PHASE I STUDIES

Phase I trial of vinflunine and pemetrexed in refractory solid tumors

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Summary *Background* Vinflunine is a novel vinca alkaloid with promising single agent clinical activity. Pemetrexed has at least additive activity with other vincas. A phase I trial was undertaken to assess the safety of vinflunine and pemetrexed in patients with refractory solid tumors. *Methods* A standard 3-patient cohort dose escalation scheme was used to determine the dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) of the vinflunine/pemetrexed combination. Pemetrexed 500 mg/m^2 was given with vinflunine 280 mg/m^2 (cohort 1), 300 mg/m^2 (cohort 2) or 320 mg/m^2 (cohort 3) on day 1 of a 21-day cycle. *Results* 19 patients were enrolled, median age 58 years (range 32 to 77) and had a median of 3 (range 1–6) prior therapies. DLT occured 1 of 6 pts in cohort 1 (thrombocytopenia, hyponatremia), 2 of 10 pts in cohort 2 (febrile neutropenia, hyponatremia, hyperbilirubinema; febrile neutropenia), and 2 of 3 pts in cohort 3 (febrile neutropenia, hypokalemia; febrile neutropenia). 1 pt in cohort 2 died prior to completion of cycle 1 likely from disease progression. Most common grade 3/4 adverse events were neutropenia (7), leukopenia (5). Febrile neutropenia occurred in 4 patients (21%). No objective responses were seen. Two patients (breast and lung) had prolonged stable disease for 25 and 20 cycles respectively. *Conclusions* Based on this experience we recommend vinflunine 300 mg/m^2 and pemetrexed 500 mg/m^2 in combination every 3 weeks for future study. At these doses, the combination of vinflunine and pemetrexed was tolerable in this heavily pretreated population. Hematologic toxicity, including febrile neutropenia, was prominent.

Keywords Phase I · Vinflunine · Pemetrexed · Vinca alkaloid

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Introduction

Vinflunine is a novel, synthetic vinca alkaloid with promising preclinical and early clinical activity for solid tumors, including lung cancer. Like other drugs in its class, vinflunine's anticancer activity results disruption of microtubule dynamics leading to G2+M phase arrest and subsequent apoptosis. A lower affinity for tubulin compared with other vincas, however, may result in a lower rate of neurotoxicity for vinflunine than other vincas [1].

In preclinical studies, vinflunine has shown activity in against lung, colon, prostate, breast, ovarian, and bladder cancers [2]. As a single agent, vinflunine has demonstrated activity in anthracycline and taxane pre-treated breast cancer [3], platinum-refractory bladder cancer [4], mesothelioma

[5], and as second-line therapy of non-small cell lung cancer [6]. The principal toxicities of single agent vinflunine are hematologic toxicity, principally neutropenia [7]. Constipation and mucositis also have been frequently reported.

Pemetrexed is a multitargeted folate antagonist with Food and Drug Administration (FDA) approval for use in malignant pleural mesothelioma, and in combination with cisplatin for first-line therapy and as single agent as maintenance and second-line therapy of non-small cell lung cancer (NSCLC). Pemetrexed also has clinical activity against a variety of other cancers including colorectal, breast, bladder, and pancreas cancers [8]. In the early experience with pemetrexed, hematologic toxicity was dose limiting and profound. However, since routine supplementation with vitamin B12 and folate in patients treated with pemetrexed has been instituted, hematologic toxicity is modest, with a 5% incidence of grade 3 or 4 neutropenia observed in a phase III trial comparing pemetrexed and docetaxel in second-line therapy of non-small cell lung cancer [9].

Thus, vinflunine and pemetrexed appear to offer overlapping spectra of activity with differing toxicity. Furthermore, in xenograft models pemetrexed has shown at least additive activity with vinorelbine [10], suggesting combinations with vincas may provide substantial clinical activity. Given that pemetrexed is a substrate for the multi-drug resistance transporter [8], combining it with vinflunine, a drug with low levels of MDR induction [1], may offer enhanced activity.

We undertook a phase I trial of the combination of vinflunine and pemetrexed in patients with refractory solid tumors to assess the safety of this combination for further testing.

