S. M. Gartler, *Biochem. Biophys. Res. Commun.* **3**, 127 (1960).
5. A. Vorbrodt, T. Wilczok, K. Schneiberg, T. Gorki, *Neoplasma* **10**, 355 (1963); G. F. Rabotti, *Exp. Cell Res.* **31**, 562 (1963); T. Tsumita and M. Iwanaga, *Nature* **198**, 1088 (1963).
6. R. Weil, *Virology* **14**, 46 (1961).
7. E. Winocour, *ibid.* **19**, 158 (1963).
8. R. Dulbecco and G. Freeman, *ibid.* **8**, 396 (1959).
9. R. D. Hotchkiss, *Methods Enzymol.* **3**, 692 (1957); the pneumococcal-transforming DNA was kindly supplied to us by Dr. S. M. Beiser of Columbia University, New York.
10. ———, *Proc. Nat. Acad. Sci. U.S.* **40**, 49 (1954).
11. M. Chorąży, A. Bendich, E. Borenfreund, D. J. Hutchison, *J. Cell. Biol.* **19**, 59 (1963).
12. A. C. Aisenberg, *Cancer Res.* **21**, 1560 (1961).
13. T. Wilczok and A. Bendich, unpublished observations.
14. R. M. Herriott, J. H. Connolly, S. Gupta, *Nature* **189**, 817 (1961).
15. An increase in the infectivity of polyoma DNA after incubation in vitro with fresh rabbit serum has been observed (E. Borenfreund, M. Krim, A. Bendich, unpublished observations).
16. R. Dulbecco and M. Vogt, *Proc. Nat. Acad. Sci. U.S.* **50**, 236 (1963).
17. R. Weil and J. Vinograd, *ibid.*, p. 730.
18. R. Weil, *ibid.* **49**, 480 (1963).
19. Y. Ito, *ibid.* **47**, 1897 (1961).
20. S. Zamenhof, G. Leidy, E. Hahn, *J. Exp. Med.* **98**, 373 (1953).
21. L. Crawford, *Virology* **22**, 149 (1964).
22. ———, R. Dulbecco, M. Fried, L. Montagnier, *Proc. Nat. Acad. Sci. U.S.* **52**, 148 (1964).
23. B. W. Catlin, *Science* **131**, 608 (1960); *J. Bacteriol.* **79**, 579 (1960); E. Ottolenghi and R. D. Hotchkiss, *Science* **132**, 1257 (1960).
24. R. M. Herriott, *Science* **134**, 256 (1961).
25. V. P. Butler, S. M. Beiser, B. F. Erlanger, S. W. Tanenbaum, S. Cohen, A. Bendich, *Proc. Nat. Acad. Sci. U.S.* **48**, 1597 (1962); B. F. Erlanger and S. M. Beiser, *ibid.* **52**, 68 (1964).
26. We thank Dr. M. Krim for carrying out the infectivity assays and Dr. S. M. Beiser for the transformation assays and for the pneumococcal-transforming DNA. Supported by funds from the National Cancer Institute (grant CA03190-09), the Health Research Council of the City of New York (contract U-1096, IIA), AEC (contract AT [30-1], 910), and grant T128D from the American Cancer Society and First National City Bank Grant for Research from the American Cancer Society. A.B. is recipient of a PHS research career award (3-K6-CA-22,533-01S1) from NIH. A part of this investigation was conducted during the tenure of T.W. as a Damon Runyon Cancer Research Fellow. E.B. is recipient of a PHS research career development award (5-K3-CA-16,720-02) from NIH.
* Present address: Institute of Oncology, Armii Czerwonej 15, Gliwice, Poland.

