Drugs 2001; 61 (7): 909-918
0012-6667/01/0007-0909/$27.50/0

Chemoprevention of Breast Cancer with Fenretinide

Rosalba Torrisi,[1] *Andrea Decensi,*[1] *Franca Formelli,*[2] *Tiziana Camerini*[3] and *Giuseppe De Palo*[3]

1 Chemoprevention Unit, European Institute of Oncology, Milan, Italy
2 Chemoprevention Unit, National Tumour Institute, Milan, Italy
3 Department of Clinical Prevention, National Tumour Institute, Milan, Italy

Abstract

Chemoprevention of cancer represents a challenge for oncology during this new millennium. Substantial advances have been accomplished in the last decade, especially for primary and secondary prevention of breast cancer. In addition to tamoxifen, raloxifene and other selective estrogen receptor modulators, retinoids are among the most promising agents, given their ability to inhibit mammary carcinogenesis in preclinical models.

Fenretinide, the synthetic amide of retinoic acid, inhibits cell growth mostly through the induction of apoptosis with mechanisms which may partly involve the retinoid receptors. Because it has a favourable toxicological profile, fenretinide has been extensively investigated in clinical trials. A large randomised phase III trial for secondary breast cancer prevention has been recently carried out in Italy. Results showed a reduction of second breast malignancies in premenopausal women. In addition, a significant decrease of circulating insulin-like growth factor (IGF)-1, a known risk factor for premenopausal breast cancer, was observed after 1 year of fenretinide administration in premenopausal women with breast cancer.

Ongoing studies on the validation of the circulating IGF-1 as a surrogate endpoint biomarker of fenretinide activity and on the effectiveness of the combination with low dose tamoxifen may provide further insight into the future clinical application of fenretinide.

The last decade of the 20th century has accomplished substantial improvements in the fight against breast cancer. Adjuvant systemic treatment is able to reduce mortality by 20 to 25% in early stage breast cancer[1,2] and, even in metastatic disease, a slight improvement in survival has been gained with new chemotherapeutic drugs and regimens.[3] In addition, systematic mammographic screening has been shown to reduce mortality by 25 to 30% in women aged 50 to 69 years.[4]

Finally, the leading challenge of the 1990s, i.e. the goal of preventing the occurrence of breast cancer through pharmacological intervention, has been accomplished,[5] and this has disclosed new perspectives for the whole strategy of cancer control in the new millennium.[6]

The term chemoprevention was first used by Sporn and Newton[7] who defined it as 'the prevention of cancer by the use of pharmacological agents that inhibit or reverse the process of carcinogenesis'. Since the late 1970s considerable advances have been achieved in this field, which rather than

being a promising area of oncology, has now become a reality.

Chemoprevention focuses on intervening in the processes involved in the cascade of carcinogenic events to prevent final progression to neoplastic disease, and it is aimed at preventing or treating premalignant cells.[8]

Focusing on breast cancer, the antiestrogen tamoxifen has been proven effective in reducing breast cancer incidence in healthy at-risk women in a large randomised trial.[5] The results of the National Surgical Adjuvant Breast and Bowel Project (NASBP)-P1 study have led to the US Food and Drug Administration (FDA) approval of tamoxifen for breast cancer risk reduction in women at increased risk. Since a 33% reduction in breast cancer incidence was observed after the first year of tamoxifen administration, the debate is still open on whether tamoxifen prevents or delays the occurrence of a fully transformed phenotype or treats the occult disease.[6] Whatever its action is, tamoxifen represents the only drug which has definitively shown a preventive activity in breast cancer. Another selective estrogen receptor modulator (SERM), raloxifene, has shown a promising trend in reducing breast cancer incidence in postmenopausal women treated for osteoporosis.[9] These results, generated from a secondary analysis, served as a rationale for designing an ongoing phase III randomised trial, evaluating the activity of raloxifene in breast cancer prevention compared with tamoxifen [the Study of Tamoxifen And Raloxifene (STAR) trial].[10]

However, to a greater extent than other drugs, the use of retinoids in oncology has met with varying degrees of success.

