Prognostic Implications of Tumor Cell DNA and RNA Content in Multiple Myeloma

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Flow cytometric studies of bone marrow DNA and RNA content were conducted in 71 previously untreated patients with multiple myeloma. There was a progressive increase in response rate with rising plasma cell RNA content. The DNA-derived ploidy level also affected chemotherapy sensitivity: only one of 11 patients with either hypodiploidy or biclonal DNA stemlines responded. DNA-RNA–defined marrow plasmacytosis was the only tumor mass-related variable adversely affecting remission induction. Survival was longer in patients with low tumor burden and favorable DNA features. The availability of objective and quantitative pretreatment variables associated with both initial response and survival should permit a risk-based selection of patients for novel treatment approaches.

AS IN MANY NEOPLASMS, there are no clinical features in myeloma that predict the likelihood of chemotherapy sensitivity. Specifically, variables used for the assessment of clinical tumor mass stage, such as hemoglobin, calcium, and M-protein concentration, although associated with survival,[1] do not influence initial treatment response. Knowledge of prognostic factors is important in order to allow the evaluation of novel treatments in patients with a poor prognosis. In myeloma, considerable effort has been devoted to the examination of cytokinetics as a potential prognosticator for response. However, unexpectedly, a low tritiated thymidine grain count as an indicator of slow DNA synthesis was associated with a higher response rate,[2] whereas the plasma cell labeling index affected only survival duration.[3,4] Attempts to test myeloma cell chemotherapy sensitivity by means of an in vitro cloning assay have been only partially successful.[5] In fact, the nature of the myeloma tumor stem cell remains elusive, and the relative chemotherapy resistance in this disease may be related to a high degree of terminal tumor cell differentiation, which has recently been invoked by Goldie and Coldman[6] to account for a high proportion of resistant cells.

We have performed flow cytometric studies of the nucleic acid content of plasma cells from many patients with myeloma. In approximately 80% of cases, an abnormal DNA stem line was identified, usually with 10% to 15% more DNA than normal hemopoietic cells.[7-9] In addition, cellular RNA content was markedly increased in nearly all patients,[4,8,9] as expected in cells producing immunoglobulin. We recently described the association of high plasma cell RNA content with chemotherapy sensitivity to both initial and salvage therapy.[10,11] In this paper, we summarize our expanded experience on the association of cellular RNA content and response in myeloma. Among 71 untreated patients, we confirm that a low tumor cell RNA content signified chemotherapy resistance, whereas survival was affected mainly by tumor burden.

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MATERIALS AND METHODS

Seventy-one previously untreated patients with symptomatic myeloma received chemotherapy with vincristine, doxorubicin, cyclophosphamide, and glucocorticoid combinations as described previously.[12] Pertinent patient characteristics are summarized in Table 1 and were similar to those described in other large series from this and other institutions. Tumor response was defined as >75% reduction in serum M-protein concentration and/or disappearance of Bence Jones proteinuria. In the two patients with nonsecretory myeloma, clearance of marrow plasmacytosis, disappearance of soft-tissue masses, and correction of anemia were required. Survival was computed from the institution of treatment using life table analysis.

All patients had at least 10% plasma cells with adequate studies of DNA and RNA content by flow cytometry. DNA and RNA analysis involved staining of bone marrow aspirates with acridine orange and subsequent measurement of at least 10,000 cells in a flow cytometer.[13] The RNA index was derived from the ratio between mean RNA content of tumor $G_{1/0}$ cells (exhibiting discrete DNA and RNA features) and normal hemopoietic $G_{1/0}$ cells. In three instances in which a discrete tumor population could not be distinguished from normal cells and concurrent cytometric analysis of cytoplasmic kappa and lambda light chains revealed monoclonal staining in at least 10% of cells,[9] the RNA index was computed on the cell population that showed monoclonal light chain reaction. The DNA index was determined from the ratio of modal channel numbers of tumor and normal hemopoietic cells in $G_{1/0}$ phase of the cell cycle.[7] There were six patients exhibiting two separate DNA stem lines with increased RNA content as typically observed in multiple myeloma; these were considered to represent biclonal DNA stem lines. Each of these DNA stem lines contained a minimum of 10% of all marrow cells. The tumor cell nature in the two patients with a diploid DNA subpopulation was confirmed by monoclonal light chain reaction in the cytoplasm. In DNA-biclonal myeloma, only the RNA index of the dominant tumor cell DNA stem line was used in the analysis. We also determined the proportion of plasma cells with abnormal DNA-RNA features henceforth referred to as marrow plasmacytosis.[8]

Prognostic factor analysis was carried out to determine the role of DNA-RNA–derived variables in relationship to standard clinical and laboratory parameters for response to chemotherapy and survival. The latter included clinical tumor mass stage[1] and its individual contributing components, age, immunoglobulin characteristics, and β_2-microglobulin (β_2M). Chi-square tests and log rank tests were used to assess the statistical significance of simple comparisons of response rates and survival curves, respectively. In order to assess the significance of one characteristic after accounting for the effects of one or more others, we used multiple regression analysis based on the logistic model for response rate[14] and the proportional hazard model for survival time.[15] In the latter analyses, P values were based on likelihood ratio chi-square statistics.