22 January 1965

## Myeloma Proteins and the Clinical Response to Melphalan Therapy

Abstract. *Objective improvement after therapy with melphalan occurred in all patients producing only Bence Jones kappa proteins, in half of the patients with myeloma serum proteins, and in none of those producing only Bence Jones lambda proteins.*

Melphalan, 3-{p-[bis(2-chloroethyl) amino]phenyl}-L-alanine hydrochloride, therapy induces objective improvements in about 50 percent of myeloma patients (*1*). The Myeloma Section of the Southwest Cancer Chemotherapy Study Group has analyzed (*2*) the responses of 132 myeloma patients treated with this drug. None of the following features correlated significantly with the response to therapy; age; sex; race; the interval between the onset of symptoms and initiation of melphalan therapy; the morphological classification of the predominant marrow plasma cell as mature, immature, or blast; or the presence or absence of Bence Jones protein (BJP) in the urine. However, objective improvement was more frequent in patients producing myeloma proteins containing kappa ($\kappa$) L-chains (*3*) than in those with lambda ($\lambda$) L-chains ($p < .01$). It is our purpose to compare two groups of myeloma patients divided on the basis of the antigenic type of L-chain contained in the myeloma protein (54$\kappa$, 37$\lambda$), to show the similarity of the two groups with respect to their status prior to therapy and the melphalan-induced leukopenia, and to show the marked difference in the incidence of objective improvement resulting from melphalan therapy.

Proteins produced by malignant plasma cells may be classified on the basis of structural and antigenic properties (*4*). The reduction of disulfide linkages with mercaptans and subsequent alkylation of the free -SH groups splits $\gamma$-globulin into L (light) and H (heavy) polypeptide chains. The BJP's present in the urine of some myeloma patients contain only L-chains with $\kappa$ or $\lambda$ antigenic determinants. Myeloma serum proteins combine one or the other type of L-chain with a gamma ($\gamma$) or an alpha ($\alpha$) H-chain. The antigenic properties and structural features of the myeloma protein are stable, heritable characteristics of the plasma-cell tumor clone (*5*). Since the structural and antigenic properties of a myeloma protein are determined by the type of plasma cell affected by the malignant transformation, classifying patients with myeloma by the type of myeloma protein would yield a homogeneous grouping of patients for comparison of the clinical response to melphalan therapy.

All the available serum and urinary proteins of myeloma patients treated with melphalan were typed by immunoelectrophoresis with rabbit or goat antiserums to $\gamma_2$-globulin (IgG) and $\gamma_1$A-globulin (IgA) and rabbit antiserums to $\kappa$- and $\lambda$-BJP (*3*). In all the patients with myeloma proteins in both serum and urine, the antigenic type of L-chain in the serum protein was identical with that of the BJP. The IgG myelomas (*6*) were considered as one group. The records of the patients were reviewed to establish the status before treatment, the toxic effects of melphalan on leukocytes, and the occurrence of objective improvement (*7*).

Patients were classified as being objectively improved (responders) by the melphalan therapy if one or more of the following criteria were satisfied: (i) a decrease in the myeloma serum protein to 50 percent, or less, of the value before study; (ii) a decrease in the amount of urinary protein excreted per 24 hours to 50 percent, or less, of the value prior to study; (iii) an increase of 2.0 g percent, or more, in hemoglobin; (iv) shrinkage of palpable plasmacytomas of 50 percent or more; and (v) decrease in serum calcium from more than 6.0 to 5.0 meq/lit. or less.

The foregoing criteria were selected arbitrarily, but with the belief that the changes are probably clinically significant. The effect of melphalan therapy was not evaluated if the changes in the myeloma proteins in serum or urine were not determined, or if the patient died or was otherwise lost in less than 3 weeks.

Two different dosages of melphalan were used. Initially 0.2 mg/kg per day (schedule A) was administered until the leukocyte count fell below 3000/mm$^3$, or platelets fell below 100,000/mm$^3$. Because this dosage frequently caused severe leukopenia, we administered a dose of 0.5 to 2.0 mg/kg (in accordance with the patient's tolerance) over a 4-day period (schedule B). Doses causing moderate leukopenia were repeated as the marrow recovered, 6 to 10 weeks later.

Most of the patients were treated with schedule B, and all patients started on melphalan have continued to receive repeated courses of the drug at 6- to 10-week intervals (Table 1).

