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PHASE I STUDIES

Phase 1 trial of Anvirzel™ in patients with refractory solid tumors

Tarek Mekhail · Hanspreet Kaur · Ram Ganapathi · G. Thomas Budd · Paul Elson · Ronald M. Bukowski

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Summary Anvirzel™ is an aqueous extract of the plant Nerium oleander which has been utilized to treat patients with advanced malignancies. The current study reports a phase 1 trial to determine the maximum tolerated dose (MTD) and safety of Anvirzel™ in patients with advanced, refractory solid tumors. Patients were randomized to receive this agent by intramuscular injection at doses of 0.1, 0.2, 0.4 ml/m^2/day with subsequent patients receiving 0.8 or 1.2 ml/m^2/day sequentially. Eighteen patients were enrolled and completed at least one treatment cycle of three weeks. Most patients developed mild injection site pain (78%). Other toxicities included fatigue, nausea, and dyspnea. Traditional dose limiting toxicity was not seen, but the MTD was defined by injection volume as 0.8 ml/m^2/day. No objective anti-tumor responses were seen. Anvirzel™ can be safely administered at doses up to 1.2 ml/m^2/day, with the amount administered intramuscularly limited by volume. The recommended phase II dose level is 0.8 ml/m^2/day.

Keywords Advanced malignancy · Oleandrin · Cardiac glycosides · Antineoplastic activity

T. Mekhail (✉) · R. Ganapathi · G. T. Budd · P. Elson · R. M. Bukowski
Taussig Cancer Center, Cleveland Clinic Foundation, Cleveland, Case Comprehensive Cancer Center,
9500 Euclid Ave., Cleveland, OH 44195
e-mail: mekhait@ccf.org
Tel.: 216-445-1785
Fax: 216 444-9464

H. Kaur
Ireland Cancer Center, University Hospitals, Case Comprehensive Cancer Center

Introduction

Plant extracts are frequently evaluated as potential anticancer agents. Generally, active constituents are isolated and examined for their biologic properties. Given the complexity of botanical products, whole plant extracts are commonly employed.

Anvirzel™ is a sterile filtered, lyophilized hot water extract of the plant Nerium oleander. This is an ornamental plant with evergreen-like leaves and colored flowers that is indigenous to the Indo-Pakistan subcontinent [1]. This botanical has been utilized for medicinal purposes in Mediterranean and South Asian countries. Conditions treated with extracts of this plant include dermatitis, superficial skin tumors and congestive heart failure [2].

The aqueous extract (Anvirzel™) contains a variety of cardiac glycosides including oleandrin, odorside, neritaloside and the aglycone oleandrigenin [3, 4]. Recently, a LC/tandem mass spectrometry method has been developed for the characterization and quantification of these glycosides and their detection in human plasma following an intramuscular injection of Anvirzel™ [5]. Two of the glycosides in Anvirzel™, Oleandrin and Oleandrigenin inhibit the catalytic activity on the Na, K-ATPase pump and may therefore inhibit fibroblast growth factor-2 (FGF-2) export [6, 7]. Smith et al. [6] showed the inhibition of FGF-2 export in prostate cancer cell lines in a concentration and time-dependent manner. Pathak et al. [8] demonstrated cytotoxic activity associated with Anvirzel™ and oleandrin in human cancer cells, with the latter being more potent. Oleandrin has been shown to block tumor necrosis factor (TNF)-induced activation of nuclear transcription factor-B (NFκ-B), Activa-

tor Protein-1 (AP-1), and related kinases in a variety of cell lines, and induce apoptosis in human prostate adenocarcinoma cell lines [2, 9]. Suppression of NFκ-B and AP-1 may be the molecular basis for the activities of oleandrin including inhibition of tumor promotion and progression. Finally, anecdotal reports from Ireland and Turkey have suggested administration of Anvirzel™ to patients with malignancies was associated with improvement. In view of these data, and interest in this extract as a potential anticancer agent, a phase I clinical trial in patients with refractory malignancies was conducted. No significant toxicity was noted, and the dose administered was limited by volume. No evidence of antitumor activity was found.

