Published in final edited form as:
Cancer Prev Res (Phila). 2009 January ; 2(1): 22–26. doi:10.1158/1940-6207.CAPR-08-0100.

Phase II Chemoprevention Trial with High Dose Fenretinide for Oral Pre-Malignant Lesions

William N. William Jr.[1], **J. Jack Lee**[2], **Scott M. Lippman**[1], **Jack W. Martin**[3], **Hai T. Tran**[1], **Anita L. Sabichi**[4], **Edward S. Kim**[1], **Lei Feng**[2], and **Vassiliki A. Papadimitrakopoulou**[1,5]

[1]Department of Thoracic/Head & Neck Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, TX

[2]Department of Biostatistics, The University of Texas M. D. Anderson Cancer Center, Houston, TX

[3]Department of Dental Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, TX

[4]Clinical Cancer Prevention, The University of Texas M. D. Anderson Cancer Center, Houston, TX

Abstract

Background—In a previous phase II trial, we demonstrated that fenretinide 200 mg/day had limited activity in retinoid-refractory leukoplakia (34% response rate), possibly due to the lack of achievement of high serum levels which would be required to elicit retinoid-receptor independent apoptosis in pre-malignant cells. We therefore designed this single-arm, phase II trial to investigate whether fenretinide at a higher dose would improve the leukoplakia response rate from our previous study's historical control.

Patients and Methods—Patients with high-risk leukoplakia were treated with 4 three-week cycles of fenretinide (900 mg/m^2 orally twice daily, days 1–7). At week 12, objective clinical responses were determined and blood samples were collected for serum drug level assessment. The original sample size was 25 patients to detect a 55% response rate (90% power, one-sided 10% type I error rate). A futility analysis was planned after accrual of the first 16 patients to allow for early trial closure if ≤4 patients responded.

Results—Fenretinide was well tolerated, with only one grade 3 toxicity (diarrhea) observed. However, only 3 of the initial 15 patients (20%) had a partial response, leading to early trial termination due to lack of efficacy. Serum levels of fenretinide rose from 0 (baseline) to 0.122 μM ± 0.093 (week 12), indicating that high serum levels of the drug were achieved during the initial days of the cycle.

Conclusions—Despite high serum levels, fenretinide for oral leukoplakia, at the dose and schedule studied herein, failed to improve historical response rates.

[5]Corresponding author: Department of Thoracic/Head & Neck Medical Oncology, The University of Texas M. D. Anderson Cancer Center 1515, Holcombe Blvd, Box 432 Houston, TX, 77030 Phone: (713) 792-6363 / FAX: (713) 792-1220 vpapadim@mdanderson.org.

Keywords

fenretinide; oral pre-malignant lesion; leukoplakia; chemoprevention

Introduction

Fenretinide is a synthetic retinoid with demonstrated (albeit limited) clinical activity in patients with retinoid-refractory leukoplakia (1). Additionally, at the doses of 200 mg/day, adjuvant fenretinide treatment for one year has recently been demonstrated to prevent new lesions or recurrences in patients with surgically resected leukoplakia in a randomized, placebo-controlled study (2).

The activity of fenretinide in pre-malignat lesions is postulated to occur due to modulation of cell growth and/or differentiation, or induction of apoptosis. A dual, dose-dependant, mechanism of action has been demonstrated to mediate the anti-neoplastic effects of fenretinide – low doses of the drug (i.e., 1 μM) were capable of eliciting cell differentiation in vitro, whereas higher doses (i.e, 3–10 μM) were required for the apoptosis-inducing effects (3–6). Of note, the induction of differentiation was not observed in mutant F9 murine embryonal carcinoma cells lacking expression ofretinoic acid receptor gamma and retinoid X receptor alpha (RAR /RXR $^{-/-}$), while apoptosis was demonstrated following exposure to fenretinide in both wild-type and receptor-negative, mutant cells (3). Taken together, these results indicate that fenretinide-induced apoptosis is independent of retinoid receptors, in contrast to the retinoid receptor-dependant cell differentiation effects.