Methods

Eligibility

Patients aged 18 or older with histologically confirmed solid tumors refractory to standard therapies, or for which no standard therapy exists, were eligible for enrollment on this phase I trial. Patients were also required to meet the following criteria: >3 month life expectancy; Eastern Cooperative Oncology Group (ECOG) performance status 0–2; measurable or evaluable disease by Response Evaluation Criteria in Solid Tumors (RECIST); at least 4 weeks from chemotherapy, investigational agent, or surgery; and adequate organ function (bilirubin≤1.5 mg/dL; transaminases≤3 times upper limit of normal (ULN), or≤5 times ULN if known liver metastasis; creatinine clearance≥60 ml/min by Cockcroft-Gault; platelet≥ 100,000/μL; absolute neutrophil count ≥ 1500/μL). Patients with creatinine clearance between 45 and 80 mg/min were

instructed to hold NSAID therapy. Patients with brain metastases were permitted only if they had undergone CNS directed therapy at least 3 months earlier and had both clinically and radiographically stable disease for at least 8 weeks.

Because of potential interaction with vinflunine, P450 CYP3A4 inducing or inhibiting drugs had to be discontinued or patients had to be on stable doses for a minimum of 2 weeks prior to enrollment; strong inhibitors of P450 CPY3A4 (e.g. ketoconazole, ritonovir) were not permitted. To ensure safety with pemetrexed, patients with clinically significant pleural effusion or ascites not amenable to drainage were excluded. Other exclusion criteria included: prior allergic reaction to a vinca alkaloid; New York Heart Association class III or IV heart failure, unstable angina, or myocardial infarction within 6 months; poorly controlled hypertension; uncontrolled infection or severe illness; pregnancy, refusal to use contraception, or breastfeeding.

All patients gave written informed consent prior to any study procedures or treatment. This study was approved by the Institutional Review Board at the University of North Carolina at Chapel Hill.

Trial design

Dose escalation proceeded according to a standard 3-patient cohort dose escalation schema in which three patients are enrolled at each dose level. If none of these three patients experiences a DLT, the three subsequent patients are enrolled at the next higher dose level. However, if one of the first three patients enrolled at any dose level experiences a dose limiting toxicity, then three additional patients are enrolled at that same dose level. If one of these additional patients experiences a DLT (or 2 of 6) then this dose level has exceeded the maximally tolerated dose (MTD). The MTD and the dose recommended for phase II trials is then defined as the dose level below which one third of patients experience dose limiting toxicity. In this study, an additional four patients were enrolled to the MTD to further assess safety at this dose level.

Dosing and administration

Dose level 1 consisted of pemetrexed 500 mg/m^2 and vinflunine 280 mg/m^2 IV on day 1 of every 21 day cycle. Dose level 2 consisted of pememtrexed 500 mg/m^2 and vinflunine 300 mg/m^2, and dose level 3 pemetrexed 500 mg/m^2 and vinflunine 320 mg/m^2. Pemetrexed was diluted in mL 0.9% NaCl and given IV over 10 min followed by vinflunine as an infusion of 500 mL of 0.9% NaCl over 20 min intravenously.

Dexamethasone 4 mg PO BID was given the days before, of, and after pemetrexed. All patients also received

supplementation with vitamin B12 (1000 mcg IM beginning within 14 days of the first pemetrexed dose then every 3 cycles) and folate (350–1000 mg PO daily beginning 7 days before the first pemetrexed dose and continuing until 21 days after the final dose). All patients received ondansetron 24 mg PO before chemotherapy on day 1 of each cycle.

Toxicity and dose modification

Toxicity was graded according to the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events version 3.0 (CTCAE v3). Dose limiting toxicities were defined as any of the following occurring during cycle one of treatment: any grade 3 or 4 toxicity except fatigue and constipation; grade 3 or 4 constipation lasting≥7 days; grade 3 rash unresponsive to supportive care; grade 4 fatigue; ANC $<0.5\times10^9$ cells/L lasting >5 days or associated with fever or infection; platelet count $<25\times10^9$ cells/L of any duration or $<50\times10^9$ cells/L with major bleeding. After a DLT, treatment was held until toxicity had resolved to entry criteria. Treatment was resumed with vinflunine at the next lower dose level and pemetrexed at 75% of the original dose. For grade 3 or 4 non-hematologic toxicity and grade 4 hematologic toxicity occurring in subsequent cycles, pemetrexed and vinflunine were resumed at 75% of the prior dose. Toxicities not resolving within 14 days from the planned start of the subsequent cycle resulted in removal from study. Two dose reductions were permitted before a patient was removed from study.