The better responses in the patients having $\kappa$ L-chains in the myeloma protein were primarily due to the fact that all patients producing only a $\kappa$-BJP were objectively improved, whereas all those producing only a $\lambda$-BJP failed to respond. The differences between the incidence of response in the patients with $\gamma_2$ $\kappa_2$- as against those with $\gamma_2$ $\lambda_2$-

Table 1. Relation of clinical response to the type of H- and L-chain of the myeloma protein (ns, not significant).

| H-chain | L-chain (responders/total evaluated) | |
|---|---|---|
| | Kappa ($\kappa$) | Lambda ($\lambda$) |
| Gamma ($\gamma$) | 19/31 | 7/17 |
| Alpha ($\alpha$) | 3/8 | 2/9 |
| BJP only | 11/11 | 0/9 |
| Not evaluated | 3 | 2 |

| Comparison | $X^2$ | $P$ |
|---|---|---|
| All $\kappa$ vs. all $\lambda$ | 13.39 | $<.01$ |
| $\kappa$ BJP vs. other $\kappa$ | 7.14 | $<.01$ |
| $\lambda$ BJP vs. other $\lambda$ | 4.15 | $<.05$ |
| All $\gamma$ vs. all $\alpha$ | 3.07 | ns |
| $\gamma_2\kappa_2$ vs. $\gamma_2\lambda_2$ | 1.78 | ns |
| BJP only vs. $\gamma + \alpha$ | 0.32 | ns |
| $\alpha_2\kappa_2$ vs. $\alpha_2\lambda_2$ | 0.18 | ns |

and the comparison of $\alpha_2\,\kappa_2$- with $\alpha_2\,\lambda_2$- were not significant. The response of patients producing only a $\kappa$-BJP was significantly better than that of patients producing $\kappa$ L-chains linked to a $\gamma$ or $\alpha$-H-chain ($p < .01$), whereas, in the $\lambda$ group, responders were significantly less frequent among patients producing only a BJP ($p < .05$). The differences noted in the other comparisons were not significant.

On the basis of the response to melphalan, the patients divide into the three groups shown with the estimates of their survival in Table 2. The differences are significant ($t$ test) for the comparison of the survival of the nonresponders with the intermediate group (from onset of symptoms, $p < .02$; from diagnosis, $p < .01$; from start of melphalan, $p < .05$). With the sample size available for comparing the survival of the responders and nonresponders, the differences are not significant, although for the survival from the onset of symptoms the difference is close to being significant at $p < .05$. There are no significant differences between survivals of the responder and intermediate groups. Since less than 50 percent of the patients are dead in the responder and intermediate groups, these estimates of survival are unstable, and, in view of the small sample size of the responder and nonresponder groups, these estimates of survival should not be interpreted as being well established.

Table 2. Median survival (in months) of myeloma patients treated with melphalan. "Median" is the geometric mean, estimated by the maximum likelihood fitting to a log-normal distribution ($\delta$); figures in parentheses represent the 95-percent confidence interval of the geometric mean.

| Responders ($\kappa$ BJP only) | Intermediate $\{\gamma_2\kappa_2, \gamma_2\lambda_2\}$ $\{\alpha_2\kappa_2, \alpha_2\lambda_2\}$ | Nonresponders ($\lambda$ BJP only) |
|---|---|---|
| *From onset of symptoms* | | |
| 68 (43–109) | 55 (45–67) | 23 (18–31) |
| *From diagnosis* | | |
| 57 (31–105) | 48 (37–61) | 18 (14–23) |
| *From start of treatment* | | |
| 48 (20–111) | 36 (28–46) | 12 (8–17) |
| *Number dead/total number in group* | | |
| 4/12 | 25/70 | 6/9 |

Prior to melphalan therapy, patients producing myeloma proteins with $\kappa$-L-chains did not differ significantly from those with myeloma proteins containing $\lambda$-L-chains with respect to age, sex, race, the interval between the onset of symptoms and the start of melphalan therapy, the hemoglobin concentration, the incidence of hypercalcemia, or the incidence of renal insufficiency. These same comparisons were also made for the group of patients producing only BJP's, and no significant differences were demonstrated.

