Timed Sequential Chemotherapy of Cytoxan-Refractory Multiple Myeloma With Cytoxan and Adriamycin Based on Induced Tumor Proliferation

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Malignant plasma cell proliferation and induced humoral stimulatory activity (HSA) occur in vivo at a predictable time following drug administration. Sequential sera from 11 patients with poor-risk multiple myeloma (MM) undergoing treatment with Cytoxan (CY) 2400 mg/sq m were assayed for their in vitro effects on malignant bone marrow plasma cell tritiated thymidine (3HdTdR) incorporation. Peak HSA was detected day 9 following CY. Sequential changes in marrow malignant plasma cell 3HdTdR labeling indices (LI) paralleled changes in serum activity, with peak LI occurring at the time of peak HSA. An in vitro model of chemotherapy demonstrated that malignant plasma cell proliferation was enhanced by HSA, as determined by 3HdTdR incorporation assay, 3HdTdR LI, and tumor cells counts, and that stimulated plasma cells were more sensitive to cytotoxic effects of adriamycin (ADR) than were cells cultured in autologous pretreatment serum. Based on these studies, we designed a clinical trial to treat 12 CY-refractory poor-risk patients with MM in which ADR (60 mg/sq m) was administered at the time of peak HSA and residual tumor cell LI (day 9) following initial CY, 2400 mg/m (CY, ADR). Eight of 12 (67%) responded to timed sequential chemotherapy with a >50% decrement in monoclonal protein marker and a median survival projected to be >8 mo duration (range 4–21 mo). These clinical results using timed sequential CY, ADR compare favorably with results obtained using ADR in nonsequential chemotherapeutic regimens.

The value of sequential administration of antitumor agents and the increased activity of these drugs against a proliferation tumor has been demonstrated in vivo in rodents and humans. We have conducted trials in rat and man bearing acute leukemia based on laboratory models, which suggest that the pharmacologic effect of cycle-active drugs is enhanced by the proper timing of the sequence, a time determined by detecting maximal induced tumor proliferation and alteration in humoral factors allowing initial drug perturbation.

Previous studies in our laboratory have demonstrated that patients with multiple myeloma (MM) are capable of producing drug-induced humoral stimulatory activity (HSA) that temporally coincides with an increase in tumor labeling index (LI) in vivo and which increases the proliferation of both normal bone marrow cells and malignant plasma cells in vitro. That malignant plasma cells demonstrate an increased growth fraction following chemotherapy has been noted by others as well. Humoral inhibition of tumor and normal marrow cell proliferation is detected in pretreatment sera of patients with MM, a finding similar to that noted in patients with acute leukemia and solid tumor. This tumorassociated inhibitory activity (TAIA) may in part explain the indolent proliferative index of malignant plasma cells and suppressed normal hematopoiesis noted at clinical presentation. The induction of HSA and coincidence of increased malignant plasma cell growth following initial drug in vivo, as well as the effects of HSA on PC proliferation in vitro, suggest that functionally TAIA can be overcome. This article details laboratory and clinical studies conducted to develop principles of sequential chemotherapy of MM. These trials are based on our previous data relating HSA to malignant plasma cell proliferation in vivo and in vitro and demonstrate an attempt to guide the use of drugs in an optimal sequence to increase antitumor effect.

MATERIALS AND METHODS

Patient Selection and Therapy

Eleven consecutive patients with newly diagnosed poor-risk MM with >90% malignant plasma cells in their marrows were admitted for therapy to The Johns Hopkins Oncology Center between May 1977 and July 1978. All of these patients received cyclophosphamide (CY), 2400 mg/sq m, given as a single intravenous infusion over 4 hr. Twelve consecutive patients with poor-risk MM who had previously been treated with multiple courses of CY 2400 mg/sq m as a single bolus or mg/sq m/day × 4 days and judged refractory were admitted to The Johns Hopkins Oncology Center between August 1978 and November 1979. All of these patients received 2 cycles of timed sequential combination chemotherapy with CY 2400 mg/sq m on day 1, given as a single intravenous infusion over 4 hr followed by Adriamycin (ADR) 60 mg/sq m on day 9, given as a single rapid intravenous infusion. The second cycle of CY, ADR was begun on day 35 after the initiation of the first cycle.

