

High-Dose VP-16-213 and Autologous Bone Marrow Transplantation for Refractory Malignancies: A Phase I Study

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VP-16-213, a congener of epipodophyllotoxin, is a useful chemotherapeutic agent especially against small-cell carcinoma of the lung, germ cell carcinoma, and lymphoma. The standard dose of this drug is limited by myelosuppression. Autologous transplantation of cryopreserved bone marrow assures the restoration of hematopoiesis after marrow ablative cytotoxic therapy. By using this technique, VP-16-213 was dose-escalated using a Fibonacci scheme from the previous highest dose administered to humans (1,500 mg/m²) to 2,700 mg/m² (900 mg/m² per day for three consecutive days). At 2,700 mg/m², severe extramed-

ullary toxicity of the mucous membranes was observed in three of three courses. At the next highest dose (2,400 mg/m²), two of 18 courses (11%, $p < 0.01$) resulted in severe mucositis, thus defining this dose as the maximally tolerated dose based on extramedullary toxicities. As anticipated, myelotoxicity was severe but based on the kinetics of marrow recovery, VP-16-213 in these doses appeared not to be marrow ablative. Based on responses observed in this study, high-dose VP-16-213 should be explored in phase II studies or used in combination chemotherapy.

VP-16-213, a semisynthetic congener of epipodophyllotoxin, underwent routine phase I evaluation to determine optimal dose and schedule of administration.¹ In these studies, VP-16-213 was demonstrated to have myelosuppression as its dose-limiting toxicity with other toxicities infrequent or mild. Careful scrutiny of these phase I studies revealed that the degree of myelosuppression obtained was in general of modest degree and short duration. For example, Radice et al reported a 43% incidence of leukopenia (378 of 877 patients) but only 23% had nadirs between 1,000–2,000/ μ L and only 10% had nadirs less than 1,000/ μ L.² Thrombocytopenia was observed in 14% of patients (109 of 807) but appeared not to be severe since in most instances the nadirs were greater than 50,000/ μ L. In addition, the most severe degrees of myelotoxicity were seen in patients with lymphoma and possible marrow involvement. From these studies, the “standard dose” of VP-16-213 was determined to be 300–500 mg/m² over one to five days repeated every two to three weeks.

Standard phase I studies define a dose which is safe as able to be administered to patients who often have physiologic compromise secondary to malignancies, and can be used in combination chemotherapy.³ Studies of this sort, however, rarely define the maximally tolerated dose of a drug especially if the dose-limiting toxicity is myelosuppression. Obviously, by accepting

greater degrees and durations of cytopenia (as is done in the treatment of acute leukemia) doses may be escalated beyond “standard limits.” From various in vitro and in vivo studies, there is evidence that meaningful dose-response relationships can occur at dose levels above the standard dose.⁴ VP-16-213 is an ideal drug for dose augmentation study because standard doses produce only moderate toxicity and preliminary in vitro data suggest a steep dose-response curve (author’s unpublished observations).

Autologous bone marrow transplantation after long-term marrow cryopreservation is a proven technique which can restore hematopoiesis after marrow ablative therapy.⁵ By harvesting marrow before cytotoxic therapy and transplantation after drug clearance, the dose of cytotoxics may be increased without regard to marrow toxicity. This technique allows the evaluation of drugs

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Submitted March 14, 1983; accepted June 27, 1983.

Supported in part by the Kleberg Foundation; Dr. Wolff is an American Cancer Society Clinical Junior Faculty Fellow.

Presented in part at the Eighteenth Annual Meeting of the American Association for Cancer Research, held in April, 1982 in St. Louis, Mo.

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0732-183X/83/0111-0006\$1.00/0

beyond the dose limitations of myelotoxicity. However, relative limitations exist since hematopoietic recovery after transplantation requires approximately three weeks and patients may have severe cytopenias for the interval between cytotoxic suppression of endogenous hematopoiesis and transplanted marrow regrowth.

In this study, VP-16-213 was dose-escalated beyond previously used doses using a modified Fibonacci schema.⁶ Autologous bone marrow transplantation of previously cryopreserved marrow was performed to assure bone marrow reconstitution. The goals of this study were to administer VP-16-213 in doses that have not been explored previously due to myelotoxicity restraints and to define a maximally tolerated dose defined solely by extramedullary toxicities.