Melphalan-induced leukopenia was compared in four ways in the two groups of patients. There were no significant differences between the two groups in respect to the total dose required to produce leukopenia with schedule A or B, the interval between the onset of therapy and the day on which the lowest leukocyte count was observed, the dose rate for the total period of observation, or the effect of 1.0 mg/kg in 4 days on the leukocyte response. In addition, no difference was demonstrated in the severity of leukopenia in patients producing only $\kappa$- or $\lambda$-BJP.

Thus, the group of patients producing $\kappa$-L-chains was similar to those producing $\lambda$-L-chains with respect to the status prior to melphalan and the melphalan-induced leukopenia.

If some extracellular factor were acting to make the melphalan more toxic and potent in myeloma patients producing $\kappa$-L-chains, one would also expect to encounter more severe leukopenia in these patients. Or, if the plasma cells in patients producing $\lambda$-L-chains were protected from the action of melphalan by some extracellular factor, such as the myeloma protein, which might inactivate the alkylating agent by reacting with it, one would expect to observe less severe leukopenia in these patients. However, there was no significant difference in the melphalan-induced leukopenia of myeloma patients producing $\kappa$- and $\lambda$-L-chains, and thus the higher incidence of melphalan responses in $\kappa$- patients probably cannot be explained by an extracellular factor.

The observations reported herein suggest that plasma-cell differentiation which allows the synthesis of only $\kappa$-BJP's permits the full expression of the factor or factors which determine melphalan responsiveness. The factors are not expressed in plasma-cell tumors producing only $\lambda$-BJP, and are expressed in only half the tumors producing the other kinds of myeloma proteins. The concept that tumor-cell differentiation may influence the response to chemotherapy needs to be explored further, and should be considered in future therapeutic trials in myeloma.

DANIEL E. BERGSAGEL*
*Department of Medicine*
PHILIP J. MIGLIORE
*Department of Pathology*
KENNETH M. GRIFFITH
*Southwest Cancer Chemotherapy Study Group Headquarters, University of Texas M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Avenue, Houston*

**References and Notes**

1. D. E. Bergsagel, C. C. Sprague, C. Austin, K. M. Griffith, *Cancer Chemotherapy Rep.* **21**, 87 (1962).
2. Myeloma Section, Southwest Cancer Chemotherapy Study Group, D. E. Bergsagel, chairman, unpublished data.
3. *Bull. World Health Organ.* **30**, 447 (1964). In accordance with the recommendations of this international committee, the following terminology is employed: kappa (type I) and lambda (type II) L-chains; gamma and alpha H-chains identify the heavy polypeptide chains associated with IgG ($\gamma$, $7S\gamma$, $6.6S\gamma$, $\gamma_2$, $\gamma_{ss}$), or IgA ($\beta_2$A, $\gamma_1$A) myeloma globulins, respectively.
4. R. Grabar, R. Fauvert, P. Burtin, L. Hartmann, *Rev. Franc. Etudes Clin. Biol.* **1**, 175 (1956); J. F. Heremans, M. T. Heremans, A. H. F. Laurell, C. B. Laurell, L. Märtensson, J. Sjorquist, J. Waldenström, *Acta Med. Scand. Suppl. 367* (1961); L. Korngold and R. Lipari, *Cancer* **9**, 183 (1956); M. Mannik and H. G. Kunkel, *J. Exp. Med.* **116**, 859 (1962); D. E. Olins and G. M. Edelman, *ibid.*, p. 635; G. M. Edelman and J. A. Gally, *ibid.*, p. 207.
5. M. Potter and J. L. Fahey, *J. Nat. Cancer Inst.* **24**, 1153 (1960).
6. W. D. Terry and J. L. Fahey, *Science* **146**, 400 (1964).
7. D. E. Bergsagel, C. C. Sprague, S. W. Ross, *Cancer Chemotherapy Rep.* **21**, 69 (1962).
8. R. Wette, unpublished.
9. Supported by grant CA 03195-07 from the Cancer Chemotherapy National Service Center, NIH, and in part by grants CA 06939 and CRT 5114.
* Present address: Chief of Medicine, Ontario Cancer Institute, Toronto.

12 February 1965