Patients and methods

Drug

Anvirzel™ was supplied as a sterile powder in 10 mL butyl-rubber stoppered vials by Ozelle Pharmaceuticals, Inc. (Anvirzel was manufactured by Ozell pharmaceuticals 11825 1H10 west, suite 213, San Antonio TX 78230). Each vial contained 150 mg of Nerium oleander extract, 500 mg mannitol, 50 mg sodium ascorbate, 2 mg ascorbic acid, 10 mg methylparaben sodium and 1 mg propylparaben sodium. Standardization and Quality Control were performed by the Ozell pharmaceuticals. Studies were undertaken to determine the reproducibility of Oleander extract preparations.

The product was reconstituted by aseptically adding 10 mL of Bacteriostatic Water for Injection, USP and gently swirling the contents. A 0.5 mL dose of Anvirzel™ contained 7.5 mg of oleander extract. This volume of Anvirzel™contains 10 to 20 μg of oleandrin, and 25 to 55 ug of oleandrigenin (Investigator Brochure).

This agent was administered daily as an intramuscular (IM) injection, with patient instruction permitting self-administration.

Study design and dose selection

Based on previous experience the maximum tolerated dose (MTD) of Anvirzel was expected to be $\geq$1.0 ml/day, however, Anvirzel™ had not previously been studied in a controlled clinical trial and therefore, to allow for the possibility of toxicity at lower doses, nine patients were initially randomized to receive 0.1, 0.2, or 0.4 ml/m^2/day as a single injection. If no dose limiting toxicity was observed among these patients dose escalation in increments of 0.4 ml/m^2/day was carried out until dose limiting toxicity was observed in $\geq$2 of 3–6 patients. Patients at these higher doses received two separate injections (secondary to volume) of Anvirzel™. Patients experiencing $\geq$ Grade 2 toxicity continued Anvirzel™ with a 50% dose reduction. The maximum tolerated dose (MTD) was defined as the dose of Anvirzel™ that produced $\geq$ Grade 3 toxicity (NCI Common Toxicity Criteria, Version 2) in $\geq$2 of 3–6 patients during first cycle of treatment. Anecdotal reports of Anvirzel™ activity used the intramuscular route of administration. The same route was therefore used for this study. It was anticipated that since the intramuscular route of administration was being utilized, that volumes required at higher levels would produce pain at local injection sites, and would probably limit drug administration. Standard WHO response criteria were utilized [10]. In responding and stable patients, treatment was continued for a maximum of 8 cycles (six months).

Patient eligibility

Patients with histologically proven advanced solid tumors refractory to standard therapy were eligible. There was no specific required time that must have elapsed since prior chemotherapy or radiotherapy, but recovery from toxicities of prior treatment was required ($\leq$ Grade 2, NCI CTC version 2). Patients with either measurable (WHO criteria [10]) or evaluable disease were eligible. Eligibility criteria also include: age $\geq$18 years, Zubrod performance status $\leq$2, and an expected survival of $>$6 weeks. Adequate hematological status defined as a hemoglobin $\geq$9.0 gm/dl, white blood cell count $\geq$3000/ mm^3, absolute neutrophil count $\geq$1200/ mm^3, and platelets $\geq$100,000/ mm^3 was required. Adequate renal and hepatic function were also required and defined as a serum creatinine of $\leq$2.5 mg/dL, a calculated creatinine clearance $\geq$30 mL/min, and a total serum bilirubin $\leq$2.0 mg/dL. Normal serum sodium (132–148 mmol(L), potassium (3.5–5 mmol/L), calcium (8.5–10.5 mg/dL), magnesium (1.6–2.0 mg(dL), and albumin (3.5–5 g/dL) were also required.