MATERIALS AND METHODS

Patient Eligibility

Patients were eligible for participation provided that they had a biopsy-proven advanced refractory neoplasm. Refractory in this study indicated no effective standard chemotherapy was available and that the extent or type of disease warranted the administration of chemotherapy. In addition, patients had to be less than 65 years old, ambulatory, have normal bone marrow morphology without histologic evidence of tumor, normal peripheral blood counts (that is, granulocytes greater than 1,500/ μ L and platelets greater than 125,000/ μ L), have undergone no concomitant chemotherapy, have no major organ system dysfunction not directly attributable to tumor, and give informed consent before marrow harvest.

Marrow Harvest, Cryopreservation, and Reinfusion

While under general or regional anesthesia, multiple percutaneous aspirations were performed simultaneously from both posterior iliac crests. Total marrow volume extracted was approximately 10 mL/kg of body weight. In the operating room, the marrow was immediately anticoagulated with heparin (10 units/mL), mixed, and filtered through stainless steel mesh (pore diameter, 0.2 and 0.3 mm). After placing in standard blood bags, the bulk of the plasma was removed by centrifugation at 4,000 rpm for 10 minutes at 20°C. The remaining marrow was resuspended and placed in siliconized glass test tubes (16 \times 150 mm). After centrifugation at 1,050 g for 15 minutes at 20°C, the buffy coat of marrow was aspirated through 18-gauge spinal needles, pooled, and counted. Just prior to freezing, the marrow was mixed with dimethyl sulfoxide (pyrogen free supplied by the National Cancer Institute, Bethesda, Md) to a final concentration of 10% and autologous plasma (final concentration, 20%) and placed in polyolefin freezing bags (Delmed, Canton, Mass). The bags of marrow were compressed between aluminum plates and frozen using a controlled rate freezer (Cryo-Med, Mt. Clemens, Mich) at 1°C/min from 0° to -80°C and stored in the liquid or vapor phase of liquid nitrogen. For reinfu-

sion, the frozen marrow was transported to the patient's room in liquid nitrogen, rapidly thawed in a 37°C water bath, and injected intravenously over two to five minutes. The required minimal cell dose per transplantation was 0.5×10^8 nucleated marrow cells/kg, an extrapolated dose from canine studies that we felt assured restoration of hematopoiesis.⁷

VP-16-213 Administration

VP-16-213 supplied by the National Cancer Institute was diluted in normal saline to a maximum concentration of 1 mg/mL and infused at a maximum rate of 500 mg (or 500 mL of normal saline) per hour. Solutions were prepared hourly and doses per infusions were equalized. For example, a daily dose of 1,200 mg was administered as three consecutive hourly infusions of 400 mg of VP-16-213 in 500 mL of normal saline. VP-16-213 was administered over three days with one third of the total dose given each day. Bone marrow transplantation was performed 72 hours after the last VP-16-213 administration. This time interval was chosen to be greater than five half lives based on pharmacologic data to ensure clearance of the parent drug and presumed potential cytotoxic metabolites.⁸ The starting dose of VP-16-213 in this study was chosen to be 1,500 mg/m² (500 mg/m² per day) representing the highest dose previously administered.^{9,10}

The format of this study was to escalate VP-16-213 in 20% increments (300 mg/m² or 100 mg/m² per day) by treating four patients at each dose level. Based on the statistical approach of Lee et al,¹¹ dose escalation to the next level was performed when two or less patients at a dose exhibited severe or fatal toxicity. Patients were observed six weeks before proceeding to the next dose level. This approach should limit severe or fatal toxicity at the highest dose to an incidence of 20% or less. When unacceptable toxicity was observed, dose escalation was halted. Approximately 14 patients were planned to be treated at the previous level better defining the maximal tolerable dose. Each patient could be retreated at the dose level that they had previously received but could not receive higher doses. This approach was undertaken to distinguish between acute or cumulative toxicity. To be evaluable for toxicity and tumor response patients had to survive at least four weeks after autologous bone-marrow transplantation. Patients were managed in regular hospital beds at Vanderbilt University Hospital, Nashville, Tenn, with no special isolation procedures. Pretreatment evaluation consisting of physical exam, routine hemogram, chemistries, chest roentgenogram, electrocardiogram, and computerized tomographic scans were repeated at frequent intervals to manage patients and to evaluate possible toxicities.

Informed consent approved by the Institutional Review Board was obtained before marrow collection and administration of chemotherapy. For statistical analysis, Fisher's exact test (one tailed) was employed. Response was calculated from initiation of therapy according to standard oncologic definitions.

