.8–7.6, 95%

CI) for PFS. Multivariate analysis including all four genetic risk factors confirmed that t(4;14) and p53 deletions were independent adverse factors for OS ($P = 0.0061$, 0.0002, respectively) and PFS ($P = 0.0066$, 0.0009, respectively).

Discussion

High-dose chemotherapy with ASCT represents the current standard of care in younger MM patients.^{7–9} A careful prognostic evaluation of these patients is warranted to identify those who derive the most benefit from ASCT as well as those who might be potential candidates for innovative treatments. We have shown that MM patients undergoing ASCT can be classified by their underlying

Table 1 Clinical and pathological features of MM patients

Age, median (range)	53 (31–71)
Sex, F:M	47:76
<i>Stage, N (%)</i>	
I–II	46 (38.7)
III	73 (61.3)
<i>Presence of bone lesions (n = 113)</i>	
None	41 (36.3)
Yes	72 (63.7)
Hb (g/l)	107 (52–192)
Calcium (mmol/l)	2.39 (1.59–4.21)
C-reactive protein (mg/l)	3.5 (2.0–122.0)
Beta-2 microglobulin (mmol/l)	233.5 (116.0–4910)
Creatinine (μ mol/l)	88.5 (51.0–1759.0)
<i>Isotype</i>	
IgA	26 (20.6)
IgD	4 (3.2)
IgG	71 (56.4)
Light chain only (κ or λ)	19 (15.1)
Nonsecretory	6 (4.8)
t(4;14) (n = 123)	15 (12.2)
t(11;14) (n = 122)	16 (13.1)
p53 deletion (n = 105)	10 (9.5)
13q14 deletion (n = 104)	39 (37.5)

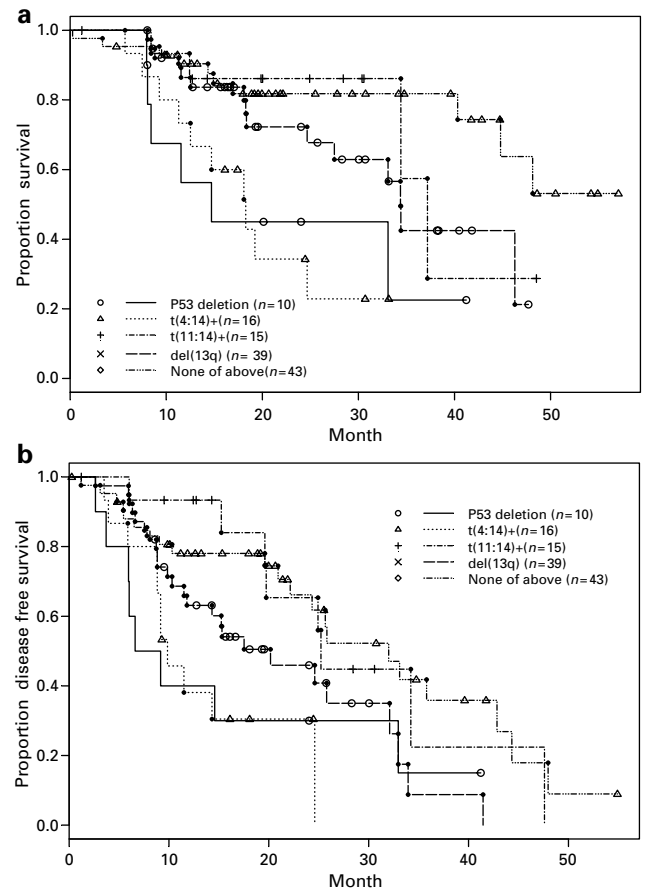


Figure 1 (a) Overall survival according to the genetic aberrations in MM. (b) Progression-free survival according to the genetic aberrations in MM.