Exclusion criteria included: symptomatic and untreated central nervous system (CNS) metastases or any serious intercurrent illnesses. The following cardiac conditions precluded entry: a history of Wolff-Parkinson-White Syndrome, myocardial infarction within 6 months of study entry, unstable, crescendo, or angina at rest, pacemaker placement, history of atrial fibrillation, sustained or symptomatic unsustained ventricular tachycardia, atrial-ventricular (AV) heart block greater than first degree, and a history of digitalis toxicity. Patients receiving concurrent chemotherapy or radiotherapy were ineligible. Patients receiving the following medications were also ineligible: other investigational drugs, digoxin/digitoxin, verapamil, amiodarone, propafenone, indomethacin, itraconazole, or alprazolam. Patients receiving concurrent hormonal therapy were excluded unless it had been administered continuously for at least 3 months with objective evidence of tumor progression. Pregnant or lactating women, or those refusing to utilize contraception while

on study and for 1 year afterward were ineligible. Informed consent in accordance with Food and Drug Administration (FDA) and institutional guidelines (Institutional Review Board) as obtained in all patients.

Study criteria

Baseline studies included a complete medical history, physical examination, performance status, 24-h Holter monitor, complete metabolic panel (serum sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose, bilirubin, total protein, albumin, alkaline phosphatase, alanine aminotransferase) aspartate aminotransferase, complete blood count (hemoglobin, white blood cell count, platelets and differential), urinalysis, 24 h creatinine clearance, electrocardiogram (EKG) with one minute rhythm strip, chest radiograph, and imaging studies as appropriate to determine disease status. Patients were evaluated daily during week 1 of cycle 1, then on days 8 and 15. Patients were then seen every 3 weeks (day 1 of each cycle). Follow up studies included a complete blood count, urinalysis and a complete metabolic panel on day 1 of each cycle and at completion of treatment. A 24-Holter monitor was required at the end of week 3 (cycle 1) and at the last visit. EKGs were obtained at baseline, week 1, 3, 12, and at the last visit. Evaluable or measurable disease (WHO criteria), were required. Chest radiographs and appropriate imaging studies were obtained after every two cycles and at the time of Anvirzel™ discontinuation to determine disease status.

Results

Eighteen patients were enrolled between April, 2000 and January, 2001, and all were eligible. Nine patients were randomized to receive, either 0.1, 0.2, or 0.4 ml/m^2/day (Group 1), six patients received 0.8 ml/m^2/day (Group 2), and three 1.2-ml/m^2/day of Anvirzel™ (Group 3). All patients received at least one treatment cycle, with a median of 3 cycles (range 1–8) administered. Three patients (two with renal cell and one with medullary thyroid cancer) completed 8 cycles. Patient characteristics are outlined in Table 1. Seventeen of eighteen patients had received prior chemotherapy (94%) and 50% had either colorectal or renal cell carcinoma. The one individual with medullary carcinoma of the thyroid, was previously treated, and had stable disease after 6 months (8 cycles) of Anvirzel™ (0.8 mL/m^2/day).

The toxicity seen is summarized in Table 2. The most common side effect was injection site pain. Injection site pain was generally mild (78%), but in two patients (11%) receiving 0.2 ml/m^2 and 0.8 ml/m^2 of Anvirzel™ was reported as moderate. This resolved without sequelae. Six patients (33%) also reported transient erythema at the site of Anvirzel™ injection. The majority of patients (67%) noted mild to moderate fatigue; one patient experienced severe fatigue. Chills were seen in ten patients (56%), and were more common at the higher dose levels of Anvirzel™ (see Table 2). Gastrointestinal toxicity seen was also mild to moderate (Grade 1 and 2) in severity, and included nausea, vomiting and diarrhea. Gastrointestinal symptoms were most common in patients receiving 0.8 and 1.2 ml/m^2/day of Anvirzel™, occurring in 89% and 55% of these patients, respectively.

Table 1 Patient Characteristics

No. patients	18
Sex (male/female)	9/9
P.S. 0/1/2	6/8/4
Median age (range)	56.7 (18–72) years
Prior therapy	
Chemotherapy	17/18
Radiation therapy	9/18
Diagnosis	
Colorectal cancer	5
Renal cell carcinoma	4
Ovarian cancer	2
Miscellaneous	7 *

*Miscellaneous included on bladder (1), melanoma (1), non-small cell lung carcinoma (1), and others (4).