Toxicity assessment, including laboratory work, in cycle one occurred on day 8, 15 and day 22/day 1 of cycle two. In subsequent cycles, toxicity assessment was performed prior to each treatment, every 21 days. Tumors were assessed for response every 2 cycles with computed tomography (CT) or magnetic resonance imaging (MRI), and categorized according to the RECIST criteria. Treatment was continued until evidence of disease progression, toxicity, patient preference, or investigator's judgment.

CYP3A4 phenotyping

Vinflunine is a liver cytochrome P450 CYP3A4 substrate. Given the known interpatient heterogeneity of CYP3A4 phenotype, we sought to explore the relationship between CYP3A4 activity and vinflunine toxicity. Midazolam clearance has been used by others as a surrogate marker of CYP3A4 activity [11]. As such, a midazolam pharmacokinetic study was conducted in conjunction with vinflunine administration as follows. Vinflunine was infused over 20 min followed immediately by an intravenous 1 mg bolus dose of midazolam over 1 min, with the line flushed between administrations in 12 patients studied. In the

remaining 7 patients, the midazolam probe dose and sampling began 3 h prior to vinflunine dosing.

Venous blood samples (5 mL) were collected from patients prior to midazolam and at 5 min, 30 min, 1, 2, 3, 4, 6, and 8 h after the end of the midazolam infusion. Samples were collected in EDTA tubes, centrifuged to separate plasma, and stored at −80 C until plasma analysis. Sampling was also performed for planned vinflunine pharmacokinetic analysis, however due to errors in processing, the samples could not be assayed by available techniques.

Plasma concentrations of midazolam were determined using a modified, validated reverse-phase HPLC method with mass spectrometric detection as previously described [12].

Individual midazolam plasma concentrations were used to estimate the following pharmacokinetic parameters using WinNonlin 4.0 (Pharsight Corp., Mountain View, CA): area under the concentration-time curve through infinity (AUC), area under the concentration time curve through the last measurable time point (AUC_t); and whole blood clearance (Cl). Association between midazolam clearance (e.g. CYP3A4 activity) and cycle one nadir neutrophil count is explored using Spearman's rank correlation for nonparametric data.

Results

Nineteen patients were treated with the combination of vinflunine and pemetrexed. Median age of the patients was 58 years (range 32–77) (Table 1). Eight patients had colorectal cancer, 3 breast, 2 each had lung, cholangiocarcinoma, and pancreas cancer, and one patient sarcoma. Patients had received a median of 3 prior therapies (range 1–6). All but one patient had an ECOG PS of 0 or 1.

A total of 93 cycles were administered. DLT occurred in 1 of 6 patients treated at dose level 1 (grade 4 platelets and grade 3 hyponatremia) (Table 2). The first patient treated at dose level 2 suffered progressive anorexia and asthenia and died from presumed disease progression. Though not considered a DLT, the dose level was expanded to 6, and none of the additional patients had DLT. Two of 3 patients treated at dose level 3 experienced DLT (grade 4 hypokalemia and febrile neutropenia; febrile neutropenia). To further explore safety, an additional 4 patients were enrolled to dose level 2, 2 of whom experienced DLT (febrile neutropenia with hypotension, grade 3 hyponatremia and hyperbilirubinemia; febrile neutropenia) for a total of 2 DLTs in 10 patients at vinflunine 300 mg/m^2 and pemetrexed 500 mg/m^2.

The most commonly experienced toxicities were leukopenia ($n=14$, 74%) and neutropenia ($n=10$, 53%) (Table 3). Constipation, fatigue, and anemia all occurred in 9 patients

Table 1 Patient characteristics, $N=19$

Median age	58 (range 32–77)
Sex (n)	
male	10
female	9
Cancer type (n)	
breast	3
colon	8
lung	2
pancreas	2
cholangiocarcinoma	2
sarcoma	1
Median # prior therapies	3 (range 1–6)
ECOG PS (n)	
0	9
1	9
2	1

(47%). The most common grade 3/4 toxicities were neutropenia (7, 37%), leukopenia (5, 26%), and febrile neutropenia (4, 21%). Grade 3 hyponatremia and abdominal pain occurred in 2 patients and grade 4 hypokalemia occurred in 2 patients. Noted vinca toxicities of constipation and peripheral neuropathy were generally mild. Of nine patients with constipation, only one patient experienced grade 3 constipation. Only two patients experienced any neuropathy, both a grade 2. Mucositis, a common adverse effect of pemetrexed, was seen in 5 patients. All mucositis events were grade 1 or 2.