1. Retinoids

Retinoids, the natural and synthetic derivatives of vitamin A, are known to play a crucial role in cellular and tissue differentiation. Retinoids are capable of suppressing tumour promotion and modifying some properties of fully transformed malignant cells, presumably by activating and/or repressing specific genes.[11]

Two families of receptors have been identified, retinoic acid receptors (RARs) and retinoid X receptors (RXRs); each family includes 3 subtypes α, β, and γ, which have cell-context–dependent expression patterns. Retinoid receptors dimerise into RAR-RXR heterodimers and RXR homodimers and bind to specific DNA sequence-retinoic acid response elements (RAREs), acting as ligand-dependent transcriptional regulators for retinoic acid responsive genes.[11] In contrast to RARs, RXRs are able to heterodimerise with other members of the nuclear receptor superfamily such as the vitamin D receptor, the thyroid receptor and the newly discovered 'orphan' receptors peroxisome proliferator-activated-receptor-γ (PPAR–γ) and farnesoid X-activated receptor (FXR).[12]

Recently, members of a new class of selective ligands of RXR, called rexinoids, have been found to be more active as chemopreventive agents in preclinical models, while lacking the classic toxicological profile of retinoids such as teratogenicity and mucocutaneous toxicity.[12] In addition to the nuclear receptors and RAREs, specific cellular binding proteins (CRBPs) bind retinoids with high affinity and regulate their metabolism, although their role in retinoid signalling remains controversial.[13]

Retinoid receptors are expressed in normal and malignant epithelial breast cells and are critical for normal development. The mechanism by which retinoids inhibit breast cell growth has not been completely elucidated, but it is likely to involve multiple signal transduction pathways. In fact, in addition to binding to the nuclear receptors, several retinoids are able to interact with the activator protein-1(AP-1) transcription pathway, which is activated upon growth factor signalling.[14] As far as RAR-dependent mechanisms are concerned, great attention has been paid to the role of RAR-β as a negative regulator in the carcinogenic process of different tumours.[15-17] Importantly, its expression in breast cancer is up-regulated by retinoids independently of estrogen receptor status and it is involved either in the inhibition of the AP-1 activity of some retinoids and in the retinoic acid (RA)-

induced expression of IGF binding protein 3 (IGFBP-3) in breast cancer cells.[17-20] *In vivo*, RAR-β expression is progressively lost in human breast carcinogenesis and may play a role in the progression from ductal carcinoma in situ (DCIS) to invasive tumour.[6,17,18]

Since knowledge of other retinoid activity in breast cancer prevention derives mainly from preclinical models, this review focuses on fenretinide, which has been the most investigated retinoid in this setting. Literature on fenretinide available in the MEDLINE database has been reviewed.

2. Fenretinide

The synthetic amide of retinoic acid, fenretinide or N-(4-hydroxyphenyl) retinamide (4-HPR), was synthesised in the late 60s, and its biological activity assayed by Sporn et al.,[21] who also showed the preferential accumulation of this drug in the breast instead of the liver. The inhibition of chemically-induced mammary carcinoma in rats by fenretinide was first described in 1979.[22] Since then, as a result of promising *in vitro* data and a favourable toxicity profile compared with other retinoids, fenretinide has been extensively studied in chemoprevention trials targeting different organs.[23]

2.1 Mechanism of Action and Preclinical Models

Fenretinide has been found to have significant chemopreventive activity in a large variety of *in vitro* and *in vivo* systems. Although its mechanism of action is not well understood, it may act through mechanisms partly distinct from other retinoids. Current experimental data suggest that fenretinide may function by both receptor-dependent and -independent mechanisms.[24-26] A characteristic feature of fenretinide is the ability to inhibit cell growth through the induction of apoptosis rather than differentiation, an effect which is strikingly different from the parental compound all-trans retinoic acid.[27] The mechanism underlying the apoptotic effect of fenretinide is not well understood, but is likely to be cell-dependent and include the up-regulation of reactive oxygen species (ROS), RAR-β and transforming growth factor (TGF)-β expression and/or apoptosis-related genes.[16,28-30]

Additional mechanisms, which may be relevant both for the therapeutic and for the preventive use of fenretinide, are currently under investigation. For example, 4-HPR was shown to decrease telomerase activity, a biomarker of breast cancer development and progression, in N-methyl-N-nitrosourea (MNU)–induced mammary tumours, which was not simply a consequence of changes in cell proliferation.[31] Interestingly, fenretinide down-regulated c-erbB-2 protein and mRNA in overexpressing breast cancer cell lines (BCCL) and induced apoptosis in HER-2 neu–transformed BCCL,[32,33] a phenotype which is known to be tamoxifen-resistant.

