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# Evaluation of a Cooperative Group Human Myeloma Protocol Using the MOPC 104E Myeloma Model[1]

**Raymond Hiramoto, Vithal Ghanta, and John R. Durant**

*Departments of Microbiology [R. H., V. G.] and Medicine [J. R. D.] and Cancer Research and Training Center [R. H., J. R. D.], University of Alabama Medical Center, Birmingham, Alabama 35294*

## SUMMARY

Data are presented on the response rates, maximum rate of cell kill, and survival rates for individual and groups of mice with MOPC 104E myeloma treated with a variety of chemotherapeutic agents and combination regimens used in clinical human myeloma. The tumor immunoglobulin M measurements are used to evaluate the therapeutic effects of drugs. Prednisolone and mescaline, when given as single drugs, showed no therapeutic action and the animals died of tumor as in the controls. The immunoglobulin M values are very similar and ranged between 8,125 and 13,410 μg/mouse. Prednisolone and melphalan given in combination indicated therapeutic effect. 1,3-Bis(2-chloroethyl)-1-nitrosourea-cyclophosphamide-prednisolone combination caused tumor regression but was toxic as shown by the immunoglobulin M values and percentage of survival. The complications and potential uses of this system, which utilized only 40 animals in 64 days, are discussed.

## INTRODUCTION

We have previously reported the development of a mouse system using MOPC 104E myeloma that permits the accurate measurement of total cell kill over a range of 4 logs and is sensitive according to our present technique to the presence of between $10^5$ and $10^6$ tumor cells in the mouse (7, 9). The system permits the assessment in individual mice of various therapeutic endeavors, thus providing a means of learning the correct time to change a program that is no longer effective and to separate the effects of tumor cell load from toxicity when comparing survival (7).

In order to test the model, we elected to perform in mice a clinical trial as comparable as possible to one now being conducted in man by the Southeastern Cancer Study Group. After treating only 40 mice in 8 groups followed over 64 days we show that in 2 combination drug regimens equal response rates were produced but the survival in the 1 regimen was superior to that in the other, probably due to less chronic toxicity.

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## MATERIALS AND METHODS

### The Mouse Model

MOPC 104E is an ascites-producing mouse myeloma obtained from Dr. Michael Potter of NIH, Bethesda, Md. It produces a monoclonal IgM that has the unique property of reacting with bacterial dextran B1355, whereas normal mouse IgM does not. Sheep red blood cells conjugated with dextran are lysed in the presence of tumor IgM and complement (4). Thus it was possible to set up a quantitative means using radial hemolysis in gel for measuring IgM in mouse serum (10, 11). The method is accurate and reproducible to a level of 1.3 μg IgM per mouse. This technique was used to measure the synthesis rates of IgM by the MOPC 104E cells in culture (6). By determining the doubling time of the tumor and half-life of IgM *in vivo*, we can construct cell kill curves, rates of growth per hr, and total tumor cell mass at whatever time intervals we choose (9).

Forty BALB/c female mice (6 weeks old) obtained from Laboratory Supply Co., Indianapolis, Ind., were maintained on Labblock feed (Wayne Feed Co., Chicago, Ill.) and water *ad libitum*. Tumor cells were prepared as described earlier (9). Each mouse was given $5 \times 10^6$ tumor cells i.v. and the entire group of mice was separated into 8 different treatment programs to be described below. Each animal was weighed on the day of injection, a microhematocrit was performed on blood from the tail vein, and the total plasma volume was determined from these 2 measurements (5, 8). Twenty to 40 μl of plasma were collected for IgM determinations at 4-day intervals thereafter until the animal died or the IgM level returned to the base line. The plasma collected was used in radial-hemolysis-in-gel plates as described, and the results were converted to total μg IgM per mouse and plotted on semilog paper.

The percentage rate of growth, maximum tumor cell load, ratio of tumor weight to animal weight, percentage of tumor cell kill per hr, maximum kill rate, and percentage of total cell kill were then calculated for each regimen.