Efficacy

No objective responses were seen. Two patients, however, experienced prolonged stable disease. One patient with stage IV breast cancer who had received prior taxane and anthracycline therapy had stable disease for 25 cycles. She

Table 2 Dose limiting toxicities

Dose level	# DLTs	DLT type

1, $n=6$
  vinflunine 280 mg/m^2
  pemetrexed 500 mg/m^2
	1	1. Grade 4 thrombocytopenia+
		  grade 3 hyponatremia

2, $n=10$
  vinflunine 300 mg/m^2
  pemetrexed 500 mg/m^2
	2	1. Grade 4 febrile neutropenia,
		  grade 3 hyponatremia, grade
		  3 hyperbilirubinemia
		2. Grade 3 febrile neutropenia

3, $n=3$
  vinflunine 320 mg/m^2
  pemetrexed 500 mg/m^2
	2	1. Grade 3 febrile neutropenia,
		  grade 4 hypokalemia
		2. Grade 3 febrile neutropenia

Table 3 Most commonly experienced adverse effects

AE	All Grades Number (%)	Grade 3 Number	Grade 4 Number
A. Adverse effects per patient. $N=19$ patients			
Leukopenia	14 (74%)	3	2
Neutropenia	10 (53%)	4	3
Fatigue	9 (47%)	1	0
Hemoglobin	9 (47%)	0	0
Constipation	9 (47%)	1	0
Hyponatremia	8 (42%)	2	0
Nausea	7 (37%)	0	0
ALT elevation	7 (37%)	1	0
Hypokalemia	6 (32%)	0	2
Mucositis	5 (26%)	0	0
AST elevation	5 (26%)	0	0
Platelets	5 (26%)	0	1
Vomiting	5 (26%)	0	0
Hyperbilirubinemia	4 (21%)	1	0
Febrile neutropenia	4 (21%)	3	1
Abdominal pain	4 (21%)	2	0
Mouth/jaw pain	4 (21%)	0	0
Anorexia	3 (16%)	1	0
Hyperglycemia	3 (16%)	0	0
ALK elevation	3 (16%)	0	0
Rash	3 (16%)	0	0
Sensory Neuropathy	2 (11%)	0	0
Bone pain	2 (11%)	0	0
B. Adverse effects per cycle. $N=93$ administered cycles.			
Leukopenia	19 (20%)	5	2
Fatigue	17 (18%)	1	0
Hemoglobin	17 (18%)	0	0
Neutropenia	14 (15%)	5	3
AST elevation	13 (14%)	0	0
Constipation	12 (13%)	1	0
ALT elevation	11 (12%)	1	0
Hyponatremia	10 (11%)	3	0
Nausea	8 (9%)	0	0
Abdominal pain	8 (9%)	3	0
Hypokalemia	6 (6%)	0	2
Thrombocytopenia	6 (6%)	1	1
Vomiting	6 (6%)	0	0
Hyperbilirubinemia	5 (5%)	1	0
Mucositis—oral	5 (5%)	0	0
Febrile Neutropenia	4 (4%)	3	1
ALK Elevation	4 (4%)	0	0
Anorexia	3 (3%)	1	0
Hyperglycemia	3 (3%)	0	0

withdrew from study because of persistent fatigue and myalgias. Another patient with stage IV lung cancer and treated brain metastases who had received four prior lines of therapy had stable disease for 20 cycles. She withdrew from study because of cumulative fatigue without evidence of disease progression. Of the 19 study patients reasons for withdraw were progressive disease (12), toxicity (5), transportation (1), physician discretion (1).

CYP3A4 phenotyping

Midazolam clearance was measured in all patients as a surrogate for CYP3A4 phenotypic activity. Nadir ANC was available for 15 of 17 patients. Nadir ANC was weakly correlated with midazolam clearance: there was a trend towards higher nadir ANC in patients with faster midazolam clearance (i.e. those with more active CYP3A4), Spearman rho=0.24 (Fig. 1).