Given the potential cardiotoxicity of Anvirzel™, close monitoring of cardiac symptoms and EKG tracings was required. Three patients had non-specific T-wave changes on their base line EKG. Their EKGs were not changed during treatment. One patient had asymptomatic complete right bundle branch block at baseline, and two patients had left anterior hemiblock, one of these patients had transient interventricular conduction delays. None of these abnormalities were changed during treatment. One patient with base line normal sinus rhythm, left atrial enlargement and rare ventricular ectopic and supraventericular beats at baseline, reported an episode of palpitation two weeks after starting treatment. This patient underwent cardiac evaluation including repeat EKG and Holter monitor. No documentation of any new abnormality was obtained. Echocardiogram was normal.

One patient receiving 0.8 ml/m^2/day developed left ventricular hypertrophy with secondary repolarization of uncertain significance. This patient was a 45 year old patient with recurrent germ cell tumor who had previously received radiation treatment to the mediastinum. He had coronary artery disease at the age of forty, thought to be secondary to the prior radiation. He underwent coronary artery bypass graft five years prior to study enrollment. His base-line EKG showed normal sinus rhythm, left atrial enlargement and myocardial changes. Baseline Holter monitor revealed isolated rare ventricular ectopic beats and rare

Table 2 Toxicity of Anvirzel™

		Group 1		Group 2	Group 3
Dose Anvirzel™ mL/m²/day	0.1	0.2	0.4	0.8	1.2
No. of patients	3	3	3	6	3
Injection site pain					
Grade 1	3	2	3	4	2
Grade 2	0	1	0	1	0
Injection site erythema					
Grade 1	0	0	1	2	3
Fatigue					
Grade 1–2	2	2	3	3	2
Grade 3	0	1	0	0	0
Chills and Rigors					
Grade 1–2	0	0	2	6	2
Nausea					
Grade 1–2	1	2	0	2	2
Grade 3	0	0	0	1*	0
Vomiting					
Grade 1	0	2	0	1	2
Diarrhea					
Grade 1	0	2	0	1	0
Constipation					
Grade 1	1	0	0	4	1
Atrioventricular block					
Grade 3	0	0	0	1*#	0
Dyspnea	1	1	0	4	0

*Same patient

#Grade 3 Complete right bundle branch block was present at base line a did not changed during treatment.

ectopic supra-ventricular beats. EKG changes occurred at the end of first cycle of treatment. The patient developed no clinical evidence of congestive heart failure and his echocardiogram showed normal ejection fraction. This finding resulted in six patients being treated at 0.8 ml/m²/day of Anvirzel™.

A traditional MTD was not reached. The volume required for injection of Anvirzel™ at 1.2 ml/m² was generally over 2.0 ml. Since only a single formulation was available, and I.M. injection of volumes over 3.0 ml would not be possible, the study was terminated. Overall, Anvirzel™ was well tolerated and had an acceptable safety profile.

Eighteen patients were evaluable for response. No evidence of tumor regression was seen. Three patients (renal cell carcinoma—two, medullary thyroid carcinoma—one) had prolonged stable disease (>six months), and completed eight cycles of therapy. All three patients had evidence of progressive disease prior to study enrollment. Additionally, the performance status of treated patients remained stable during the trial.

Discussion

The purpose of the current trial was to investigate the toxicity of Anvirzel™ and determine the MTD in patients with advanced and refractory solid tumors. Our results demonstrate this plant extract is well tolerated, and that dose escalation was limited by the injection volumes required. Patients on the current trial experienced only mild to moderate side effects. One patient developed severe fatigue that was felt to be secondary to disease progression. The gastrointestinal symptoms seen, appeared more common at the two highest dose levels of Anvirzel™ utilized.