Sera Collection

Prior to therapy and 3 times a week until the peripheral white blood cell count (WBC) recovered, 20 ml of whole blood were collected from each patient and allowed to clot, and the sera were separated under sterile conditions and stored immediately at –70°C. All sera from each patient were then analyzed simultaneously. Normal control sera were obtained from 20 volunteers and...
pooled.\textsuperscript{10,11,32} Pooled drug-induced stimulatory serum (HSA), used in the in vitro cell culture experiments, was obtained from 10 patients with MM at the determined time of peak serum stimulation.\textsuperscript{11} Each of these sera stimulated $^3$HTdR incorporation by normal bone marrow cells, ranging \textasciitilde\textasciitilde 120\%\textasciitilde\textasciitilde 150\% of $^3$HTdR incorporation in normal serum. Sera containing in vivo levels of ADR were obtained 30 min after the end of a rapid intravenous infusion of drug\textsuperscript{31} and stored at \textasciitilde\textasciitilde 70\°C until use.

**Bone Marrow Cell Suspensions**

Bone marrow cells were obtained by routine needle aspiration from normal volunteers and from patients with MM who had \textasciitilde\textasciitilde 90\% malignant plasma cells in their marrow, as judged morphologically by multiple bone marrow aspirates and biopsies. Aspirated marrow cells were collected in Roswell Park Memorial Institute Medium (RPMI) 1640 and monodispersed by drawing through a 25-gauge needle. Suspensions of normal marrow cells and malignant marrow plasma cells were studied in separate experiments.

**In Vitro Cell Cultures**

Cells were obtained from 7 consecutive patients whose bone marrow contained \textasciitilde\textasciitilde 90\% malignant plasma cells. Cells from each patient were studied individually. Replicate cultures at a total volume of 1 ml were initiated in 5-ml plastic Falcon 2063 tubes with cells in RPMI 1640 media and the specific serum to be tested (HSA or autologous MM pretreatment (MM Pre)) at 30\%. Although the number of cells in the initial cultures on day 0 varied within a narrow range (1.25\textasciitilde\textasciitilde 1.50 \times 10^6 cells/ml) for each experiment relative to the volume and cell density of the bone marrow aspirate, the initial number of cells for each of the 7 experiments was constant. The concentrations of sera and cells used in these experiments were within the optimal range for cell growth of control cultures.\textsuperscript{24} On day 1 and day 2, after incubation at 37\°C in a humidified atmosphere containing 5\% CO\textsubscript{2}, the serum and media supernatant was decanted from all cultures and fresh media with 30\% specific serum (HSA or MM Pre) was added, restoring the total volume to 1 ml. The cell button was dispersed by gentle agitation. On day 3, the time of predicted peak proliferation in vitro of plasma cells cultured in HSA,\textsuperscript{15} serum and media supernatant were decanted from all cultures and fresh media with 30\% specific serum (HSA \textasciitilde\textasciitilde MM Pre) was added, restoring the total volume to 1 ml. Incubation was continued through day 4. Each parameter of proliferation was measured in triplicate cultures.

$^3$HTdR Incorporation Assay

This method has been described previously.\textsuperscript{13,32} Results for assays of sequential sera are expressed for the purpose of comparison as the percent cpm of $^3$HTdR incorporated into the trichloracetic acid-insoluble precipitate in the posttherapeutic serum relative to the incorporation in normal serum and pretreatment serum. In the in vitro cell culture experiments, measurements were made daily in cultures for 4 days. $^3$HTdR, 1.0 \muCi/ml, was added 18 hr prior to each daily DNA extraction.\textsuperscript{10,11,32} Results are reported as cpm $^3$HTdR incorporated by the malignant plasma cells.

Preparation of $^3$HTdR Microautoradiographs

This method has been described previously.\textsuperscript{10,13,32} Aliquots of aspirated bone marrow cells from patients with \textasciitilde\textasciitilde 90\% malignant plasma cells in their marrows were obtained prior to the first course of therapy and at intervals thereafter. By studying these marrows, which had a high density of tumor cells, confusion of labeled plasma cells with labeled normal elements was avoided. Differential morphological evaluation confirmed that normal marrow elements did not significantly contribute to the cell population. Aliquots of bone marrow were added to 3.5 ml of heparinized RPMI 1640. 1.5 ml patient's serum from that day and $^3$HTdR 0.1 \muCi/ml. These cultures were incubated for 1 hr 15 min at 3\°C in a 5\% CO\textsubscript{2} atmosphere. In the in vitro culture experiments, $^3$HTdR (0.1 \muCi/ml) was added daily to cultured in HSA, MM Pre, and ADR on days 0 through 3 and incubated for 18 hr. Following incubation, cells were washed and lightly cytocentrifuged (at 1500 g for 15 min) onto slides coated with gelatin. Autoradiographs were prepared with Kodak NTM-2 photographic emulsion exposed for 21 days, developed, and stained with Giemsa. The $^3$HTdR labeling index (LI) was determined by counting the number of cells per 1000 that contained 5 or more grains overlying the nucleus. Background labeling was estimated by the number of grains present in a cell-free area equivalent to the area of the plasma cell nucleus. Multinucleate plasma cells were counted as one nucleus when one or all nuclei were labeled. Greater than 90\% of all plasma cells scored contained approximately 25 grains. Results are reported as percent of labeled plasma cells. Our SE of this method is \textasciitilde\textasciitilde 1\%.