RESULTS

From March 1981 to March 1982, 28 patients were treated (18 males and 10 females) with a median age of 39.5 years (range, 18-65 years). Twenty-five patients had undergone prior cytotoxic chemotherapy, one patient had prior cranial

Table 1. Patient Characteristics

Tumor Types	No. with Prior Rx	No. Without Prior Rx
Small cell carcinoma of the lung (SCCL)	3	...
Non-SCCL	1	1
Adenocarcinoma of the gastrointestinal tract	4	1
Breast carcinoma	3	...
Hodgkin's lymphoma	1	...
Acute lymphocytic leukemia	1	...
Glioblastoma multiforme	4	...
Germ cell carcinoma	6	...
Prostatic carcinoma	1	...
Adenocarcinoma of choroid plexus (brain)*	1	...
Ovarian carcinoma	1	...
Total	26	2

*Prior radiotherapy only.

radiation therapy, and two patients were previously untreated. Patient characteristics are described in Table 1.

Table 2 describes the dose escalations and evaluability of courses. As noted, only three patients were entered at the highest dose of 2,700 mg/m² since all three patients developed severe prohibitive toxicity. Thirty-four (94%) of 36 courses were evaluable. The two nonevaluable patients died within 10 days of transplantation from sepsis associated with severe neutropenia. Acute toxicities to high-dose VP-16-213 consisted of fever, chills, nausea, and vomiting. As had been previously described with standard dose VP-16-213, fever and chills are occasionally associated acutely with the drug infusion. In our study 25% of patients had fever and/or chills which occurred only during the first day of infusion. Hypotension was not observed. Nausea and vomiting occurred in almost half of the patients but was mild and well controlled by antiemetics.

Table 2. Dose Escalations

Total Dose (mg/m ²)	No. of Patients	No. of Courses	No. of Evaluable Courses
1,500	4	5	4
1,800	4	4	4
2,100	4	5	5
2,400	13	19	18
2,700	3	3	3
Total	28	36	34

Table 3. Patients With VP-16-213 Mucositis

Dose (mg/m ²)	Degree of Mucositis			
	Mild	Moderate	Severe	Fatal
1,500
1,800	...	1/4 (25)
2,100	...	2/5 (40)
2,400	4/18 (22)	12/18 (67)	2/18 (11)*	...
2,700	3/3 (100)	...
Total	4/36 (11)	15/36 (42)	5/36 (14)	0/36

NOTE. Mild mucositis was described as pain without ulceration, the patient was able to drink and eat most foods; moderate mucositis was described as painful ulcerations, pain controlled by local means, and the patient was able to drink; severe mucositis was described as painful ulcerations lasting more than one week and requiring narcotic analgesic for more than one week. Data are no. of episodes/total no. of courses (% of courses).

* $p < 0.01$.

Compensated metabolic acidosis was seen in 43% of the patients with serum bicarbonates dropping to a nadir of 16–18 mM/L during the week after chemotherapy. The complex solvent system consisting of polyethylene glycol, Tween-80, citric acid, and absolute ethanol were presumed responsible for the acidosis. The acidosis was not accompanied by symptoms or signs and was completely reversible in all patients. Three patients developed mild disorientation during drug administration that was transient and appeared consistent with intoxication by the large volumes of ethanol included with the chemotherapy.

Clinically important side effects of VP-16-213 consisted of myelosuppression and mucositis. The mucositis was dose related and proved to be the extramedullary toxicity which defined the maximally tolerated dose of 2,400 mg/m². The extent of mucositis and its relationship to dose is illustrated in Table 3.

As anticipated, myelosuppression was prominent and prolonged in this study. As shown in Table 4, high-dose VP-16-213 produced a prolonged duration of cytopenias at all dose levels. However, the magnitude of cytopenias was not dose related. Peripheral blood count recovery (that is, > 500 neutrophils/ μ L and > 20,000 platelets/ μ L) occurred before three weeks after transplantation in all patients except one who required 23 days to manifest a platelet count greater than 20,000/ μ L.

Objective but very transient responses to VP-

Table 4. Hematopoietic Toxicity

Dose (mg/m ²)	No. of Days with WBC <1,000/ μ L	No. of Days with Neutrophils <500/ μ L	No. of Days with Platelets <20,000/ μ L
1,500	6.5 (0-8)	8 (7-11)	1 (0-11)
1,800	10.5 (7-14)	12.5 (9-15)	12 (7-23)
2,100	7.5 (6-9)	9 (6-11)	4 (2-7)
2,400	9.5 (0-14)	11 (8-16)	5.5 (0-11)
2,700	10 (9-14)	11 (9-12)	7 (6-8)

NOTE. Data are median (range); WBC = white blood cells.