Oleander ingestion by humans [1, 11] has been reported to produce cardiac toxicity such as bradycardia and be associated with nausea, vomiting and abdominal pain. These effects are thought to be secondary to the glycoside oleandrin present in this plant. Two oleander glycosides have been detected in Anvirzel™ [6]. They include oleandrin and oleandrigenin, both of which may have cytotoxic effects *in vitro* [8]. In the current trial, cardiac monitoring was performed with EKG and Holter monitoring. No significant toxicity was found, however, one patient receiving 0.8 ml/m²/day developed left ventricular hypertrophy with secondary repolarization of uncertain significance. The majority of side effects reported by patients, were mild and may have in part been secondary to underlying disease. The potential anti-tumor effects of Anvirzel™ have been attributed to a variety of underlying mechanisms, and related to the effects of oleandrin and/or oleandrigenin. These have included growth factor

inhibition [6], direct cytotoxic effects [8], inhibition of NFκB [2, 9] or induction of apoptosis [2, 9]. Anti-tumor effects and symptomatic improvement were not seen in the current trial. The population of patients treated was quite varied, and had significant amounts of prior therapy. The current trial does however demonstrate that this agent is well tolerated and produces only mild toxicity.

Clinical evaluation of natural products for anti-neoplastic activity and toxicity is complex. In the current trial, drug administration was limited by its formulation and the intramuscular route. Pharmacokinetic studies when the active ingredient is known can provide additional information. Anvirzel™ is a complex agent with several possible cardiac glycosides present. Preliminary studies to determine oleandrin and olenadrigenin levels in selected patients receiving Anvirzel were conducted. Detectable levels of both glycosides were found in patients receiving from 0.1 to 0.4 mL/m^2/day (data not shown). Detailed pharmacokinetics, however were not performed by the sponsor of the study.

In conclusion, the current trial demonstrated Anvirzel™ can be safely administered to patients with solid tumors. No objective tumor regressions were seen, however the patient population treated represented individuals with refractory disease. Antitumor activity may be demonstrated in less heavily pretreated patients. The recommended dose for phase II studies is 0.8 ml/m^2/day based on injection volume.

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References

1. Langford SD, Boor PJ (1996) Oleander toxicity: An examination of human and animal toxic exposures. Toxicology 109(1):1–13
2. Manna SK, Sah NK, Newman R, Cismerps A, Aggarwal BB (2000) Oleandrin suppresses activation of nuclear transcription of factor-B, activator protein-2 and c-Jun NH2-terminal kinase. Cancer Res 60:3838–3847
3. Abe F, Yamaguchi T (1978) Digitoxigenin oleandroside and 5α-Adynerin in the leaves of Nerium odorum. Chem Pharm Bull 26:3023–3027
4. Abe F, Yamaguchi T (1979) Oleasides-novel cardenolies with an unusual framework on Nerium. Chem Pharm Bull 27:1604–1610
5. Wang X, Plomley JB, Newman RA, Cisneros A (2000) LC(MS(MS analyses of an oleander extract for cancer treatment. Anal Chem 72(15):3547–3552
6. Smith JA, Madden T, Vijjeswarapu M, Newman RA (2001) Inhibition of export of fibroblast growth factor-2 (FGF-2) from the prostate cancer cell lines PC3 and DU 145 by Anvirzel and its cardiac glycoside component, oleandrin. Biochem Pharmacol 62(4):469–472
7. Jortani SA, Helm RA, Valdes R (1996) Inhibition of Na,K-ATPase by oleandrin and oleandrigenin, and their detection by digoxin immunoassays. Clin Chem 42:1654–1658
8. Pathak S, Multani AS, Narayan S, Kumar V, Newman RA (2000) Anvirzel, an extract of Nerium oleander, induces cell death in human but not murine cancer cells. Anticancer Drugs 11(6):455–463
9. McConkey DJ, Lin Y, Nutt LK, Ozel HZ, Newman RA (2000) Cardiac glycosides stimulate Ca increases and apoptosis in androgen-independent, metastatic human prostate adenocarcinoma cells. Cancer Res 60(14):3807–3812
10. WHO (1979) Handbook for Reporting Results for Cancer Therapy. WHO, Geneva
11. Gupta A, Joshi P, Jortani SA, Valdes R Jr, Thorkelsson T, Verjee Z, Shemie S (1997) A case of nondigitalis cardiac glycoside toxicity. The Drug Monit 19(6):711–714