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A phase II trial of pyrazine diazohydroxide in patients with disseminated malignant melanoma and no prior chemotherapy – Southwest Oncology Group study

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Summary

Malignant melanoma is rapidly increasing in the United States. Metastatic disease responds poorly to currently available chemotherapy. Pyrazine diazohydroxide (PZDH) is a new agent inhibiting DNA synthesis that is active in mouse tumor models and human xenografts and lacks cross resistance with multiple standard agents. In this phase II trial, patients with no prior chemotherapy or immunotherapy for metastatic disease and performance status (SWOG) of 0–1, were treated with pyrazine diazohydroxide at a dose of 100 mg/m^2/day by IV bolus injection over 5–15 minutes for 5 consecutive days every 6 weeks. There were 23 eligible patients entered on this trial with 74% having PS of 0 and 91% having visceral metastases. There were no confirmed anti-tumor responses. The overall response rate is 0% (95% CI 0%–15%). Median overall survival is six months (95% CI 5-8 months). The most common toxicities were hematologic and consisted of lymphopenia, thrombocytopenia, anemia, and leukopenia. Fatigue, and nausea and vomiting were the next most common toxicities. Pyrazine diazohydroxide by this dose and schedule has insufficient activity in the treatment of disseminated malignant melanoma to warrant further investigation.

Introduction

There has been a steady increase in malignant melanoma in the United States over the last century with now at least 47,000 new cases diagnosed per year [1]. Metastatic disease responds poorly to chemotherapy. Dacarbazine is the standard agent with response rates of 14% to 20% in multiple series and median response durations of 4 to 6 months [1]. There is no definite evidence that dacarbazine produces any substantial survival benefit and current controlled clinical trials show no definite superiority for combination therapy [1]. The biologic agents of interferon-α and interleukin 2 each produce response rates around 15% and are being tested in combinations [1]. With the lack of overall survival benefit for patients with metastatic disease treated with known chemotherapy, patients with metastatic melanoma are candidates for clinical trials [1].

Pyrazine diazohydroxide (PZDH) was selected by the National Cancer Institute for clinical development because it was more chemically stable at physiologic pH than its parent compound, pyridine -2- diazohydroxide, and had shown significant antitumor activity *in vivo* against a variety of murine and human tumors [2]. PZDH appeared to inhibit DNA syn-

thesis by the intracellular formation of the pyrizinyldiazonium ion which then reacts with a nucleophile to cause DNA adduct formation and single strand DNA breaks [2]. The antitumor activity was first observed in the intraperitoneal P388 leukemia prescreen. Maximum increased life span (ILS) values ranging from 87–261% in 17 experiments (median 158%) were obtained following daily i.p. administration of PZDH in the optimal dose range of 25–38 mg/kg for nine days [2]. In additional intraperitoneally implanted mouse tumor models with B16 melanoma and L1210 leukemia, the administration of PZDH daily for 9 days produced maximum increased life span values ranging from 55%–69% and 36%–85% respectively, in several experiments [2,3]. Intermittent treatment on days 1, 5, 9, and 13 in the intraperitoneally implanted M5076 sarcoma model produced similar results [2,3]. Of importance was the finding that activity against the L1210 leukemia was significantly retained when the tumor and drug injection sites were separated (s.c. tumor and i.p. PZDH) [2].

PZDH at a 100 mg/kg dose administered intraperitoneally on days 1, 5, and 9, caused complete regression of the human MX-1 mammary tumor placed under the renal capsule of nude mice [3]. Using the same dose and schedule, the life span of nude mice bearing the intraperitoneally implanted human LOX amelanotic melanoma was extended by 150% [3]. In the i.p. LOX human melanoma model, four different i.p. schedules, (day 5 only, q3h $\times$ 8 day 5, days 5, 9, and 13, and qd 5–13), were equivalent with respect to percent increased life span and cell kill [4]. The i.v. route produced similar results [4]. P388 and L1210 leukemia cell lines resistant to melphalan were also resistant to PZDH *in vivo*, but sensitivity to PZDH was retained in P388 lines that were resistant to doxorubicin, vincristine, cisplatin, cyclophosphamide, and methotrexate [4]. By quantitative *in vivo* bioassay, there appeared to be a 2 log greater cell kill in pulmonary metastases then against the primary subcutaneous LOX melanoma tumor in the same mouse [4]. In nude mouse models where tumor was implanted subcutaneously in the flanks, PZDH was given at 100 mg/kg i.p. on days 1, 8, and 15. The breast cancer model MAXF 401 regressed completely 28 days after the start of therapy, a partial remission was achieved in the gastric cancer GXF 97, and a minor regression was observed in the large-cell lung cancer [5].