Viable Tumor Cell Counts (TCC)

The effect of the varied sera on TCC in the in vitro cell culture system was determined for replicate cultures in each serum type daily throughout the culture period. Viability (assessed by trypan blue dye exclusion) in HSA and MM Pre remained \textasciitilde\textasciitilde 95\% throughout the 4 days of culture.

**RESULTS**

High Dose CY-Perturbations in Malignant Plasma Cell LI and Sequential Serum Activity Following Drug (Fig. 1)

Bone marrow plasma cells and sequential sera were obtained from 11 patients with \textasciitilde\textasciitilde 90\% marrow involvement by tumor prior to therapy with CY, 2400 mg/sq m, given on day 1 and during the period of drug-induced bone marrow aplasia and recovery. The pretreatment 1-hr malignant plasma cell LI ranged from 2.3 to 13.7, with 9 of 11 patients having an LI less than that prior to therapy. The LI from each patient increased significantly, with peak LI detected 8--9 days following CY (range 8.1--30.9) with return toward pretreatment levels by days 15--18.

This peak in tumor cell LI coincided temporally with peak drug-induced HSA (Fig. 1). Sequential sera were analyzed for their effects on 18-hr $^3$HTdR incorporation by normal bone marrow cells. Previous studies have demonstrated the similar effects of such sera on normal and malignant hematopoietic cells.\textsuperscript{1,2,10,13,17,24} Sera obtained on days 9--12 following CY stimulated in vitro normal marrow cell proliferation (131\% above pretreatment values, $p < 0.01$, range 114\%--183\%). Mean 1-hr plasma cell LI increased from pretreatment values of 7.5 \pm 1.3 to 16.3 \pm 2.7 \textasciitilde\textasciitilde 0.001) on day 9. A return toward pretreatment levels by days 15--21 of both the LI and serum activity occurred after the peak in proliferation noted on day 9.
decrease in $^3$HtdR incorporation (37,000–6800 cpm, $p < 0.0005$); $^3$HtdR LI (25.8–6.3, $p < 0.0005$); and TCC ($2.13 \times 10^6$–$0.91 \times 10^6$ cells/ml, $p < 0.001$). This suppressive effect of ADR on proliferating plasma cells was not evident for cells initially cultured in ADR or in the day 4 MM Pre → ADR cultured. In those cultures maintained in HSA or MM Pre (HSA → HSA, MM Pre → MM Pre), there was no significant decreases in any of the day 4 measurements ($p > 0.25$).

The mean LI and TCC of the 7 malignant plasma marrow cells are depicted in Fig. 3. LI paralleled the results of the $^3$HtdR incorporation assay for each of the 7 individual experiments (correlation coefficient, 0.83; data not shown). Mean day 1 LI of cells cultured in MM Pre was 7.6 ± 0.7, while the LI of cells cultured in HSA was 13.2 ± 1.1. Cells cultured in ADR had a mean LI 9.1 ± 0.8. During the first 3 days of culture, cultures in HSA attained a mean LI of 19.8 ± 0.8, while the LI of cells cultured in MM Pre did not change. With the addition of ADR to the stimulated plasma cells on day 3 (HSA → ADR), LI decreased from 19.8 ± 0.8 to 5.3 ± 0.3 on day 4. In contrast, in those cells initially cultured in MM Pre, addition of ADR (MM Pre → ADR) was not asso-

In Vitro Model of Chemotherapy (Figs. 2 and 3)

Previous studies in our laboratory have determined that malignant plasma cells respond in vitro to HSA with proliferation that is maximal on day 3 of culture, while cells cultured in autologous MM Pre are cytostatically retarded.$^{13}$ Based on these findings, we developed an in vitro model of chemotherapy, similar to our model in acute leukemia,$^{10}$ to determine if stimulated human malignant marrow plasma cells are more sensitive to the effects of cycle-active antitumor agents than are plasma cells maintained in an autologous pretreatment milieu.