Based on the aforementioned pre-clinical data, we have previously conducted a phase II clinical trial of fenretinide 200 mg/day for 12 weeks in patients with retinoid-refractory leukoplakia (1). The primary hypothesis of that trial was that through, in part, retinoid receptor-independent mechanisms, a short-term treatment course with fenretinide would be able to eliminate pre-malignant clones via apoptosis, thus producing sustained durable responses on patients with leukoplakia that had never responded to or that had progressed during treatment with a natural retinoid. In that study all clinical responses (observed in 34% of patients) were partial, often short-lived, and correlated with previous response to natural retinoids (1). These results indicated that the activity of fenretinide observed was most likely due to retinoid receptor-mediated mechanisms. Additionally, the serum levels of fenretinide achieved during that study (i.e., 0.230 μM) were at least 10-fold lower than the concentrations required in vitro for induction of apoptosis (i.e., > 3 μM) (6–8), thus corroborating the hypothesis that the limited clinical activity observed with fenretinide 200 mg/day probably did not involve high-dose, retinoid receptor-independent apoptotic effects.

In an ongoing phase I trial at that time, Jasti et al. had demonstrated that the dose of fenretinide of 900 mg/m^2 orally twice daily on days 1–7 every 21 days was tolerable and resulted in peak serum levels of the drug of 3–5 μM (9). Based on the in vitro data described above, fenretinide, at this concentration, would theoretically elicit retinoid receptor-independent apoptosis in oral pre-malignant cells in vivo. Therefore, we designed this single-arm, phase II study to test whether the administration of fenretinide 900 mg/m^2 twice

daily to patients with oral leukoplakia would result in higher response rates than what was observed in our previous study with fenretinide 200 mg/day.

Methods

This was an open-label, single-arm, phase II trial conducted at The University of Texas M. D. Anderson Cancer Center. The study was approved by the Institutional Review Board and was conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines.

Patient Eligibility

Main eligibility criteria included: presence of high risk, bidimensionally measurable or evaluable OPL (as indicated by the presence of dysplasia – any grade, localization to the floor of the mouth, ventrolateral tongue, or soft palate complex, and/or being extensive / symptomatic), adequate liver, kidney and bone marrow function, Zubrod performance status < 2, no history of cancer within the preceding 6 months (except for non-melanoma skin cancer), and no exposure to retinoid or carotenoid supplements within the preceding 3 months.

Treatment Plan

Prior to study entry, patients were required to sign an informed consent statement indicating knowledge of the experimental nature of the treatment. Baseline evaluation included a complete history and physical examination, serum chemistry and hematologic tests, biopsy and measurement of oral lesions. Patients were then treated with four cycles of fenretinide 900 mg/m^2 orally twice daily days 1 through 7, repeated every three weeks. Toxicity during treatment was regularly assessed using the National Cancer Institute Common Toxicity Criteria version 2.0 modified to include retinoid-specific toxicities (10). At the end of the treatment period, oral lesions were measured and biopsied again. Optional blood collection for pharmacokinetics analyses (i.e., measurements of serum levels of fenretinide and its major metabolite, N-(4-methoxyphenyl) retinamide [4-MPR]) were performed at baseline (pre-treatment) and at 14 days after the last dose of fenretinide (week 12). Details of blood sample processing and analysis methodology were previously described (1).

Study Endpoints and Statistical Analysis

The primary endpoint of the study was determination of clinical response rate (complete and partial response) at the end of the 3-month treatment period. Response was evaluated as follows: complete response (CR) - gross inspection revealed no evidence of a lesion; partial response (PR) – the size of a lesion or of the sum of the measurementsof all lesions decreased by at least 50 percent; stable disease (SD) - lesion sizes increased by less than 25percent or decreased by less than 50 percent; disease progression (PD) - increase of at least 25 percent in the sumof the measurements of all lesions or appearance of any new lesion (10). Simon’s minimax two-stage design was applied – we planned to accrue 16 patients in the first stage; if there were 4 or less responders, the study would be terminated and the treatment would be considered inefficacious. If there were 5 or more responders, additional patients would be added to the study in the second stage to achieve a total of 25

patients. At the end of the trial, the treatment was considered to be efficacious if there were 11 or more responders. The study had 90% power with a one-sided 10% type I error rate assuming the null response rate would be 30% and a response rate of 55% or higher would be of interest (25 % improvement over previously reported of approximately 30% with low-dose fenretinde (1)). Additional endpoints included toxicity, pharmacokinetics assessments, and histological response at month 3.