Because PZDH shows an acid-catalyzed breakdown to the active agent, it is hypothesized that it exhibits selective toxicity towards hypoxic solid tumor cells. This was tested *in vitro* using A204 human rhabdomyosarcoma cells to determine the drug concentration required to produce a 50% inhibition of cell growth (IC 50) under different culture conditions. With a one hour drug exposure at pH of 7.4, the IC 50 was 61 μg/ml while at pH of 6.0 it was 31 μg/ml [6]. Under hypoxic conditions in the presence of glucose, stimulating production of lactic acid, the IC 50 at pH 7.4 was 22 μg/ml [6].

Preclinical toxicology and pharmacokinetic studies have been performed in CD2F1 mice, Fischer 344 rats, and beagle dogs. In mice, a single dose LD 10 is 166 mg/kg (498 mg/m^2) and a five daily dose LD 10 is 60 mg/kg/day (180 mg/m^2/day) [2]. The dose limiting toxicity in both rats and dogs is myelosupression with decreases in leukocytes, and at higher doses, red cells and platelets and bone marrow atrophy. Mild GI toxicity and testicular atrophy also occurred [2]. In mice and dogs, pharmacokinetic analysis showed a rapid, monophasic, dose-dependent elimination pattern. Elimination half-lives ranged from 5.8 to 7.3 minutes and the rate of plasma drug clearance ranged between 29.9 and 55 ml/min/kg [2]. Following an i.v. bolus of 14C labeled pyrazine-2-diazohydroxide, 79% of the radioactivity was excreted in the urine in 24 hours, 3% in the feces, 0.4% in the expired air, and 18% remained in the carcass with the highest levels of radioactivity in the liver and kidney [7].

In humans, pharmacokinetic studies of 14 patients treated at doses between 100 mg/m^2 and 487 mg/m^2 have confirmed that PZDH rapidly disappears from plasma with an average elimination half life of 11.2 minutes [2]. There was a decrease in clearance and in the volume of distribution as the dose was escalated suggesting that there was a saturable component to the elimination of PZDH [2]. Pharmacokinetic analysis performed on 28 patients receiving between 18 mg/m^2/d $\times$ 5 days and 100 mg/m^2/d $\times$ 5 days of PZDH revealed that at the lowest dose, drug could only be detected for 30–90 minutes (assay detection limit = 10 ng/ml) [8]. Compartmental modeling of the four lowest dose levels (18–56 mg/m^2/d $\times$ 5) was consistent with a two-compartment model, while modeling of the three higher doses (75–133 mg/m^2/d $\times$ 5) revealed a third phase to the decay curve. The AUC increased progressively with dose and there was no evidence of dose-dependent pharmacokinetics [8]. For the 10 patients who received 100 mg/m^2/d $\times$ 5, the mean AUC was 105.4 μg min/ml and the rate of clearance was 1.96 l/min. Peak plasma concentration varied widely with a range from 4.1–80.2 μg/ml (mean 18.8

and standard deviation 22.9 μg/ml) with a rapid drop off [8]. Body surface area (BSA) was only moderately correlated with clearance. BSA apparently correlated with elimination but not with distribution [8].