2.2 Breast Cancer Chemoprevention

Since fenretinide is selectively accumulated in the breast,[34] it appeared particularly attractive to evaluate fenretinide as a chemopreventive agent in breast cancer. Women with early breast cancer are at increased risk of a contralateral cancer, with a risk which is approximately 8/1000, that is 5 to 6 times that of the general population in the same age range.[35] In the 1980s these women were not candidated to adjuvant systemic therapy and, thus, they appeared a suitable population to test the efficacy of fenretinide for secondary breast cancer prevention. A phase I dose-ranging study was completed and the 200mg daily dose was chosen as the safest dose for prevention, as one case of a pathological electroretinogram after 24-weeks administration was observed with the 300 mg/day dose.[36,37] Higher doses, up to 400mg, have been used in women with metastatic cancer in combination with tamoxifen, with no evident toxicity on liver and lipid profile, but with an increased incidence of nyctalopia.[38,39]

The phase I study also provided important information on the pharmacokinetics of fenretinide.

Fenretinide administration induced a dose-related linear decrease of plasma retinol levels, which was associated with diminished dark adaptation. In order to minimise this adverse effect, a 3-day treat-

ment interruption at the end of each month was introduced to increase plasma retinol levels, thus allowing the partial recovery of retinal storage. Studies of the mechanisms responsible for retinol reduction have indicated that fenretinide shows a high binding affinity to retinol binding protein (RBP), thus interfering with the RBP-retinol-transthyretin complex formation and the secretion of retinol from the liver.[40] Additional mechanisms suggesting a specific effect of fenretinide on ocular turnover of vitamin A have been advocated to explain dark adaptation impairment associated with the administration of this retinoid.[41] Older and heavier women showed the higher fenretinide-induced decrease in retinol levels.[42]

Long term (5 years) daily administration of fenretinide 200mg resulted in an average plasma concentration of 350 μg/L (i.e. approximately 1 μmol/L), which remained constant throughout the 5-year treatment period.[43] Concentrations of N-(4-methoxyphenyl)retinamide (4-MPR), the major metabolite of fenretinide, were similar to those of the parental drug. Retinol levels were reduced by an average 65% and this reduction was constant during the 5-year treatment period.[43] After 5 years' administration, plasma fenretinide concentrations were at the limits of detection at 6 and 12 months after drug discontinuation, whereas the concentrations of 4-MPR were approximately 5 times higher. Baseline retinol levels recovered after 1 month.[43]

2.3 Phase III Breast Cancer Trial

A phase III, multicentre, randomised trial coordinated by the National Tumour Institute of Milan was started in 1987. Patients with stage I (T_{1-2} N_0) breast cancer, aged 30 to 70 years, who had undergone breast cancer surgery within the previous 10 years and who had received no systemic adjuvant therapy were eligible for inclusion. Women were randomly assigned to receive either no treatment or fenretinide given orally at a dose of 200 mg/day for 5 years. A placebo control was not incorporated into the study design because of the large capsule size and the objective nature of the main end-point. A 3-day drug interval at the end of each month was recommended, in order to allow retinol recovery and to minimise dark adaptation impairment.[44]

The principal end-point was the occurrence of contralateral breast cancer as the first malignant event. Another main end-point was the incidence of ipsilateral breast cancer reappearance, defined as local recurrence in the same quadrant or the occurrence of a second breast malignancy in a different quadrant from the primary tumour. The use of this endpoint was considered appropriate for a preventive intervention, because it is at least in part due to the progression of premalignant or early-malignant cells. The occurrence of distant metastases (including regional relapse) and death were recorded, but they were not considered efficacy end-points as fenretinide was not thought to be active in the late phases of breast carcinogenesis.[44]

The study was powered at 90% to detect a 50% reduction in the incidence of contralateral breast cancer, with a 5% type I error probability level (for a 2-sided test), and a 3-year linear lag to obtain a full intervention effect. A 10% dropout rate was anticipated.[44]

Accrual started on March 1987 and was closed prematurely on July 1993, below the expected sample size of 3500, after the US National Cancer Institute alert which recommended the administration of adjuvant systemic treatment in node-negative breast cancer patients. 2972 patients entered the study, 2867 of whom were assessable, giving a 87% power to detect the expected difference. The 2 groups were well balanced for all patient and tumour characteristics.