Results

Patient characteristics and treatment

Between February 2003 and April 2004, 21 patients were registered in this study. Six patients were found to be ineligible due to history of cancer within 6 months (3), non-measurable disease (2) or refusal of treatment (1). Therefore, 15 patients initiated and completed treatment with the study drug. Their baseline characteristics are described in Table 1. All 15 patients received the 4 planned cycles of fenretinide with no dose reductions. Self-reported compliance (confirmed by capsule count) was > 90% in 13/15 patients.

Toxicity

Treatment was well tolerated, with only 1 patient experiencing grade 3 toxicity (diarrhea lasting < 24 hours). No grade 4 toxicities were observed. The complete list of adverse events occurring in more than 1 patient (regardless of grade) is outlined in Table 2.

Efficacy

All 15 patients were evaluable for clinical response assessment at the end of 3 months. There were no complete responses. Three patients (20%) had a partial response, whereas stable disease was observed in 11 patients (73%). Only 1 patient (7%) progressed during treatment, in terms of clinical response. Histologically, 2 patients (13%) had downgrading of the dysplasia, 7 patients (47%) had no histological change, and 6 patients (40%) had worsening of the degree of dysplasia. None of the patients developed invasive cancer within the 3-month follow-up period. After the initial 15 patients had been evaluated, we recognized that the pre-specified minimum clinical response rate during the first stage of the trial (i.e., 5 out of 16 patients) would not be achieved. This prompted early termination of the study due to lack of efficacy of the investigational agent.

Fenretinide and 4-MPR serum levels

Fourteen patients had both baseline and post-treatment blood samples collected for analysis of drug serum levels. As expected, fenretinide was undetectable in all 14 samples at baseline. The mean (± standard deviation) serum level of fenretinide increased to 0.122 μM ± 0.093 post-treatment (i.e., 14 days after the last dose of fenretinide). Similarly, 4-MPR (a major fenretinide metabolite) was undetectable in all 14 samples at baseline and increased to 0.643 μM ± 0.398 (mean ± standard deviation) post-treatment.

Discussion

In the present study, we found that high-dose fenretinide (i.e., 900 mg/m^2 twice daily on days 1 through 7 repeated every 21 days for four cycles) for oral leukoplakia was well tolerated, but elicited objective responses in only 3 out of 15 patients (20%). The drug failed to meet the pre-specified criteria for efficacy during the first stage of the study (i.e, 5 responses in the initial 16 patients) and the trial was, therefore, terminated early.

Retinoids are the most widely studied class of agents for prevention of oral pre-malignant lesions. The landmark study by Hong et al. established the activity of high-dose 13-cis retinoic acid in patients with leukoplakia, albeit with intolerable toxicities (11). Attempts at improving the therapeutic index of retinoids with the use of low-dose 13-cis-retinoic acid or retinyl palmitate were unsuccessful, either due to low long-term efficacy (retinyl palmitate) or low long-term tolerance (13-cis-retinoic acid) (10, 12). The development of synthetic retinoid compounds, such as fenretinide, was a promising new strategy to overcome the limitations of previous studies, given their tolerability and (possibly more effective) alternate mechanisms of action; in vitro studies had demonstrated a potential of these agents for overcoming acquired or de novo retinoid-resistance, through both retinoid receptor-dependent and independent effects (3, 13–15). Indeed, promising results were obtained in a randomized study of fenretinide versus placebo in patients with resected leukoplakia, demonstrating fewer relapses or new lesions in patients exposed to the study drug (2). Our phase II study of low-dose fenretinide (i.e., 200 mg/day) in patients with retinoid-refractory leukoplakia indicated that the serum levels of fenretinide achieved were not high enough to elicit retinoid receptor-independent, apoptotic-inducing effects, which might explain the limited clinical activity (i.e., response rates) observed (1). This prompted the design and conduct of the present study, focusing on an alternate dose and schedule.