Table 1. Patient Characteristics

Number of patients	71
Median age (yr)	60
Male (%)	61
Black (%)	23
High tumor mass (%)	42
Immunoglobulin type	
IgG (%)	55
IgA (%)	22
Only Bence Jones protein (%)	20
Nonsecretory (%)	3
Results of treatment	
Response (%)	56
Median survival (mo)	30

RESULTS

Table 2 summarizes, by univariate analysis, the impact of pretreatment parameters on response and survival in 71 previously untreated patients. A major role of RNA and DNA features was apparent. There was a stepwise increase in response rate with rising RNA index from 18% for patients with low values (<4) to 60% for those with intermediate values (4 to 6) to 76% when the RNA index exceeded 6. The low RNA index group of 17 patients included seven who displayed hypodiploid or biclonal DNA stem lines (Fig 1).

Analysis by ploidy level revealed an above-average response rate of approximately 65% for most patients displaying either a single diploid (12 patients) or a hyperdiploid DNA stem line (48 patients), regardless of the degree of hyperdiploid abnormality. In contrast, none of the five patients with hypodiploidy responded (Fig 1). There were six patients with biclonal DNA abnormalities, in which the dominant DNA stem lines were hyperdiploid in three, diploid in two, and hypodiploid in one patient. None of the five patients with hyperdiploid or diploid DNA content exhibited a hypodiploid cell subpopulation. The ploidy levels in a given patient with biclonal abnormalities differed by 1.1- to 1.9-fold. The only response was observed in the one patient who had an RNA index >6 associated with a dominantly hyperdiploid DNA stem line.

Table 2. Pretreatment Prognostic Factors in Untreated Myeloma

Pretreatment Characteristic	No. of Patients	Percentage Responding	P	Median Survival (mo)	P
RNA index					
<4	17	18		15	
4-6	25	60	.001	42	.06
>6	29	76		30	
DNA index					
Diploid	12	67		28	
Hyperdiploid	48	65		36	
			.002		.001
Hypodiploid	5	0		9	
Biclonal	6	17		7	
Marrow plasmacytosis (%)					
<20	23	70		34	
20-40	27	63	.02	27	.01
>40	21	33		15	
β_2-microglobulin (mg/L)					
<4	16	63		40	
4-6	12	58	.14	30	.007
>6	23	39		17	
Tumor mass					
Low	13	77		36+	
Intermediate	28	54	.14	30	.14
High	30	50		18	

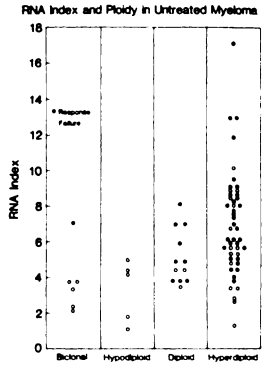

Fig 1. RNA index and ploidy in untreated myeloma. There was a preponderance of low RNA index values among patients with biclonal and hypodiploid DNA stem lines; the only responder among these 11 patients had a high RNA index of 7.0. There was no difference in response rate among the more favorable diploid and hyperdiploid categories. A progressive increase in response rate from low (<4) to intermediate (4 to 6) to high RNA index values (>6) is readily appreciated.

Among the tumor mass-related variables, a high degree of DNA-RNA–defined marrow plasmacytosis adversely affected treatment response. The prognostic importance of the plasma cell RNA index applied similarly in low and high marrow tumor infiltrate groups (Table 3). Combined consideration of RNA index and marrow plasmacytosis, defined as the relative RNA index, or RRI (ratio of RNA index and percentage of tumor cells), further magnified the differences provided by analysis of the individual variables.

In a multiple regression analysis not including RRI, RNA index was the characteristic most strongly associated with response ($P < .001$), followed by DNA index ($P = .01$), whereas marrow plasmacytosis showed only borderline significance ($P = .06$) after adjusting for RNA and DNA index (Table 4). Consideration of the RRI parameter in the multiple regression analysis led to a stronger association with response than RNA index itself, with chi-squares of 18.40 and 14.55, respectively. Adjusting for RRI also reduced the significance of the DNA index to $P = .03$. Neither tumor

Table 3. Response in Previously Untreated Myeloma According to RNA Index and Marrow Plasmacytosis

	Marrow Plasmacytosis (%)					
	≤30			>30		
RNA Index	No. of Patients	Percentage Responding	P	No. of Patients	Percentage Responding	P
<4	4	25		13	15	
4-6	15	73	.02	10	40	.02
>6	16	88		13	62	

Table 4. Multiple Regression Analysis of Pretreatment Variables Affecting Response and Survival

Endpoint	Parameter	Unfavorable Effect	P
Response	RNA index	Low	<.001
	DNA index	Hypodiploid or biclonal	.01
Survival	β_2-microglobulin	High	.001
	DNA index	Hypodiploid or biclonal	.01

mass nor B_2M was significantly related to response rate, either before or after accounting for the other features.