### Treatment Regimens

**Untreated Control.** Regimen 1.

**Single-Drug Cancer Chemotherapy Regimen.** Regimen 2,

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single dose of CP,[2] 200 mg/kg i.p.; Regimen 3, single dose of BCNU, 39 mg/kg i.p.; Regimen 4, single dose of prednisolone, 235 mg/kg i.p.; Regimen 5, single dose of melphalan (Alkeran), 19 mg/kg i.p.

**Single-Drug Noncancer Chemotherapeutic Regimen.** Regimen 6, single dose of mescaline, 250 mg/kg i.p.

**Drug Combinations.** Regimen 7 consisted of prednisolone, 12 mg/kg/day for 7 days; CP, 132 mg/kg; BCNU, 25 mg/kg. Regimen 8 consisted of prednisolone, 12 mg/kg/day for 7 days; melphalan, 1 mg/kg/day for 3 days.

For the multiple-drug regimen the dosages were converted from the human schedules (1) according to the method of Freireich *et al.* (3). Prednisolone was substituted for prednisone because of the insolubility of the latter compound. Therapy was initiated 19 days after tumor implantation. All the drugs were dissolved in 0.9% NaCl solution except melphalan which was dissolved in acid-alcohol solvent (Burroughs Wellcome and Co., Research Triangle Park, N. C.).

## RESULTS

The measured total μg of IgM per mouse over time is shown (Charts 1 to 4). The mescaline- and prednisolone-treated group is not shown because it paralleled exactly the untreated control. By Day 25 all the control animals were dead after a logarithmic increase in total IgM (Chart 1). Prednisolone showed essentially the same result except that the curve was shifted slightly to the left with greater amounts of IgM at death. CP treatment (Chart 2) resulted in a return to base-line levels of IgM and a 4-log cell kill in all animals. Chart 3 shows the individual responses to melphalan, which had an effect on IgM production in only 2 animals. In Mouse 5, IgM production reached a plateau but the mouse died with a considerable tumor cell load still present. One animal, Mouse 1, responded as did all the mice given CP. BCNU was as effective as CP except that 2 animals died before a therapeutic effect had time to occur. The striking findings are noted in combination therapy. In Chart 4, the prednisolone-melphalan combination, given at lower total doses of each and in a slightly different schedule, produced a 4-log reduction in IgM production, comparable to that with CP. However, the BCNU-CP-prednisolone regimen produced a similar cell kill but all the animals died, usually at a time when tumor cell mass was too small to account for the death of the animal.

Table 1 shows the rate of tumor growth prior to therapy for each treatment group, the peak calculated tumor cell load, and the percentage of the weight of the animal due to tumor at maximum tumor size. It can be seen that the percentage rate of growth for each group was comparable (2.4 to 2.9%/hr) with peak tumor cell loads varying from $4600 \times 10^6$ in the prednisolone group to $510 \times 10^6$ in the CP group. The prednisolone-treated group had the highest percentage of tumor load to body weight (29.4%). In Table 2 the effect of therapy on the tumor is shown. The highest

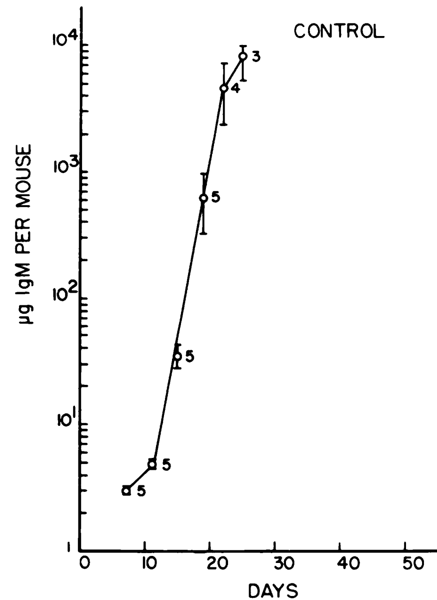


Chart 1. Average tumor growth curve for the control (untreated) group. IgM produced by the tumor in μg IgM per mouse is plotted against time. *Numbers*, number of animals alive and averaged for that point. *Bar*, mean ± S.E.