A number of phase I trials conducted in patients with solid tumors justify the use of fenretinide at higher doses (i.e., 900 mg/m^2 twice daily) (9, 16, 17). In the experience reported by Villablanca et al. in children, for example, the mean peak and trough levels of fenretinide during cycle 1 (at the dose of 1860 mg/m^2/day, on days 1 through 7) were 6.59 and 5.99 μM, respectively (17). Formelli et al. demonstrated that after a single dose of fenretinide of 600 or 1700 mg/m^2, the mean fenretinide maximum serum concentrations (C_{max}) were 2.6 and 4.3 μM, respectively (16). Therefore, the dose of 900 mg/m^2 twice daily for 7 days is adequate to achieve serum levels ≥3 μM for at least 72 hours, which would be considered sufficient to produce retinoid-receptor independent apoptotic effects (6–8), and consequently, elimination of clonal pre-malignant cells. This is further corroborated by the mean fenretinide and 4-MPR levels obtained in the samples from our trial (0.122 and 0.643 μM, respectively). Although these concentrations are lower than previously reported in the literature, the timing of sample collection in our study was different (i.e., 14 days after the last dose of treatment). Based on the reported termination half-life of 14 – 25 hours for fenretinide (16, 18, 19) and assuming linear pharmacokinetics, extrapolation of current data to time of maximal concentration demonstrates that fenretinide levels during the initial days of the cycle in our patient population, are comparable to the reported concentration range achieved with high-dose fenretinide (9, 16, 17).

Despite the administration of high doses of fenretinide, we did not observe improved response rates in this trial, as compared to historical control. In this study, 47% of patients had surgery in the past for treatment of leukoplakia and 40% of patients received retinoids prior to enrolment. This illustrates that the population studied had persistent / recurrent oral pre-malignant lesions, most likely to harbor genetic abnormalities and, conceivably, less likely to regress spontaneously or respond to retinoids. Nonetheless, the primary hypothesis of this study was that high-dose fenretinide would be able to elicit retinoid-receptor independent effects and, therefore, the study drug would theoretically be active even in retinoid-refractory leukoplakia. The reasons for lack of efficacy are unknown, but may be due to a low fenretinide concentration in the target tissue per se. Indeed, although the ratio of tissue / serum levels of fenretinide are usually high following oral administration of the drug, Colombo et al. recently demonstrated that, in ovarian cancer patients, the actual concentration inside the cancer cells are 50-fold lower than the intracellular concentrations found in cells exposed in vitro to the same drug levels (20). Thus, other factors present in vivo may prevent accumulation of the drug in tumor cells, and consequently impair its anti-neoplastic actions.

In summary, fenretinide in the dose and schedule studied herein has not been found to be an effective chemoprevention strategy in patients with oral pre-malignant lesions. Similarly, randomized studies of this agent for chemoprevention of breast and bladder cancer have also failed to demonstrate efficacy (21, 22).

Given the limitations of retinoids for cancer chemoprevention, as illustrated by this and other previous trials (1, 10–12), our group is now focusing on the development of molecular-targeted agents for patients with oral pre-malignant lesions. As an example, we are currently conducting a placebo-controlled randomized study of erlotinib with cancer development as the primary endpoint. These agents might confer a better therapeutic index and could potentially become a useful strategy for chemoprevention in the future (23).

Acknowledgments

The authors are indebted to the assistance provided by the research nurses and data managers. This work was supported by a the National Cancer Institute, Department ofHealth and Human Services grants P01 CA052051 and P30 CA016672(The University of Texas M.D. Anderson Cancer Center support grant).