Table 2 Overall survival (OS) and progression-free survival (PFS) according to genetic abnormality

Abnormality	N	Median OS (months)	Relative risk (95% CI)	P-value	Median PFS (months)	Relative risk (95% CI)	P-value
p53 del	10	14.7	4.5 (1.5–13.1)	0.0025	7.9	2.5 (1.1–5.8)	0.0248
t(4;14)	15	18.3	4.8 (1.8–12.7)	0.0005	9.9	3.4 (1.5–7.8)	0.0019
t(11;14)	16	37.2	1.5 (0.5–4.8)	0.5231	25.2	1.1 (0.5–2.5)	0.7954
13q del	39	34.4	2.3 (1.0–5.2)	0.0498	20.2	2.1 (1.1–3.9)	0.0178
None of above	43	Not reached	0.99		32.1	0.99	

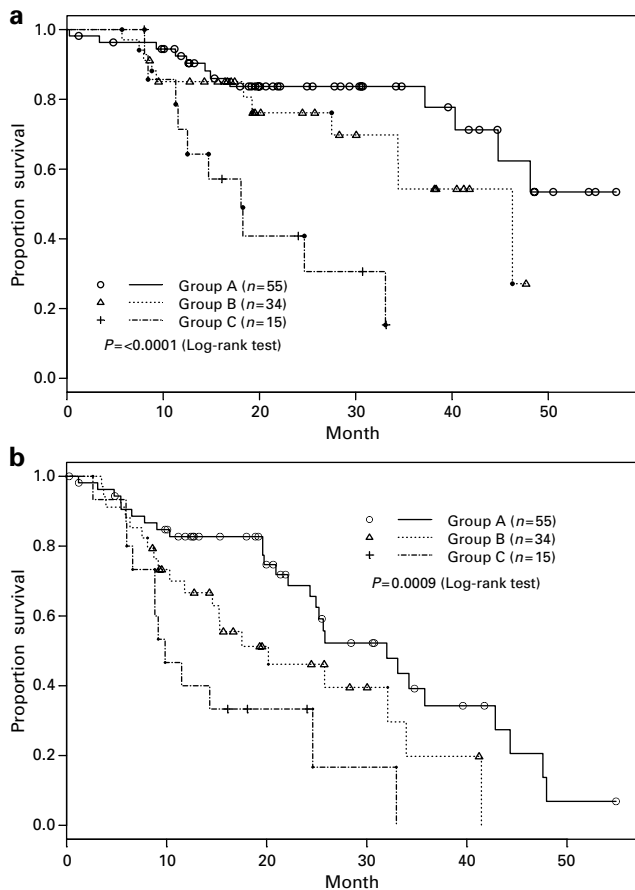


Figure 2 (a) Overall survival according to the number of genetic risk factors in MM. (b) Progression-free survival according to the number of genetic risk factors in MM. This risk groups are classified based on four genetic markers (13q deletions, t(11;14), t(4;14) and p53 deletions). Group A = none or t(11;14); Group B = any one genetic abnormality other than 11;14; Group C = any two or more genetic abnormality other than t(11;14).

genetic aberrations and these abnormalities alone can establish prognostic categories. In our proposed FISH-based classification model, patients with no genetic abnormality or only t(11;14) have the best clinical outcome, and patients with only one adverse genetic abnormality have a better outcome than patients with two or more adverse genetic abnormalities. Our data add to the knowledge of prognostic factors for MM and assists in the selection of patients for referral for ASCT as some have a very poor prognosis even undergoing this costly and not entirely risk-free procedure.

The frequencies of the four recurrent genetic changes detected in our patients are consistent with those described in large series.^{3,10–13} We and others have shown that specific genetic changes in MM patients are linked to their distinct biological features, that is, association of a t(11;14) with light chain myeloma, t(4;14) with IgA isotype,^{3,4,10} p53 deletions with higher creatinine and calcium levels.^{5,12} In this series, we found that eight of 15 (53%) patients with t(4;14) had 13q deletions, in contrast to an over 80% correlation between these two abnormalities reported

by other groups.^{10,11} Consistent with other reports, all translocations in this cohort were mutually exclusive, no patients had two coexistent translocations.