There were 3 Phase I trials of PZDH in patients. No objective responses occurred on these trials [2]. In trial 1, drug was administered once every 3 weeks i.v. at doses ranging from 15 mg/m^2 to 608 mg/m^2. The dose limiting toxicity was myelosuppression with diarrhea, nausea and vomiting, anorexia, alopecia, and decreased performance status also seen. Delayed myelosuppression was seen as the doses were escalated. The recommended phase II dose was 487 mg/m^2 i.v. bolus once every 5 weeks [2]. In trial 2, drug was administered by i.v. bolus once every 3 weeks at doses from 50 mg/m^2 to 350 mg/m^2. Toxicities included moderate to severe nausea and vomiting, anorexia, fatigue, myelosuppression and headache. At the MTD of 350 mg/m^2 the dose limiting toxicity was life-threatening hepatotoxicity seen twice, once after the first course and once after the second course. There was also a case of life-threatening pulmonary toxicity possibly unrelated to the drug [2]. In trial 3, PZDH was given i.v. at doses of $\leq$ 75 mg/m^2 every 4 weeks or at doses of $>$ 75 mg/m^2 every 6 weeks on a daily $\times$ 5 schedule. Patients were treated at doses from 18–133 mg/m^2. At the MTD of 133 mg/m^2 Grade 3 and 4 toxicities included myelosuppression which was dose limiting, elevated liver function tests, nausea and vomiting, peripheral neuropathy, and fever [2]. One death occurred on study. The recommended phase 2 dose was 100 mg/m^2/d $\times$ 5 days every 6 weeks, the dose used in this phase 2 trial.

Materials and methods

Patient population: All patients were required to have a histologically proven diagnosis of malignant melanoma that was Stage IV and not surgically curable. Patients had to have no evidence of brain metastases by CT or MRI or if they had a history of brain metastases, they had to be resected completely free of disease followed by a course of radiation therapy. They were required to have bi-dimensionally measurable disease and a Southwestern Oncology Group (SWOG) performance status of 0–1 ($\geq$ 70 Karnofsky), thus ambulatory and able to carry out light work. Patients may have received at most one prior biologic or immunotherapy regimen given in an adjuvant fashion, but no adjuvant chemotherapy and no prior chemotherapy or immunotherapy for metastatic disease. Prior surgery and/or radiation therapy was allowed provided patients had recovered from all adverse effects of prior treatments and, for prior radiation treatment, had shown objective evidence of progression of disease. Patients had to have a pretreatment granulocyte count of $\geq$ 1500 cells/μL a platelet count of $\geq$ the institutional lower limit of normal and a hemoglobin level $\geq$ 10/gm/dL serum creatinine and serum bilirubin within the institutional upper normal limits, and a serum glutamic oxaloacetic transaminase level $\leq$ 2.5 times the institutional upper limit of normal or $\leq$ 5 times the institutional upper limit of normal if the liver was involved with tumor. Patients with other serious illnesses, serious active infections, requiring therapy with other investigational drugs, or known to be human immunodeficiency virus antibody seropositive were not eligible. Pregnant or nursing women were not eligible, nor were patients with a second malignancy except for adequately treated basal or squamous cell skin cancer or *in situ* cervical cancer, adequately treated stage I or II cancer from which the patient was currently disease free, or any other cancer for which the patient has been disease free for at least five years. Women or men of reproductive potential had to agree to use an effective contraceptive method. No type of concomitant therapy for the patient's malignant melanoma was allowed.

Pyrazine diazohydroxide: Pyrazine diazohydroxide was supplied by the National Cancer Institute (NCI) as a lyophilized powder in 500 mg. vials. When reconstituted with 9.8 ml of Sterile Water for Injection USP it formed a solution of 50 mg/ml. When diluted to a concentration of 1 mg/ml for infusion it was stable for 4 hours at room temperature.