References

1. Lippman SM, Lee JJ, Martin JW, et al. Fenretinide activity in retinoid-resistant oral leukoplakia. Clin Cancer Res. 2006; 12:3109–14. [PubMed: 16707609]
2. Chiesa F, Tradati N, Grigolato R, et al. Randomized trial of fenretinide (4-HPR) to prevent recurrences, new localizations and carcinomas in patients operated on for oral leukoplakia: long-term results. Int J Cancer. 2005; 115:625–9. [PubMed: 15700313]
3. Clifford JL, Menter DG, Wang M, Lotan R, Lippman SM. Retinoid receptor-dependent and -independent effects of N-(4-hydroxyphenyl)retinamide in F9 embryonal carcinoma cells. Cancer Res. 1999; 59:14–8. [PubMed: 9892176]
4. Clifford JL, Sabichi AL, Zou C, et al. Effects of novel phenylretinamides on cell growth and apoptosis in bladder cancer. Cancer Epidemiol Biomarkers Prev. 2001; 10:391–5. [PubMed: 11319181]

5. Sun SY, Yue P, Kelloff GJ, et al. Identification of retinamides that are more potent than N-(4-hydroxyphenyl)retinamide in inhibiting growth and inducing apoptosis of human head and neck and lung cancer cells. Cancer Epidemiol Biomarkers Prev. 2001; 10:595–601. [PubMed: 11401908]
6. Ponzoni M, Bocca P, Chiesa V, et al. Differential effects of N-(4-hydroxyphenyl)retinamide and retinoic acid on neuroblastoma cells: apoptosis versus differentiation. Cancer Res. 1995; 55:853–61. [PubMed: 7850799]
7. Di Vinci A, Geido E, Infusini E, Giaretti W. Neuroblastoma cell apoptosis induced by the synthetic retinoid N-(4-hydroxyphenyl)retinamide. Int J Cancer. 1994; 59:422–6. [PubMed: 7927952]
8. Mariotti A, Marcora E, Bunone G, et al. N-(4-hydroxyphenyl)retinamide: a potent inducer of apoptosis in human neuroblastoma cells. J Natl Cancer Inst. 1994; 86:1245–7. [PubMed: 8040893]
9. Jasti BR, LoRusso PM, Parchment RE, et al. Phase I Clinical Trial of Fenretinide (NSC374551) in Advanced Solid Tumors. Proc Am Soc Clin Oncol. 2001; 20:abstr 485.
10. Lippman SM, Batsakis JG, Toth BB, et al. Comparison of low-dose isotretinoin with beta carotene to prevent oral carcinogenesis. N Engl J Med. 1993; 328:15–20. [PubMed: 8416267]
11. Hong WK, Endicott J, Itri LM, et al. 13-cis-retinoic acid in the treatment of oral leukoplakia. N Engl J Med. 1986; 315:1501–5. [PubMed: 3537787]
12. Papadimitrakopoulou VA, Hong WK, Lee JS, et al. Low-dose isotretinoin versus beta-carotene to prevent oral carcinogenesis: long-term follow-up. J Natl Cancer Inst. 1997; 89:257–8. [PubMed: 9017007]
13. Delia D, Aiello A, Lombardi L, et al. N-(4-hydroxyphenyl)retinamide induces apoptosis of malignant hemopoietic cell lines including those unresponsive to retinoic acid. Cancer Res. 1993; 53:6036–41. [PubMed: 8261419]
14. Oridate N, Lotan D, Xu XC, Hong WK, Lotan R. Differential induction of apoptosis by all-trans-retinoic acid and N-(4-hydroxyphenyl)retinamide in human head and neck squamous cell carcinoma cell lines. Clin Cancer Res. 1996; 2:855–63. [PubMed: 9816241]
15. Sheikh MS, Shao ZM, Li XS, et al. N-(4-hydroxyphenyl)retinamide (4-HPR)-mediated biological actions involve retinoid receptor-independent pathways in human breast carcinoma. Carcinogenesis. 1995; 16:2477–86. [PubMed: 7586155]
16. Formelli F, Cavadini E, Luksch R, et al. Pharmacokinetics of oral fenretinide in neuroblastoma patients: indications for optimal dose and dosing schedule also with respect to the active metabolite 4-oxo-fenretinide. Cancer Chemother Pharmacol. 2007
17. Villablanca JG, Krailo MD, Ames MM, Reid JM, Reaman GH, Reynolds CP. Phase I trial of oral fenretinide in children with high-risk solid tumors: a report from the Children's Oncology Group (CCG 09709). J Clin Oncol. 2006; 24:3423–30. [PubMed: 16849757]
18. Garaventa A, Luksch R, Lo Piccolo MS, et al. Phase I trial and pharmacokinetics of fenretinide in children with neuroblastoma. Clin Cancer Res. 2003; 9:2032–9. [PubMed: 12796365]
19. Otterson GA, Lavelle J, Villalona-Calero MA, et al. A phase I clinical and pharmacokinetic study of fenretinide combined with paclitaxel and cisplatin for refractory solid tumors. Invest New Drugs. 2005; 23:555–62. [PubMed: 16034523]
20. Colombo N, Formelli F, Cantu MG, et al. A phase I-II preoperative biomarker trial of fenretinide in ascitic ovarian cancer. Cancer Epidemiol Biomarkers Prev. 2006; 15:1914–9. [PubMed: 17035399]
21. Veronesi U, De Palo G, Marubini E, et al. Randomized trial of fenretinide to prevent second breast malignancy in women with early breast cancer. J Natl Cancer Inst. 1999; 91:1847–56. [PubMed: 10547391]
22. Sabichi AL, Lerner SP, Atkinson EN, et al. Phase III prevention trial of fenretinide in patients with resected non-muscle-invasive bladder cancer. Clin Cancer Res. 2008; 14:224–9. [PubMed: 18172274]
23. Lippman SM, Heymach JV. The convergent development of molecular-targeted drugs for cancer treatment and prevention. Clin Cancer Res. 2007; 13:4035–41. [PubMed: 17634526]