The effects of HSA and autologous MM Pre on the in vitro proliferation and subsequent sensitivity of malignant bone marrow plasma cells to ADR from 1 patient with MM and >95% marrow involvement by tumor are depicted in Fig. 2. Throughout the first 3 days of the culture period, plasma cells proliferated in HSA when compared with cells in MM Pre ($^3$HtdR incorporation $p < 0.001$; $^3$HtdR LI $p < 0.0005$; TCC $p < 0.005$). MM Pre appeared to cytostatically retard malignant plasma cell proliferation as measured by all 3 parameters ($p > 0.10$ for each parameter, day 1 through day 3). When serum containing ADR was added on day 3 to cells cultured in HSA (HSA → ADR), assays on day 4 cultures revealed a pronounced
While the TCC remained unchanged in MM Pre cultures. As for LI, day 4 TCC in HSA → ADR cultures decreased to 0.95 ± 0.07, while the TCC in MM Pre → ADR cultures did not decrease.

Timed Sequential Chemotherapy of MM Patients With CY,ADR9 (Figs. 4-6; Tables 1 and 2)

Data obtained from MM patients undergoing treatment with high-dose single bolus CY demonstrated peak HSA and residual tumor LI on day 9 of therapy (Fig. 1). Based on these findings and on the results of timed sequential chemotherapy in rat and human leukemias,1,2,8 we designed a clinical trial in CY-refractory poor-risk patients with MM in which the cycle-dependent agent ADR303 was administered at the time of peak HSA and residual tumor cell proliferation (day 9) following high-dose CY.

Sequential sera were obtained from these 12 patients and assayed for their effects on 18-hr normal bone marrow cell 3HTdR incorporation (Fig. 4). Pretreatment sera inhibited in vitro marrow cell proliferation relative to the effects of normal serum (mean 79% ± 7% of normal serum, \( p < 0.025 \)). By day 9 of CY-induced aplasia and prior to ADR administration, serum activity had increased to 112% ± 5% (range 98%-152%) of normal serum baseline. Further HSA increase to 121% ± 6% (range 111%-170%) above normal serum effect was detected on days 15-18 of...
therapy, at a time when the peripheral WBC was at a nadir. Serum activity returned to pretreatment levels by days 25–29, at the time of WBC recovery. Bone marrow plasma cells were obtained from 9 patients with >90% marrow involvement by tumor prior to and during the first course of therapy with CY1ADR9. The 1-hr pretreatment LI ranged from 3.1 to 14.9, while day 9 post-CY LI increased in all patients (range 9.2–30.0). Following ADR on day 9, the 1-hr LI decreased on days 13–15, with a subsequent second peak on days 18–22 (range 9.6–32.5), followed by a return toward baseline levels by day 29. Tumor LI were not performed during the second cycle of CY1ADR9 because of the recovery of normal proliferative erythroid and granulopoietic elements and a concomitant decrease in malignant marrow plasmacytosis to <60% of the total marrow cell population.

Mean serum activity relative to the effects of pretreatment serum and mean 1-hr malignant plasma cell LI obtained during CY1ADR9 therapy are depicted in Fig. 5. At the time of predicted peak HSA on day 9 following initial CY (123% ± 7% above pretreatment values; range 112%–168%), the mean LI had increased from 7.9 ± 1.1 to 19.5 ± 2.3. While HSA increased to 135% ± 9% (range 118%–191%) above pretreatment values on days 15–18, malignant plasma cell LI decreased on day 15 to 9.1 ± 2.1, followed by a subsequent increase in tumor LI to 18.8 ± 2.8 on day 18 and 22.0 ± 1.9 on day 22. Both serum activity and LI returned toward pretreatment levels by day 29.