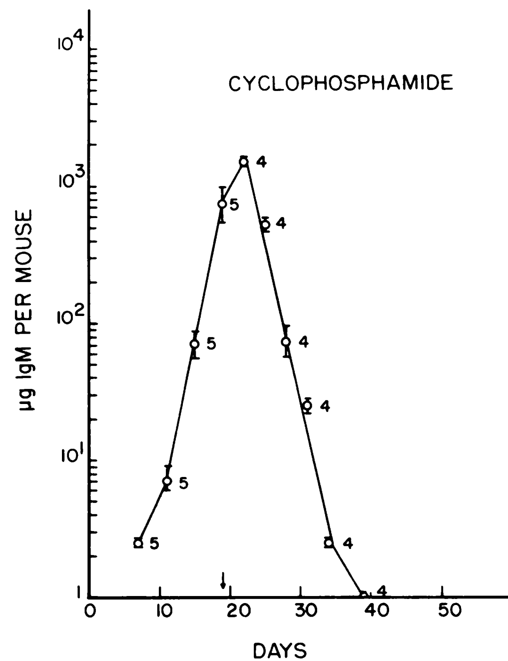


Chart 2. Average tumor growth and response to CP. Treatment given on Day 19 (*arrow*). One animal died on the day of treatment.

percentage rate of tumor cell kill was attained at 25 to 28 days by BCNU (3.6%/hr). Four-log cell kills were obtained with CP, BCNU, melphalan, and both combination regimens. Of greater interest again, however, is the failure of large doses of either melphalan or prednisolone alone to produce effective cell kill and yet the combination of each at lower doses produced at least a 4-log kill.

Groups treated with CP and BCNU individually and prednisolone-melphalan in combination had 100% survival,

[2] The abbreviations used are: CP, cyclophosphamide; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea.



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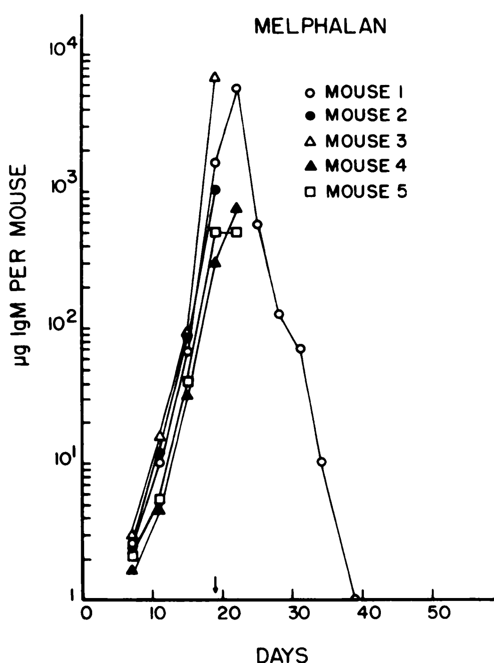


Chart 3. Individual response curve for mice bearing tumors after single treatment with melphalan on Day 19. Only Mouse 1 responded to the drug therapy.

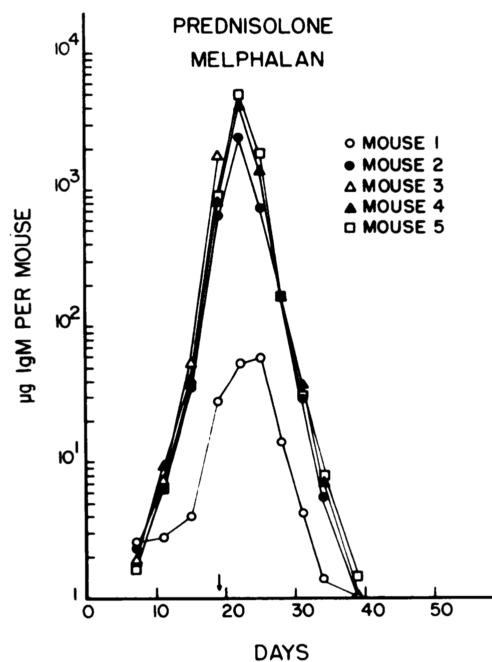


Chart 4. Individual tumor growth and response to prednisolone and melphalan combination. Therapy given on Day 19 when tumor growth was large.