Table 1

Baseline characteristics of eligible patients

Characteristic	Number of patients (%)N=15
Median age, years (range)	59 (39–77)
Gender	
Female	7 (47%)
Male	8 (53%)
Race	
Asian	1 (7%)
Black	1 (7%)
White	13 (87%)
Smoking status	
Never smoker	7 (47%)
Former smoker	6 (40%)
Current Smoker	2 (13%)
Histology	
Hyperplasia	4 (27%)
Mild dysplasia	6 (40%)
Moderate dysplasia	2 (13%)
Severe dysplasia / carcinoma in situ	3 (20%)
Primary site	
Oral tongue, lateral border	7 (47%)
Buccal mucosa	6 (40%)
Floor of the mouth	1 (7%)
Oral tongue, dorsum	1 (7%)
Prior treatment for oral pre-malignancy	
No surgery	8 (53%)
Surgery	7 (47%)
No previous chemoprevention	6 (40%)
Previous chemoprevention[§]	9 (60%)
13-cis retinoic acid	6 (40%)
Beta-carotene + retinyl palmitate	1 (7%)
Fenretinide, low dose[†]	3 (20%)
Celecoxib	5 (33%)

[§]Five patients received more than 1 chemoprevention regimen in the past. Seven patients received prior retinoids.

[†]Fenretinide 200 mg/day

Table 2

Worst toxicities by grade (1–3) per patient. Only toxicities occurring in more than 1 patient (regardless of grade) are included.

	Number of Patients (%N=15)		
Grade	**1**	**2**	**3**
Gastrointestinal (other)	9 (60%)		
Diarrhea	8 (53%)		1 (7%)
Ocular visual (other)	8 (53%)		
Triglyceride, serum high	8 (53%)		
Pain	6 (40%)		
Cholesterol, serum high	5 (33%)		
Constipation	5 (33%)		
Dermatology / skin (other)	4 (27%)		
Photosensitivity	4 (27%)		
Fatigue	3 (20%)	1 (7%)	
Glucose, serum high	3 (20%)		
Arthritis	2 (20%)		
Conjunctivitis	2 (20%)		
Headache	2 (20%)		
Hot flashes	2 (20%)		
Nausea alone	2 (20%)		
Nyctalopia	1 (7%)	1 (7%)	