The analysis of results after a median follow-up duration of 97 months has been recently reported[45] and the results are summarised in table I. No effect on contralateral breast cancer occurrence and a nonsignificant 17% reduction in ipsilateral breast cancer incidence was seen with fenretinide. However, a distinct effect was noted when the analysis was stratified by menopausal status, with a beneficial trend in premenopausal women on both contralateral and ipsilateral breast cancer and a possible reversed trend on contralateral breast can-

Table I. Number of events during the phase III Milan study[45] and results of the Cox models on the efficacy end-points (modified from Veronesi et al.,[45] with permission)

	Number of events		Hazard ratio	95% Confidence interval	Wald's statistic p-value
	fenretinide	control			
Contralateral breast cancer					
Unadjusted analysis	65	71	0.92	0.66-1.29	0.642
Adjusted analysis[a]					0.045[c]
Premenopausal women	27	42	0.66	0.41-1.07	
Postmenopausal women	38	29	1.32	0.82-2.15	
Ipsilateral breast cancer reappearance					
Unadjusted analysis	100	121	0.83	0.64-1.09	0.177
Adjusted analysis[b]					0.045[c]
Premenopausal women	58	87	0.65	0.46-0.92	
Postmenopausal women	42	34	1.19	0.75-1.89	

a Adjusted for primary tumour site, lobular histology, menopausal status and interaction between treatment and the menopausal status.

b Adjusted for primary tumour site, size and histology, type of surgery, interval from surgery to randomisation, menopausal status and interaction between treatment and menopausal status.

c p-Value for testing interaction between treatment and menopausal status.

cer in postmenopausal women. No effect of treatment on distant metastases and death was observed overall or in the 2 subgroups separately. The frequency of second primary tumours was comparable between the 2 groups (38 in the fenretinide group versus 40 in the control group) with no significant excess of a specific tumour in either of the 2 arms. However, the incidence of ovarian cancer during the 5-year intervention period was significantly lower in the fenretinide group (none versus 6 patients in the control group), whereas ovarian cancer occurred in 3 patients in the fenretinide group after treatment discontinuation.[46]

These findings support the role of fenretinide as a preventive agent, acting at different steps in the breast carcinogenic process, since it is able to decrease both ipsilateral breast cancer reappearance and contralateral cancer, which are attributable either to the proliferation of residual cancer cells or to the transformation of premalignant cells. However, the lack of effect on the occurrence of metastases suggests that the retinoid is ineffective on the progression to a more malignant phenotype, possibly as a result of the loss of retinoid receptor expression.[38] This study suggests a beneficial effect of fenretinide in premenopausal women with breast cancer. Some criticisms have been raised on this observation, since the analysis of the interaction between intervention and menopausal status was not planned at the beginning of the study and it was a *post hoc* observation.[47] Results should thus be confirmed in further studies.

The search for a qualitative interaction between fenretinide and menopausal status was prompted by a previous observation on the effect of fenretinide on plasma IGF-1 levels according to menopausal status in a subset of patients participating in the phase III trial.[48] It was shown that fenretinide administration for 12 months reduced circulating IGF-1 levels in premenopausal women, whereas no significant change was observed in postmenopausal women. When the analysis was performed using age as a continuous variable, a significant age by treatment interaction was observed, with younger women exhibiting a greater decline in IGF-1 levels (28%). A slight but significant up-regulation of serum IGFBP-3 was associated with the IGF-1 decline.[49]

Consistently, fenretinide has been shown to inhibit IGF-1–stimulated growth of BCCL and to down-regulate the IGF system in both estrogen receptor (ER)-positive and ER-negative BCCL.[50]

In addition, a large prospective study has shown that high circulating IGF-1 levels and lower levels of its major binding protein (IGFBP-3) are positively associated with the risk of developing subsequent premenopausal, but not postmenopausal, breast cancer.[51] Moreover, the observation that the modulation of IGF-1 by fenretinide reflects its clinical effect on second primary cancers[45] suggests that the decline in IGF-1 levels may at least partially account for the chemopreventive activity of the retinoid. This effect on the IGF system is peculiar to fenretinide and is not shared by other retinoids such as all-trans retinoic acid.[52]

The validation of circulating IGF-1 levels as surrogate end-point biomarker of breast carcinogenesis is currently under investigation. The change of IGF-1 levels between follow-up and baseline will be correlated retrospectively with the occurrence of a second breast malignancy in more than 600 premenopausal women participating in the Milan phase III chemoprevention trial with fenretinide.