Similar to the findings by Fonseca *et al*¹² in MM patients treated with conventional chemotherapy and Moreau *et al*³ in those receiving high-dose therapy, we confirm that patients with t(4;14) or p53 deletions had worse prognosis than those with 13q deletions. When 13q deletions coexist with t(4;14) or p53 deletions, it appears that t(4;14) or p53 deletions outweighs the prognostic impact of 13q deletions, both the OS and the PFS are inferior to those of other patients with 13q deletions. In addition, we demonstrate that in the high-risk group in which 13 of 14 (93%) patients had 13q deletions, additional genetic changes, either t(4;14) or p53 deletions conferred even worse prognosis than any one of those alone.

In conclusion, we propose a FISH-based risk stratification model for MM patients undergoing ASCT. While prospective clinical trials are in need to confirm the validity of this model integrated with current biological risk factors, our data indicates that patients with 13q deletions, t(4;14) or p53 deletions and especially those with two or more of above genetic aberrations have limited benefit from ASCT and may be candidates for innovative treatments.

Acknowledgements

This study was supported in part by a grant from the Leukemia Research Fund of Canada, Canadian Institute for Health Research and National Cancer Institute of Canada.

References

- Avet-Loiseau H, Brigaudeau C, Morineau N *et al*. High incidence of cryptic translocations involving the Ig heavy chain gene in multiple myeloma, as shown by fluorescence *in situ* hybridization. *Genes Chromosomes Cancer* 1999; **24**: 9–15.
- Bergsagel PL, Kuehl WM. Chromosome translocations in multiple myeloma. *Oncogene* 2002; **20**: 5611–5622.
- Moreau P, Facon T, Leleu X *et al*. Recurrent 14q32 translocations determine the prognosis of multiple myeloma, especially in patients receiving intensive chemotherapy. *Blood* 2002; **100**: 1579–1583.
- Chang H, Sloan S, Li D *et al*. The t(4;14) is associated with poor prognosis in myeloma patients undergoing autologous stem cell transplant. *Br J Haematol* 2004; **125**: 64–68.
- Chang H, Qi XY, Yi QL *et al*. P53 gene deletion detected by fluorescence *in situ* hybridization is an adverse prognostic factor for patients with multiple myeloma following autologous stem cell transplantation. *Blood* 2005; **105**: 358–360.
- Chang H, Li D, Zhuang L *et al*. Detection of chromosome 13q deletions and IgH translocations in patients with multiple myeloma by FISH: comparison with karyotype analysis. *Leuk Lymphoma* 2004; **45**: 965–969.
- Attal M, Harousseau JL, Stoppa AM *et al*. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Francais du Myeloma. *N Engl J Med* 1996; **335**: 91–97.
- Child JA, Morgan GJ, Davies FE *et al*. Medical research council adult leukaemia working party. High-dose chemotherapy and hematopoietic stem-cell rescue for multiple myeloma. *N Engl J Med* 2003; **348**: 1875–1883.

- 9 Barlogie B, Shaughnessy J, Tricot G *et al*. Treatment of multiple myeloma. *Blood* 2004; **103**: 20–32.
- 10 Avet-Loiseau H, Facon T, Grobois B *et al*. Oncogenesis of multiple myeloma: 14q32 and 13q chromosomal abnormalities are not randomly distributed, but correlate with natural history, immunological features, and clinical presentation. *Blood* 2002; **99**: 2185–2191.
- 11 Fonseca R, Oken MM, Greipp PR, Eastern Cooperative Oncology Group Myeloma Group. The t(4;14)(p16.3;q32) is strongly associated with chromosome 13 abnormalities in both multiple myeloma and monoclonal gammopathy of undetermined significance. *Blood* 2002; **98**: 1271–1272.
- 12 Fonseca R, Blood E, Rue M *et al*. Clinical biologic implications of recurrent genomic aberrations in myeloma. *Blood* 2003; **101**: 4569–4575.
- 13 Konigsberg R, Zojer N, Ackermann J *et al*. Predictive role of interphase cytogenetics for survival of patients with multiple myeloma. *J Clin Oncol* 2000; **18**: 804–812.