Survival was affected much more by variables related to tumor burden than by tumor cell-intrinsic features, such as the RNA index (Table 2). However, the 11 patients with hypodiploid or biclonal DNA features and a low response rate had a significantly inferior survival with a median of only nine months compared with 32 months for the remainder without these abnormalities ($P = .002$). In a multiple regression analysis of all pretreatment variables available in 51 of the 71 patients, serum β_2M and DNA index remained as the only major factors ($P = .001$ and $P = .01$, respectively) (Table 4).

DISCUSSION

Myeloma results from the proliferation of a yet-unidentified clonogenic tumor stem cell that differentiates into plasma cells that produce and usually secrete large amounts of monoclonal immunoglobulin. In this report, we demonstrate that a low RNA content as well as certain ploidy features of plasma cells (hypodiploid and biclonal DNA stem lines) are associated with resistance to chemotherapy.[10] These DNA abnormalities were present in 41% of the patients with low RNA content, suggesting that drug resistance was genetically determined in some patients. Unfortunately, there is only limited cytogenetic information available in myeloma[16,17] due to the generally low proliferative activity of plasma cells, particularly at diagnosis.[4] Yet there is recent evidence of an increasing prognostic impact of specific chromosomal aberrations in leukemia.[18] While not a substitute for modern chromosome banding studies revealing subtle structural abnormalities, ploidy analysis of $G_{1/0}$ cells by DNA cytometry is independent of cell proliferative activity and has previously been shown to adequately reflect numeric chromosomal aberrations.[19] In the current study, two separate DNA features (hypodiploidy and two DNA stem lines) were recognized as being strongly associated with chemotherapy resistance.

Because of the important role of glucocorticoids in the treatment of myeloma,[11] the association of certain nucleic acid features and chemotherapy sensitivity may be explained on the basis of glucocorticoid receptor expression. The resistance to glucocorticoids as well as to cytotoxic chemotherapy of myeloma with low RNA content and hypodiploid or biclonal DNA stem lines suggests the possibility of pleiotropic drug resistance on the basis of changes in membrane permeability.[20-22]

Noteworthy was the similarity in the relationship of RNA index and response rate for both untreated and resistant patients. Among 50 previously treated patients with melphalan resistance, 52% responded to combinations of vincristine, Adriamycin, and glucocorticoids.[11,23] Those 24 patients with a high RNA index (>6.0) had a higher response rate than the 26 patients with a lower value (75% *v* 31%; $P < .01$). Serial studies from diagnosis to relapse in 20 previously untreated patients indicate that the RNA index remained constant and did not change with the acquisition of drug resistance. A low RNA index therefore indicated primary resistance to standard agents, whereas a high RNA index signified a high likelihood of drug sensitivity in patients not exposed to optimum therapy with vincristine–Adriamycin–glucocorticoid combinations.[11] However, the chemotherapy sensitivity in patients with intermediate and high plasma cell RNA content was reduced when marked marrow plasmacytosis was present. Among 20 evaluable patients, we also observed a greater degree of RNA dispersion with rising marrow tumor infiltrate, reflecting more tumor cell heterogeneity, including drug-resistant cell populations.[24]

Survival was influenced more markedly by parameters related to tumor burden than by the RNA index even though RNA content was the dominant feature affecting response. There was, however, a subgroup of patients with unfavorable DNA features for remission induction who also experienced an unusually short survival time. Hence, longevity was compromised in patients with a high risk of initial drug resistance (hypodiploidy, biclonal DNA stem lines, or low RNA index) and in those presenting with high tumor burden. Unlike Bunn et al,[25] we did not observe a longer survival in patients with diploid tumors as opposed to those with hyperdiploid tumors. The shorter survival in patients with higher tumor mass could be attributed to a lower initial response rate even in patients with favorable RNA features (see Table 3), probably due to a higher proportion of drug-resistant tumor cells.[24] In addition, the residual tumor load in responding patients with high tumor burden may be higher and result in a shorter remission duration, a factor that is currently being investigated. Unfortunately, we do not have close enough follow-up information on our patients in remission to examine remission duration as a separate endpoint.

The availability of a highly discriminatory cellular response feature permits the exploration of novel treatment modalities, such as high-dose melphalan for patients with a response likelihood below 20%.[26] Similarly, patients with an unfavorable long-term prognosis (high pretreatment tumor mass stage) can be selected for exploration of more intensive and potentially more cytoreductive treatment in remission.

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