The mean age of these 12 patients undergoing treatment with CY1ADR9 was 53.4 yr (range 46–64 yr, Table 1). Three females and 9 males underwent this therapy. Two patients (17%) had plasma cell leukemia33 with plasmacytoma at the time of therapy. Four patients had IgG monoclonal protein abnormality, while 3 of the 12 (25%) had IgA and 5 (42%) had Bence-Jones (BJ) proteinuria as the sole protein marker. Five patients had lambda light chains detected in serum and/or urine, and 3 of the 5 patients with only BJ proteinuria excreted lambda light chains. Each patient had a minimum of 2 poor-risk factors at the time of therapy and 11 of 12 had ≥3 risk factors. All suffered impaired normal bone marrow function due to increasing disease activity, and 9 (75%) had >90% marrow involvement by tumor at the initiation of the first course of CY1ADR9. All had performance status ≥3, with 7 (58%) having performance status of 4.29 Hypercalcemia at the time of treatment was documented in 3 (25%), hypoalbuminemia was detected in 3 (25%), and renal dysfunction with serum

Table 1. Patients Treated With CY1ADR9: Disease Characteristics and Therapeutic Outcome

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>M-Protein (Class and Type)</th>
<th>Risk Factors*</th>
<th>Response (&lt;50% Protein Reduction)</th>
<th>Survival (mo)</th>
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<tr>
<td>1. 57/M</td>
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<td>a1, a2, e</td>
<td>+</td>
<td>6</td>
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<tr>
<td>2. 61/F</td>
<td>IgA, lambda</td>
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<td>7.5</td>
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<td>3. 52/F</td>
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<td>a1, a2, b, c, d, e</td>
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<tr>
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<td>BJ, kappa</td>
<td>a2, e</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>5. 64/M</td>
<td>IgG, kappa</td>
<td>a2, e</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td>6. 54/M</td>
<td>IgG, kappa</td>
<td>a1, a2, b, c, d, e</td>
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<td>8</td>
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<tr>
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<td>BJ, lambda</td>
<td>a1, a2, b, e</td>
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<tr>
<td>8. 48/M</td>
<td>BJ, lambda</td>
<td>a1, a2, b, e</td>
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<td>8</td>
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<tr>
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<tr>
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<tr>
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<td>IgG, kappa</td>
<td>a1, a2, e</td>
<td>+</td>
<td>13</td>
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</tbody>
</table>

*Risk factors: a, impaired normal bone marrow function; a1, >90% marrow involvement by tumor; a2, WBC < 3500 cells/cu mm; a3, plasma cell leukemia (with plasmacytomas); b, impaired renal function (SUN > 30 mg/dl); c, Ca++ > 12 mg/dl; d, albumin < 3 g/dl; e, performance status 3–4.27

†BJ, Bence-Jones proteinuria only.
urea nitrogen (SUN) >30 was present in 5 patients (42%).

The therapeutic outcome for each of these CY-refractory poor-risk patients is depicted in Table 1. Eight of 12 (67%) responded to timed sequential chemotherapy with a >50% decrement in monoclonal protein marker, and 2 of these 8 patients had a >75% reduction in protein. Hypercalcemia was reversed in all affected patients and did not require supplemental treatment. Hematologic suppression was ameliorated in 10 patients, as evidenced by at least a 35% decrease in marrow plasmacytosis accompanied by improvement in anemia, leukopenia, and/or thrombocytopenia following CY,ADR. In addition, performance status improved ≥1 grade in all patients and by ≥2 grades in the 8 patients who achieved a protein response.

This response rate to CY,ADR was compared with responses obtained by others using ADR singly or in combination with other antitumor agents (Table 2) in patients with refractory MM. Our 67% response rate in these 12 patients using ADR at the time of peak induced HSA and tumor cell LI following CY is significantly higher than the response rates achieved using ADR in nonsequential regimens in similar patients (p < 0.005, complex chi square). When our data are compared with results obtained using ADR nonsequentially in combination with other agents, an improved response rate following CY,ADR therapy is again demonstrated (p < 0.05, chi square).

Survival of all patients ranged from 4 to 21 mo, with a minimum follow-up of 6 mo. As can be seen in Fig. 6, 9 of 12 (75%) survived ≥7 mo and 6 (50%) are surviving ≥8 mo as of April 15, 1980. Median duration of survival following CY,ADR therapy has not yet been reached, but will be >8 mo (product limit method, Kaplan and Meier).

Survival of these CY-refractory poor-risk patients treated with CY,ADR was compared with the survival documented in 57 patients treated with CY who had become refractory to that therapy and were treated individually with various modalities, including chlorambucil, vincristine, prednisone, l-phenylalanine mustard, ADR, and/or nitrosoureas singly or in combination (R.L. Humphrey, unpublished observations). Median duration of survival in this latter group of 57 CY-refractory patients was 3.3 mo (range 0.1–86, Fig. 6, product limit method) and differed significantly from the >8-mo median survival documented in the 12 patients, similarly CY-refractory, treated with CY,ADR (p < 0.005, log rank test).