Table 1

*Rate of growth of tumor prior to therapy and peak tumor load attained for each group*

Therapy started 19 days after tumor was given.

| Group | Drug | % rate of growth/hr prior to therapy (exponential phase 11-19 days) | AFC[a] at peak (× 10⁶) | % tumor at peak[b] |
|---|---|---|---|---|
| 1 | Control | 2.9[c] | 2700 (25 days) | 13.9 |
| | *Single dose* | | | |
| 2 | CP | 2.4 | 510 (22 days) | 2.7 |
| 3 | BCNU | 2.8 | 1800 (22 days) | 10.4 |
| 4 | Prednisolone | 2.8 | 4600 (25 days) | 29.4 |
| 5 | Melphalan | 2.8 | 750 (22 days) | 4.3 |
| 6 | Mescaline | 2.4 | 1600 (22 days) | 8.4 |
| | *Combination* | | | |
| 7 | Prednisolone + CP + BCNU | 2.6 | 700 (22 days) | 4.6 |
| 8 | Prednisolone + melphalan | 2.4 | 1400 (22 days) | 8.9 |

[a] AFC, total number of tumor cells/mouse.

[b] Percentage of tumor in the body; 1 × $10^9$ tumor cells equals 1 g of tumor.

[c] In control the exponential phase of tumor growth was 11 to 22 days.

the melphalan group had 30% survival, and the untreated control group and the groups treated with prednisolone and mescaline individually had no survival. Measurable cell kill for all regimens except the BCNU-CP-prednisolone combination occurred on the day following therapy. With the latter regimen it occurred 2 days later, probably an insignificant difference. By Day 64 one animal treated with CP had relapsed. This indicates that, while our method is not yet sensitive enough to detect very small residual numbers of tumor cells, it is capable of detecting early relapse in individual mice.

## DISCUSSION

These studies are presented as a follow-up to the intial studies concerned with chemotherapy and rate of kill of



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Table 2
*Effect of therapy on the rate of growth of the tumor*
Therapy started 19 days after tumor was given.

| Group | Drug | % rate of tumor growth and kill/hr after 1 treatment | | | | | Maximum rate of kill (%/hr) | % total kill after 1 treatment |
|---|---|---|---|---|---|---|---|---|
| | | 19–22 days | 22–25 days | 25–28 days | 28–31 days | 31– days | | |
| 1 | Control | +2.88[a] | +0.72 | (All dead) | | | None | |
| | *Single dose* | | | | | | | |
| 2 | CP | +0.82 | −2.06[b] | −2.06 | −2.06 | −2.06 | 2.06 | 99.99 |
| 3 | BCNU | +1.92 | −0.74 | −3.60 | −1.92 | −1.92 | 3.60 | 99.99 |
| 4 | Prednisolone | +2.88 | +0.82 | (All dead) | | | None | None |
| 5 | Melphalan | +0.50 | −1.92 | −1.92 | −1.92 | −1.92 | 1.92 | 99.99 |
| 6 | Mescaline | +2.40 | | (All dead) | | | None | None |
| | *Combination* | | | | | | | |
| 7 | Prednisolone + BCNU + CP | +1.44 | −0.90 | −2.88 | −2.88 | −1.44 | 2.88 | 99.99 |
| 8 | Prednisolone + melphalan | +1.69 | −2.40 | −2.40 | −2.40 | −2.40 | 2.40 | 99.99 |

[a] Positive refers to the percentage of growth of tumor per hr.
[b] Negative indicates the percentage of kill of tumor cells per hr.

tumor cells. In those studies we showed that combinations of actinomycin-vincristine given continuously at near-toxic levels could not appreciably influence the course of growth of the neoplasm, but the model was accurate enough to permit continuous assessment of tumor cell load during this period of treatment. Monitoring of tumor cell number during the course of the experiment allowed us to change the program of therapy to CP and continue to assess tumor cell numbers killed by the drug (7).