16-213 occurred in one patient with acute lymphocytic leukemia and one patient with Hodgkin's disease. Useful and more prolonged objective responses were seen in patients with glioblastoma multiforme and germ cell tumors, all of which had been heavily treated with standard chemotherapy or radiation therapy prior to VP-16-213 therapy. Responses are shown in Table 5.

DISCUSSION

In this study, VP-16-213, beginning at the highest dose level previously reported was step-wise dose-escalated using a modified Fibonacci schema. Since without existing knowledge concerning the magnitude of myelosuppression it was conceivable that such augmented doses might ablate the bone marrow, patients were transplanted with their own previously cryopreserved bone marrow to ensure the restoration of hematopoiesis. We also reasoned that autologous bone marrow transplantation would ensure a finite duration of severe cytopenia even if the therapy is not completely marrow ablative. Since recovery of hematopoiesis after autologous bone marrow transplantation cannot be determined to be solely of exogenous (for example, transplant) versus endogenous source, knowledge concern-

ing the true extent of myelosuppression in this study is hidden by the transplantation.

In this study, VP-16-213 was escalated from 1,500 mg/m² to a maximum tolerated dose of 2,400 mg/m² over three days. Further dose escalation was prevented by severe and potentially fatal oropharyngeal mucositis in all three patients treated at a dose of 2,700 mg/m². Other than mucositis and the anticipated severe myelosuppression no other severe toxicities were encountered at the maximum tolerated dose of 2,400 mg/m². As had been previously reported using standard dose VP-16-213, acute episodes of fever and chills occurred on the first day of infusion. Hypotension, or other serious sequelae were not observed. Metabolic acidosis, presumably from the solvent required to deliver the augmented dose of drugs occurred in 43% of all patients but did not require therapy and resolved in all patients. Other side effects including rash, transient confusion, and liver function test abnormalities were infrequent and not severe. Since many of our patients had preexisting alopecia, accurate evaluation of that side-effect was not accomplished, but it would be expected to be frequent based on the occurrence of alopecia with standard dose VP-16-213 therapy.

Recovery from the severe myelosuppression occurred within 16 days of transplantation in all patients except one. Based on studies using autologous bone marrow transplantation with absolute marrow ablative therapy such as total body irradiation, recovery of blood counts in this study occurred before the anticipated interval. Although indirect evidence, these data suggest that VP-16-213 in this study was not marrow ablative and recovery of endogenous hematopoiesis contributed heavily to blood count recovery.

Although a phase I study, tumor responses occurred in 36% of all patients including five out

Table 5. Responses of Patients

Disease	No. of Responses/No. Treated	Complete Remission	Partial Remission	Durations (mo)
Acute lymphocytic leukemia	1/1	0	1	1
Hodgkin's lymphoma	1/1	0	1	1
Germ cell carcinoma	5/6	1	4	1+, 1+, 1.5+, 2+, 4+
Glioblastoma multiforme	3/4	0	3	5, 5, 5+
Other	0/16	0	0	...
Total	10/28	4%	32%	...

of six patients with refractory germ cell tumors. Interestingly, of the four germ-cell patients who had failed previous standard-dose VP-16-213 therapy, three patients responded suggesting a dose-response relationship to VP-16-213. Further evaluation of the therapeutic efficacy of high-dose VP-16-213 is ongoing in phase II trials.

In summary, VP-16-213 was dose-escalated in this study using autologous bone marrow transplantation of previously cryopreserved marrow. A maximally tolerated dose of 2,400 mg/m² over three consecutive days was defined. At that dose severe mucositis, lasting more than one week is expected to occur in 11% (0–25%, 95% confidence limits) of courses. Further dose escalation was associated with the more frequent occurrence of severe mucosal membrane toxicity.

With the knowledge gained from this study, this new dose of VP-16-213 should be explored in a phase II study to evaluate its therapeutic benefit. Since some patients in this study with refractory germ cell tumors who had previous standard dose VP-16-213 therapy had responses to high-dose VP-16-213, there is reason to compare the therapeutic benefit of augmented doses of VP-16-213 against standard dose VP-16-213 in tumors that are responsive to VP-16-213 at standard doses (for example, small-cell lung cancer, lymphoma, germ cell carcinoma). In addition, the information derived from this study will help to use VP-16-213 at a supra-standard dose as part of a combination chemotherapy program although additive or synergistic myelosuppression and mucositis would be possible.

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