Treatment plan: Pyrazine diazohydroxide was given by IV bolus injection over 5–15 minutes at a dose of 100 mg/m^2/day for five consecutive days of week one, followed by a five week rest period. Each cycle was thus scheduled to be six weeks duration. It was recommended that patients be aggressively pretreated with a potent antiemetic regimen (ondansetron or granisetron plus dexamethasone) to prevent development of serious nausea or vomiting. Toxicity was evaluated using the Southwest Oncology Group Toxicity Criteria in place at the time of the study. Dose reductions were required for grade 3 or 4 nausea or vomiting that occurred despite aggressive antiemetic treatment and could be done twice before removal from treatment was required. Weekly CBC and platelets counts were required after the first treatment and

again in all subsequent cycles if grade 3 or 4 myelosuppression occurred. A grade 4 level of absolute neutrophils ($<$ 500 cells/μL) or platelets ($<$ 25,000 cells/μL) required a 25% dose reduction of PZDH in subsequent cycles. G-CSF could be used during the dose reduction cycle and the dose could be escalated back to the original dose if the granulocyte count remained $\geq$ 1000 cells/μL throughout the cycle of G-CSF supported chemotherapy. Two 25% dose reductions were allowed for grade 4 absolute neutrophil count or thrombocytopenia before a patient would be removed from treatment.

Definition of response: Standard SWOG response criteria were used to define the antitumor effects that were observed. A complete response required the disappearance of all measurable and evaluable disease in all disease sites including normalization of abnormal disease-related laboratory values and disease-related symptoms with no new lesions. A partial response required a $\geq$ 50% decrease in the sum of the products of the perpendicular diameters of all measurable lesions, with no new lesions or progression of evaluable disease with all measurable and evaluable disease and sites assessed. Progressive disease was defined as (a) a 50% increase or an increase of 10 cm^2 (whichever is smaller) in the sum of the products of measurable lesions over the smallest sum observed or clear worsening of any evaluable disease, or (b) the appearance of any new lesion or the reappearance of any lesion that had disappeared, or (c) failure to return for evaluation due to deteriorating condition (unless deterioration was clearly unrelated to the cancer). Stable disease was disease that did not meet the criteria for either a complete or partial response or progression. Tumor assessment was requested at the end of every two cycles. After first documentation of a complete or partial response, a second assessment was required after 4 weeks to confirm the response.

Statistical considerations: The primary goal of the study was to evaluate the response rate in patients with advanced melanoma. A two-stage design was used for patient accrual. It was assumed that the regimen would not be of interest if the true response rate were less than 5%. It was also assumed that that a true response rate of 20% or more would be of considerable interest. Twenty eligible patients would be entered initially. If necessary, the study would be temporarily closed while response data matured. If zero responses were observed, the study would be permanently closed and the regimen concluded to be inactive. If one or more responses were observed in the first 20 patients, an additional 20 patients would be accrued. Five or more responses out of 40 would be considered evidence that the regimen warranted further study, provided other factors, such as toxicity and survival also were favorable. This design had a significance level (probability of declaring an agent with a 5% response probability to warrant further study) of 5%, and a power (probability of correctly declaring an agent with a 20% response probability to warrant further study) of 92%.

Table 1. Patient characteristics (N = 23)

	No.	%
Age, years		
Median	64.0 years	
Range	33–83 years	
Sex		
Male	17	74%
Female	6	26%
Performance status		
0	17	74%
1	6	26%
TNM classification		
M1a	2	9%
M1b	21	91%
Liver involvement		
yes	7	30%
no	16	70%
Prior adjuvant biologics		
yes	5	22%
no	18	78%
Prior radiation therapy		
yes	4	17%
no	19	83%

Results

Patient population: Twenty four patients were entered onto this study from 16 different institutions. One patient was ineligible due to insufficient baseline documentation. The characteristics of the 23 eligible patients are listed in Table 1. Median age was 64 years (range 33–83 years) and 74% were male. The majority, 74%, had a performance status of 0, while 26% had performance status of 1. Ninety one percent of patients were M1b, thus having some site of visceral metastases. Seven patients, or 30%, had liver involvement.