We would like to stress that, by following an IgM tumor marker, results of treatment trials for myeloma can be quantitated for individual mice so that one can establish not only the percentage of responders but also the extent of the response. For instance, although only 1 mouse responded to melphalan, the cell kill was very large indicating an active agent under certain conditions. In this case a model system that lumped results and reported an average percentage increase in life-span could have failed to appreciate the profound effect this agent could have.

Second, although in these initial experiments we did not measure white blood cell and platelet counts, we believe the animals in the BCNU combination group died not of acute hematological toxicity but rather of an ill-defined chronic toxicity. This is because of the very long time from treatment to time of death. Furthermore, because of measurements made from the model, we are certain that the mice died at a time when no or very little tumor remained. This, then, illustrates another advantage of the model, the ability to tell in an individual animal the difference between effects of toxicity and recurrent disease.

The model may provide quantitative evidence concerning synergism. Although the dose and schedule of melphalan and prednisolone in the combination were different from that used in the individual experiments, no evidence for any cell kill could be seen for large individual doses of either agent (except for 1 mouse with melphalan). Furthermore, a possible deleterious effect of such doses was seen for prednisolone alone because the growth curve showed a slight shift to the left and peak tumor cell loads at death were the greatest for this group. The dramatic response to melphalan-prednisolone does not seem to be due to giving a lower total dose of melphalan in a more favorable schedule, since all other data suggest that alkylating agent scheduling is best done with single high intermittent doses (14). One advantage of the model is, therefore, that with a small number of animals we can quickly study drug synergism, both therapeutic and toxic, short term and long, of a wide variety of chemotherapeutic agents.

The model is of great interest to us in that it appears reflective of the human situation, at least in the sense that it shows marked effectiveness of 2 combinations that do indeed produce high response rates in man. The Southeastern Cancer Study Group chose the BCNU-CP-prednisone program because of preclinical and clinical data concerning the efficacy of BCNU as a single agent in the treatment of human myeloma as well as studies that indicate therapeutic synergism with BCNU-CP combinations in many animal tumor systems (2). Using this animal model we may be able to provide insight into the contribution in terms of cell kill of various permutations of large numbers of drugs in combination. The final point is that the model may provide a means of studying the modification of regimens so that chronic toxicity might be anticipated and avoided without the need to await complicated autopsy reports.

As an added note of caution, we are aware that the various transplantable mouse myeloma cell lines differ in their rates of growth and responses to chemotherapeutic agents in much the same way that individual myeloma patients do. To use only 1 mouse myeloma as a model for human myeloma is comparable to using a single myeloma patient as a model for all other patients with this disease and this is not our proposal. On the other hand, it can be argued

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that even this degree of reproducibility for a single human myeloma cannot be achieved in human clinical trials because of individual differences in the tumor type, sites involved, degree of dissemination, tumor load, genetic background, age, sex, etc., in patients bearing neoplasms.

While some have taken the pessimistic view that any mouse tumor model cannot reflect a pattern of drug sensitivity characteristic for individual human tumors, the same can be argued for any human tumor studied; *i.e.*, there is no typical human tumor with a pattern of drug sensitivity characteristic for a specific individual bearing a similar kind of tumor.

Nevertheless, chemotherapy trials in animal models using specific transplantable syngeneic neoplasms cannot be abandoned for they provide us with the only reproducible means of studying therapeutics of tumor growth under drug therapy.

We believe the ramifications and possibilities of this model for studying the tumor biology of myeloma and the effects of treatment are just beginning to be realized. We further believe that, by using the sandwich assay of Salmon (12, 13), we may be able to detect ng amounts of IgM in the system. This means that we could quantitate cell kills of 7 or 8 orders of magnitude in individual mice and thus have arrived at a mouse model system as sensitive as methods in humans to assay for the B chain of human chorionic gonadotropin in malignant trophoblastic disease. If these methods could then be applied to human myeloma, as we are currently attempting, the potential benefit to the management of individual myeloma patients is obvious.

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