Survival of all patients ranged from 4 to 21 mo, with a minimum follow-up of 6 mo. As can be seen in Fig. 6, 9 of 12 (75%) survived ≥7 mo and 6 (50%) are surviving ≥8 mo as of April 15, 1980. Median duration of survival following CY,ADR therapy has not yet been reached, but will be >8 mo (product limit method, Kaplan and Meier).
DISCUSSION

These data confirm those of our previous studies in hematopoietic malignancies and demonstrate that a predictable peak in drug-induced HSA and a temporally coincident increase in tumor LI occur in vivo in patients with MM undergoing intensive treatment with high-dose CY. This HSA enhances malignant plasma cell proliferation in vitro and increases tumor cell sensitivity to cycle-active agents relative to the drug effect noted on cells maintained in a pretreatment milieu. The effect of drug-induced HSA on malignant plasma cell proliferation, as measured in vitro by 3 independent parameters, and the temporal coincidence of this serum activity with increased plasma cell growth in vivo, suggest that HSA may be involved in mediating the proliferative kinetics of these tumor cells. Data from our in vitro model of chemotherapy further support the hypothesis that the antitumor effect of a cycle-active agent in MM is enhanced against a proliferating tumor, similar to our findings in acute leukemia.

Our clinical trials of timed sequential chemotherapy in poor-risk CY-refractory patients with CY administered on day 1 followed by ADR given on day 9 at the time of predicted peak drug-induced HSA and malignant plasma cell LI were designed to test the hypothesis that this predictable increase in plasma cell growth following initial drug may increase the therapeutic efficacy of the second drug in sequence. We selected ADR as that second drug because of its cycle-dependent mechanism of action and because of its clinical usefulness as an antitumor drug in MM patients refractory to alkylating agents.

As predicted from data obtained from patients treated with single bolus CY, 2400 mg/sq m, peak HSA and tumor LI occurred on day 9. Administration of ADR was associated with a further increase in HSA days 15–18 of therapy (days 6–9 following ADR), coincident with continued bone marrow aplasia. Tumor LI on day 15, however, was decreased from day 9 values. This decrease could reflect persistence of ADR in the tumor cells themselves, as prolonged high-affinity binding to intracellular DNA and cell membranes following ADR administration in the face of rapid plasma clearance has been postulated from pharmacokinetic studies. Further, the decreased LI may reflect the cytotoxic action of ADR on the proliferating plasma cell population, with a large number of cells being “doomed to die.” Residual plasma cell LI again increased on days 18–22 of therapy, concomitant with a persistently humoral stimulatory milieu.

The clinical results of this intensive timed sequential regimen in patients with aggressive refractory MM compare favorably with results obtained in comparable patients using ADR in nonsequential combination chemotherapy regimens. In particular, our data may support the contention that ADR activity may be enhanced when given at the time of tumor proliferation, as ADR used as a single agent yields responses in 10%–25% of patients and ADR used nonsequentially in combination with other drugs yields responses in 25%–50% of patients. In our series, 67% (8 of 12) achieved a >50% reduction in protein with 2 of those 12 patients having a >75% reduction; 10 of 12 (83%) experienced hematologic improvement; and 100% had subjective (performance status) improvement. Although the number of patients treated with CY, ADR is small, this response rate following therapy in these poor-risk CY-refractory MM patients is encouraging and may relate to multiple factors, including drug dosage as well as drug timing. Data from our previous trials in patients with poor-risk MM have demonstrated that once patients become CY-refractory, median survival with various nonsequential regimens is significantly shorter (median 3.3 mo) than the median survival obtained following timed sequential chemotherapy (>8 mo).

These clinical trials were performed in a highly select group of patients whose tumor had already been demonstrated resistant to intensive chemotherapy with CY alone. Therapy with CY, ADR produces significant length and depth of bone marrow aplasia (Fig. 4) with its attendant complications. Such therapy should not be attempted without full awareness of aplasia-related toxicities and the capabilities for maximal antibiotic and blood product support during the period of maximal drug-induced bone marrow suppression.

In summary, we have developed a timed-sequential chemotherapeutic regimen in poor-risk CY-refractory patients with MM, based on in vivo and in vitro laboratory findings that suggest that malignant plasma cell proliferation and subsequent sensitivity to cycle-active antitumor agents can be enhanced by drug-induced HSA. Further clinical trials designed to test this hypothesis are being conducted at this center.

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