2.4 Tolerability

Given the foreseen long term use in healthy individuals, the other crucial issue in chemoprevention is toxicity. The 5-year administration of fenretinide in the Milan phase III trial provided a huge body of information on the long term tolerability of this retinoid.[45] In this study, however, the accurate evaluation of fenretinide toxicity was hampered by the lack of a placebo control group. Dermatological, gastrointestinal, visual and ophthalmological events were relatively frequent, but an exact quantification of the fraction actually related to treatment is not possible. The most common adverse events reported were diminished dark adaptation (cumulative incidence 19%) and dermatological disorders (18.6%), such as skin and mucosal dryness, pruritus and urticaria.[45] Less common events were gastrointestinal symptoms (13%) and alterations of the ocular surface (10.9%). Interestingly, with the exception of ocular surface disorders, the incidence of the other adverse effects decreased with time.[53] Adverse effects were significantly more frequent in postmenopausal women.[53] However, only 63 of 1432 women (4.4%) discontinued treatment for drug-related toxicity and no life-threatening events were observed.[45]

Finally, the objective assessment of laboratory values did not show any difference between the 2 arms in the number of patients with abnormal results.[45] A major concern for retinoid use is liver toxicity and increases in blood lipid levels, especially triglycerides. No difference in the 5-year cumulative incidence of abnormal liver tests or of hypertriglyceridaemia between the fenretinide and the control group were observed in the Milan phase III trial.[53] A slight excess (17.7% and 11%, respectively, in the fenretinide and the placebo groups) of abnormal lipid profile (mostly increase in triglycerides) has been recently reported in patients with superficial bladder cancer after 2 years of fenretinide administration.[54] Consistent with these long term findings, no significant increase in triglyceride levels was observed after short term combination therapy with tamoxifen, even when fenretinide was administered at a dose of 400 mg/day.[38,39,55] On the contrary, a favourable significant increase in high density lipoprotein cholesterol levels was observed, both in patients with metastatic breast cancer and in healthy women.[38,55]

Since ocular toxicity is undoubtedly the major concern with fenretinide, the pattern of occurrence of subjective and objective visual and other ophthalmological disorders were extensively investigated in the phase III Milan study as well as other studies.[56-58] The 5-year cumulative incidence of diminished dark adaptability was approximately 20%. The symptoms occurred more frequently at the start of intervention, but they tended to recover without treatment discontinuation.[56]

An objective evaluation of fenretinide-induced dark adaptation impairment was performed on a subset of patients from the phase III Milan study who had received treatment for a median of 32 months.[57] Results of a subjective evaluation through a structured questionnaire were compared with plasma retinol levels and with the results of the Goldmann-Weekers adaptometer test. Mild and moderate al-

terations of dark adaptability, specifically a prolongation of time to the cone-rod break and a higher final rod threshold were observed in 23.5 and 26.5% of women, respectively, and were associated with plasma retinol levels below 160 and 100 μg/L, respectively. Importantly, only half of the patients with positive dark adaptometry were symptomatic, thus raising the issue of the real-life implications of fenretinide-associated visual alterations.

Similar data were reported in another study[58] after a shorter duration (approximately 4 months) of fenretinide administration, which confirmed the low rate of reduced dark adaptability in patients with alterations of the Goldmann-Weekers test. In this study, 16 out of 22 (73%) patients receiving fenretinide 200 mg/day showed a significant delay of rod-cone break compared with controls, but only 4 of them reported symptoms of dark adaptation impairment. Importantly, this study also showed the complete reversibility of this effect of fenretinide on dark adaptation, with the rod-cone break measured after a median of 2 months after drug discontinuation being comparable to baseline values.[58]

3. Combination Therapy

Similarly to the therapeutic setting, where the combination of several drugs is superior to monotherapy, the concept of combining 2 or more agents with different mechanisms of action in an attempt to increase the effectiveness of therapy, while minimising the toxic adverse effects, is an attractive option for chemoprevention.