Conclusions

In this phase I study of the combination of vinflunine and pemetrexed, hematologic toxicity was dose-limiting in some patients at all dose levels. Febrile neutropenia was common, occurring in 21% of patients. Severe electrolyte abnormalities, including hyponatremia and hypokalemia, were also common. The maximum tolerated dose recommended for future study is vinflunine 300 mg/m^2 and pemetrexed 500 mg/m^2 every 21 days.

Hematologic toxicity, as seen in our trial, has been the most prominent adverse effect reported with single agent

Fig. 1 Midazolam clearance and nadir ANC, n=15. Spearman rank correlation 0.24, p=0.4

vinflunine. In the largest phase II study of vinflunine at 320 mg/m^2 in patients with advanced transitional cell carcinoma of the bladder, 67% experienced grade 3 or 4 neutropenia, and 10% had febrile neutropenia [4]. Similar rates of grade 3/4 neutropenia (65% and 71%), but lower rates of febrile neutropenia (5% and 0%) were seen in the phase II refractory breast cancer experience [3, 13]. Neutropenia was less common in a phase III study of vinflunine at 320 mg/m^2 compared with docetaxel for second line therapy of nonsmall cell lung cancer in which grade 3/4 neutropenia occurred in 32% of patients, and only 3% had an episode of febrile neutropenia [6].

Combination therapy trials with vinflunine are ongoing, and toxicity might be expected to be more profound than seen in the single agent experience. We combined vinflunine with pemetrexed, a drug known to cause only modest hematologic toxicity when given with vitamin B12 and folate supplementation. With the combination, the rate of febrile neutropenia was quite high, 21%. Our exploratory analysis of CYP3A4 phenotype and nadir ANC found a trend towards lower nadir ANC in patients with slower midazolam clearance. This suggests that hematologic toxicity from vinflunine might correlate with CYP3A4 phenotype. A larger study in which vinflunine pharmacokinetics, CYP3A4 phenotype, and toxicity can be modeled would shed light on whether *a priori* dose adjustments according to CYP3A4 phenotype might avoid the high rate of febrile neutropenia experienced by patients in this phase I trial, and the larger single agent vinflunine experience.

We saw limited clinical activity with the combination of vinflunine and pemetrexed. One patient with breast cancer and one patient with lung cancer, both of whom and had received significant prior therapy, had prolonged stable disease. This suggestion of clinical activity is supported by vinflunine's known activity to date. In the previously mentioned phase III second line non-small cell lung cancer trial, vinflunine resulted in a minimal rate of radiographic response, only 3%, but provided disease control (response + stable disease) in 40% of patients [6]. Response rates to single agent vinflunine in refractory breast cancer and transitional cell carcinoma of the bladder have been better. In two separate phase II trials of women with anthracycline and taxane refractory breast cancer, response rates were 13% and 30% [3, 13], suggesting significant activity. In the largest of the platinum refractory bladder cancer studies, vinflunine produced a partial response in 18% and disease control in 67% of patients [4].

Given this benefit from vinflunine in patients with malignancies generally resistant to most therapies, vinflunine combinations are being further evaluated in bladder cancer, breast cancer, and nonsmall cell lung cancer [1]. We believe the combination of vinflunine and pemetrexed may offer promise in nonsmall cell lung cancer, particularly if
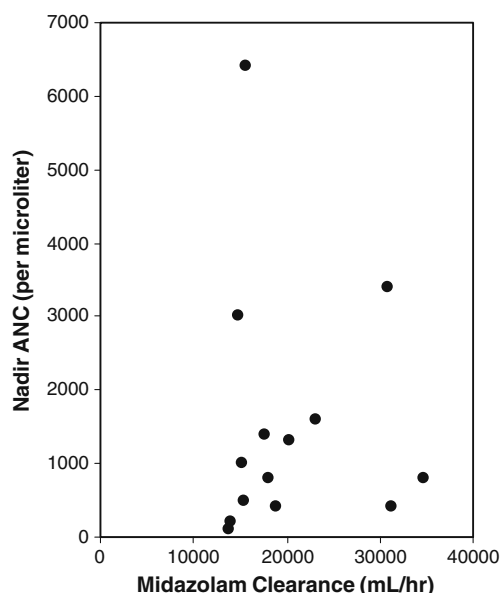
further study of CYP3A4 phenotype were to allow *a priori* dose adjustment to temper the risk of febrile neutropenia.

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