Table 2. Response (N = 23)

	Number	Percent
Complete response	0	0%
Partial response	0	0%
Unconfirmed response	1	4%
Stable/No/Response	1	4%
Increasing disease	19	83%
Early death	1	4%
Assessment inadequate	1	4%
Total	23	100%

Response and survival: Response data are listed in Table 2. All 23 eligible patients were evaluated for response. One patient had inadequate response assessment and one patient died prior to response assessment. The patient that died had a stroke that was apparently unrelated to either disease or treatment. Both of these patients are assumed to be non-responders. There were no confirmed responses. There was one unconfirmed response in a patient with a lung nodule and abdominal mass, but by the next evaluation there were new lung, liver, spleen and abdominal lesions indicating progression of disease. Thus, the overall response rate is 0% with 95% confidence interval of 0% to 15%. Twenty patients came off treatment because of progression, one came off because of toxicity, and two patients were off treatment due to death. The estimated median number of courses received was 2 (range 1–5). There were no major protocol deviations. Twenty two of the 23 eligible patients have died. The median overall survival is six months with a 95% confidence interval of 5 months to 8 months.

Toxicity: All 23 eligible patients were evaluated for toxicity (Table 3). The most common toxicities were hematologic and consisted of lymphopenia, thrombocytopenia, anemia and leukopenia. Fatigue and nausea/vomiting were also frequent. A decrease in the number of lymphocytes is recorded as a toxicity in SWOG and so is reported here. Lymphopenia occurred with high frequency and was often severe, but its clinical relevance is uncertain. There was one instance of grade 4 thrombocytopenia in a patient after 2 cycles of therapy. His platelets did not recover to within normal range within the time frame allowed by the study and he was removed from treatment. It was necessary to combine several toxicities into broad categories to make Table 3 manageable. The category of Respiratory includes dyspnea, Neurologic includes incoordination/ataxia, vision and headache, and Miscellaneous includes insomnia, erythema, alopecia, anxiety/depression, hyperglycemia, dehydration, and GI, GU, Liver, and Dermatologic other than what is specifically listed, and additional miscellaneous toxicities.

Discussion: In this clinical trial, patients with advanced malignant melanoma were treated with the promising new agent pyrazine diazohydroxide to assess response and toxicity. PZDH had demonstrated impressive *in vitro* activity in human xenograft tumor models and lack of cross resistance to multiple commonly used agents, and theoretically, it might show selective activity against hypoxic solid tumor cells. Unfortunately, as is frequently seen, activity in *in vitro* models failed to translate into this clinical area. A published report by Vogelzang et al. [9], using the same dose and schedule of PZDH as in this trial, indicated that there were no antitumor responses in 15 renal cell cancer patients and 14 colorectal cancer patients, showing lack of significant antitumor activity in these disease sites as well.

It is unlikely that this was due to an inferior schedule of administration. In the mouse tumor models, single day administration, 3 times a day administration, every 4 day administration, every 8 day administration, and daily for 9 days, whether i.p. and i.v. were equivalent with respect to increased life span and cell kill [4]. In the human phase I trials in which drug was administered once every 3 or 5 weeks, life threatening hepatotoxicity and pulmonary toxicity were seen [2] suggesting that the 5 day schedule might be better for larger phase II trials.

In the mouse models, total dose of drug administered on the different schedules varied from 128 mg/kg on the single day schedule to 513 mg/kg on the daily times 9 day schedule [4]. Even the lowest total dose of 128 mg/kg was larger than the dose the average 70 kg. $5'8''$ (1.8 m^2) person would receive at the dose and schedule used in this trial. At 100 mg/m^2/day over 5 days the total dose administered for a 1.8 m^2 individual would be 900 mg or 12.9 mg/kg assuming that they were 70 kg. Thus one reason that responses were not seen may be because patients received much smaller doses on a mg per kg basis then was used in the mouse trials.