In preclinical models there are several examples of such synergy between retinoids and other agents. For example, the activity of 9-cis-retinoic acid against MNU-induced mammary tumours in Sprague-Dawley rats is enhanced by combination with tamoxifen or raloxifene.[59] In addition, the combined administration of fenretinide and tamoxifen has proven additive and synergistic in both the growth inhibition of MCF-7 cells and the prevention of MNU-induced mammary carcinomas.[60]

The safety and the tolerability of this combination has been investigated in clinical trials firstly in patients with metastatic breast cancer and then in at-risk women.[38,39,55] In a pilot study involving 32 healthy at-risk women, oral fenretinide was administered for four 28-day cycles at the daily dose of 200mg.[55] Tamoxifen was started after the first month of fenretinide administration at the daily dose of 20mg and continued for 24 months. This schedule allowed investigation of the pharmacodynamics and the toxicity of fenretinide as a single agent and after tamoxifen administration. No interference of tamoxifen on fenretinide concentrations was observed. The incidence of fenretinide-induced nyctalopia was 6% and reversed completely after fenretinide discontinuation. Hot flashes (flushes) were reported in 84% of women after the start of tamoxifen therapy.

However, concerns regarding the feasibility of this combination have been recently raised by the high withdrawal rate (30%) observed in a US trial of fenretinide 400 mg/day and tamoxifen as adjuvant treatment in older women with breast cancer.[61] In that study, a higher incidence of grade I to II leucopenia, hypercalcaemia, nyctalopia, and pulmonary and genitourinary symptoms was noted in the combination arm. Interestingly, however, women receiving fenretinide plus tamoxifen exhibited a borderline significant reduction of hot flashes compared with those receiving tamoxifen alone.

A 2×2 randomised trial of fenretinide and low dose tamoxifen using change in IGF-1 levels as principal end-point is currently ongoing in Italy (table II). Premenopausal women with microinvasive ER-positive, well-differentiated, low proliferating breast cancer, DCIS, or who are at increased risk of breast cancer according to the Gail model, have been randomised to receive fenretinide, low dose tamoxifen (5 mg/day), the combination of both or placebo for 2 years.[62]

This study will allow multiple issues to be addressed. Firstly, to assess the activity of the combination of fenretinide and a SERM compared with each agent separately. Secondly, to clarify the role

Table II. Design of the breast cancer prevention trials currently ongoing in Italy with fenretinide[63]

Patient population (n)	Treatment arm	End-points
Premenopausal women (300)	2 years	
Microinvasive cancer [(T <1cm, N0), ER+, Ki67 <20%]	TAM 5 mg/day + P	Δ IGF-I
DCIS	4-HPR 200 mg/day+ P	Δ IGF-I/BP-3
LCIS	TAM 5mg + 4-HPR 200mg	Δ Mx density
Healthy at-risk (Gail score ≥1.67 at 5y)	P + P	Δ breast FNA (image analysis) endometrium
Healthy postmenopausal women (248)	1 year	
Menopause 6-60mo	tHRT + 4-HPR 200 mg/day	Δ IGF-I
No previous HRT	tHRT + P	Δ IGFBP-3
	oHRT + 4-HPR 200 mg/day	Δ Mx density
	oHRT + P	Δ breast FNA (image analysis)

4-HPR = fenretinide; **DCIS** = ductal carcinoma *in situ*; **ER** = estrogen receptor; **FNA** = fine needle aspirate; **HRT** = hormone replacement therapy; **IGF-I** = insulin-like growth factor 1; **IGFBP-3** = IGF binding protein 3; **LCIS** = lobular carcinoma *in situ*; **Mx** = mammographic density; **P** = placebo; Δ = change; **oHRT** = oral HRT; **TAM** = tamoxifen; **tHRT** = transdermal HRT.

of IGF-1 as surrogate end-point biomarker of breast carcinogenesis. And, finally, to explore the activity of fenretinide as single agent therapy in patients with DCIS, a pathological feature which is frequently associated with HER2/neu overexpression and, potentially, a hormone-resistant phenotype.

Women receiving hormone replacement therapy (HRT) may be considered at increased risk of breast cancer. A randomised trial aimed at investigating the ability of fenretinide to modulate well recognised surrogate end-point biomarkers of breast cancer risk, such as circulating IGF-1 and breast mammographic density, in postmenopausal women willing to start HRT is currently under way in Italy (table II).[63]

4. Conclusions

Retinoids are among the most promising agents for breast cancer prevention. In particular, fenretinide has shown an encouraging activity for prevention of secondary breast malignancies in premenopausal women, while knowledge of other retinoids is limited to that from preclinical models.

However, the role of fenretinide in breast cancer prevention needs to be further elucidated. Ongoing studies on the validation of circulating IGF-1 levels as a surrogate end-point biomarker of fenretinide activity and the effectiveness of the combination with low dose tamoxifen may provide further insight into the future clinical application of fenretinide. Since fenretinide seems to be active in HER2/neu tumours, its role in the adjuvant treatment of ER-negative and/or HER2/neu overexpressing tumours in premenopausal women in addition to chemotherapy may be usefully explored in the future. Furthermore, since it seems plausible that the dose of 200 mg/day results in serum concentrations which are lower than those active in preclinical models, the feasibility of using higher doses, possibly in conjunction with vitamin A supplementation, should be investigated.

Acknowledgements

The Chemoprotective Unit of the European Institute of Oncology is partially supported by a grant from the Italian Foundation of Cancer Research (FIRC).

References

1. Early Breast Cancer Trialists' Collaborative Group. Polychemotherapy for early breast cancer: an overview of the randomized trials. Lancet 1998; 352: 930-42
2. Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomized trials. Lancet 1998; 351: 1451-67
3. Crown J, O'Leary M. The taxanes: an update. Lancet 2000; 355: 1176-8
4. Kerlikowske K. Efficacy of screening mammography among women aged 40 to 49 years and 50 to 69 years: comparison