From [6], under optimal conditions in an *in vitro* assay with a one hour continuous exposure, the lowest concentration of drug producing a 50% inhibition of tumor cell growth was 22 μg/ml. This gives an area

Table 3. Toxicity (N = 23)

Toxicity	Any grade of toxicity		Grade 3	Grade 4
	No.	%	No.	No.
1. Lymphopenia	21	91	6	11
2. Thrombocytopenia	17	74	1	1
3. Anemia	15	65	3	0
4. Leukopenia/granulocytopenia	11	48	2	0
5. Malaise/fatigue/weakness	8	35	2	0
6. Pain	8	35	0	0
7. Nausea/vomiting/anorexia	7	30	1	0
8. Liver function abnormalities	6	26	2	0
9. Constipation	6	26	0	0
10. Renal	5	22	0	0
11. Fever/chills	5	22	0	0
12. Infection	4	17	4	0
13. Neurologic	3	13	2	0
14. Edema	3	13	0	0
15. Diarrhea	2	9	0	0
16. Stomatitis/gastritis	2	9	0	0
17. Rash/urticaria	2	9	0	0
18. Respiratory	1	4	1	0
19. Miscellaneous	10	43	1	0

under the curve of 1320 μg min/ml. From [8], Figure 2 is a representative time-concentration graph for a patient treated with PZDH at the 100 mg/m^2 dose level. Estimating from this graph, the peak concentration achieved in the blood is about 8.5 μg/ml and rapidly falls off. For the first 60 minutes, the estimated approximate area under the curve is 185 μg min/ml. Going out to 500 minutes, the end of the graph, the approximate total area under the curve is under 250 μg min/ml. For 5 days of treatment, the estimated approximate area under the curve would be less than 1250 μg min/ml, total, of *interrupted* exposure. Thus, as in the mouse data above, the *in vitro* data suggest that levels of drug achieved in patients on this schedule is less than the minimum required to produce meaningful cell kill.

The toxicity that was seen in this trial was similar in type and severity to that seen in the phase I trial reported in reference 8. Our most common toxicity was bone marrow, with decreases in all cell lines as shown in table 3. There was one thrombocytopenia grade 3 (25.0–49.9 $\times$ 10^3/μL), and one grade 4 (< 25.0 $\times$ 10^3/μL), and two leukopenia/granulocytopenias grade 3 (WBC 1.0–1.9 $\times$ 10^3/μL; granulocytes 0.5–0.9 $\times$ 10^3/μL). Predominant toxicity was grade 1 or 2 (platelets 50 $\times$ 10^3/μL – lower limit of normal; WBC 2.0–3.9 $\times$ 10^3/μL; granulocytes 1.0–1.9 $\times$ 10^3/μL). In the phase I trial, at this dose level, median platelet nadir was 87 $\times$ 10^3/μL (range 9–172 $\times$ 10^3/μL) corresponding to our grade 1, median WBC nadir was 2.5 $\times$ 10^3/μL (range 0.6–7.6 $\times$ 10^3/μL) corresponding to our grade 2, and median absolute granulocyte count was 1280/μL (range 50–5170/μL) again corresponding to our grade 2. Though the numbers of patients in the different trials are small, the types and severity of bone marrow toxicity that we saw, are similar to the median and range of bone marrow toxicities seen in the phase I trial and the two phase II trials in renal and colorectal cancer [8,9]. In the phase I trial, the variability in bone marrow toxicity was noted, but there was insufficient data to determine if it was due to pharmacokinetic variability or to other factors.

Though theoretically promising in the treatment of cancer patients because of the encouraging preclinical results, PZDH in the dose and schedule used here demonstrated no benefit for the treatment of metastatic melanoma. Although its dose might be increased some, because of its significant toxicities, it is unlikely that doses in humans equivalent to those producing anti-tumor effects in mice or *in vitro* could be safely

administered. In fact in the phase I trial, the dose of 133 mg/m^2/day produced grade 4 neutropenia in 3 of the 4 patients and grade 4 thrombocytopenia in 2 of the 4 patients in which it was tested. Although PZDH is no longer in clinical development, testing of this compound in pre-clinical models at lower doses, ones that are achievable in patients, in combination with other agents that have different toxicities, might be done to evaluate for anti-tumor responses. If promising, there might be a role for future trials of combination therapy with PZDH in the clinic.

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