of relative and absolute benefit. J Natl Cancer Inst Monogr 1997; 22: 79-86
5. Fisher B, Costantino JP, Wicherham DL, et al. Tamoxifen for prevention of breast cancer: report of the national surgical adjuvant breast and bowel project P-1 study. J Natl Cancer Inst 1998 Sep 16; 90 (18): 1371-88
6. Lippman SM, Brown PH. Tamoxifen prevention of breast cancer: an instance of the fingerpost [commentary]. J Natl Cancer Inst 1999 Nov 3; 91 (21): 1809-19
7. Sporn MB, Newton DL. Chemoprevention of cancer with retinoids. Fed Proc 1979; 38: 2528-34
8. Lippman SM, Lee JJ, Sabichi AL. Cancer chemoprevention: progress and promise. J Natl Cancer Inst 1998; 90: 1514-28
9. Cummings SR, Eckert S, Krueger KA, et al. The effect of Raloxifene on risk of breast cancer in postmenopausal women. Results from the MORE randomized trial. JAMA 1999 Jun 16; 281 (23): 2189-97
10. Rhodes DJ, Hartmann LC, Perez EA. Breast cancer prevention trials. Curr Oncol Rep 2000; 2: 558-65
11. Chambon P. A decade of molecular biology of retinoic acid receptors. FASEB J 1996; 10: 940-54
12. Sporn MB, Suh N. Chemoprevention of cancer. Carcinogenesis 2000; 21 (3): 525-30
13. Spinella MJ, Dimitrovsky E. Aberrant retinoid signaling and breast cancer: the view from outside the nucleus [editorial]. J Natl Cancer Inst 2000; 92: 438-40
14. Fanjul A, Dawson Mi, Hobbs PD, et al. A new class of retinoids with selective inhibtion of AP-1 inhibits proliferation. Nature 1994; 372: 107-11
15. Lotan R, Xu X-C, Lippman SM, et al. Suppression of retinoic acid receptor- in premalignant oral lesions and its upregulation by isotretinoin. N Engl J Med 1995; 332: 1405-10
16. Sabichi Al, Hendricks DT, Bober MA, et al. Retinoic acid receptor- expression and growth inhibition of gynecologic cancer cells by the synthetic retinoid N-(4-hydroxyphenyl) retinamide. J Natl Cancer Inst 1998; 15: 597-605
17. Xu X-C, Sneige N, Liu X, et al. Progressive decrease in nuclear retinoic acid receptor messenger RNA level during breast carcinogenesis. Cancer Res 1997; 57: 4992-6
18. Widschwendter M, Berger J, Daxenbichler G, et al. Loss of retinoic acid receptor beta expression in breast cancer and morphologically normal adjacent tissue but not in the breast tissue distant from the cancer. Cancer Res 1997; 57: 4158-61
19. Lin F, Xiao D, Kumar Kolluri S, et al. Unique anti-Activator Protein-1 activity of retinoic acid receptor . Cancer Res 2000; 60: 3271-80
20. Shang Y, Baumrucker CR, Green M. Signal relay by retinoic acid receptor and in the retinoic acid-induced expression of insulin-like growth factor-binding protein-3 in breast cancer cells. J Biol Chem 1999; 274: 18005-10
21. Sporn MB, Dunlop NM, Newton DL, et al. Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). Fed Proc 1979; 35: 1332-8
22. Moon RC, Thompson HJ, Becci PJ, et al. N-(4-hydroxypheynl) retinamide), a new retinoid for prevention of breast cancer in the rat. Cancer Res 1979, 39: 1339-46
23. Kelloff GJ, Crowell JA, Boone CW, et al.: Clinical development plan: N-(4-hydroxyphenyl)retinamide (4-HPR). J Cell Biochem 1994; 20 Suppl.: 176-96
24. 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
25. Fanjul AN, Delia D, Pierotti MA, et al. 4-hydroxyphenylretinamide is a highly selective activator of retinoid receptors [published erratum appears in J Biol Chem 1996; 271: 33705]. J Biol Chem 1996; 271: 22441-6
26. Sun SY, Li W, Yue P, et al. Mediation of N-(4-hydroxyphenyl) retinamide-induced apoptosis in human cancer cells by different mechanisms. Cancer Res 1999; 59: 2493-8
27. Lotan R. Retinoids and apoptosis: implications for cancer chemoprevention and therapy [editorial]. J Natl Cancer Inst 1995; 87: 1655-7
28. Oridate N, Suzuki S, Masahiro H, et al. Involvement of reactive oxygen species in N-(4-hydroxyphenyl)retinamide-induced apoptosis in cervical carcinoma cells. J Natl Cancer Inst 1997; 89: 1191-8
29. Herbert BS, Sanders BG, Kline K. N-(4-hydroxyphenyl) retinamide activation of transforming growth factor- beta and induction of apoptosis in human breast cancer cells. Nutr Cancer 1999; 34 (2): 121-32
30. Sun SY, Yue P, Lotan R. Induction of apoptosis by N-(hydroxyphenyl)retinamide and its association with reactive oxygen species, nuclear retinoic acid receptors, and apoptosis-related genes in human prostate carcinoma cells. Mol Pharmacol 1999; 55: 403-10
31. Bednarek A, Shilkaitis A, Green A, et al. Suppression of cell proliferation and telomerase activity in 4-(hydroxyphenyl) retinamide-treated mammary tumors. Carcinogenesis 1999 May; 20 (5): 879-83
32. Jinno H, Steiner MG, Mehta RG, et al. Inhibition of aberrant proliferation and induction of apoptosis in HER-2/neu oncogene transformed human mammary epithelial cells by N-(4-hydroxyphenyl)retinamide. Carcinogenesis 1999 Feb; 20 (2): 229-36
33. Rao GN, Ney E, Herbert NA. Effect of retinoid analogues on mammary cancer in transgenic mice bearing c-neu transfected breast cancer oncogene. Breast Cancer Res Treat 1998 Apr; 48: 265-71
34. Mehta RG, Moon RC, Hawthorne M, et al. Distribution of fenretinide in the mammary gland of breast cancer patients. Eur J Cancer 1991; 27 (2): 138-41
35. Broet P, de la Rochefordiere A, Scholl SM, et al. Contralateral breast cancer: annual incidence and risk parameters. J Clin Oncol 1995; 13: 1578-83
36. Formelli F, Carsana R, Costa A, et al. Plasma retinol reduction by the synthetic retinoid fenretinide: a one year follow-up study. Cancer Res 1989; 49: 6149-52
37. Costa A, Malone W, Perloff M, et al. Phase I trial of fenretinide (HPR) in breast cancer patients. Eur J Cancer Clin Oncol 1989; 25: 805-9
38. Zujewski J, Pai L, Wakefield L, et al. Tamoxifen and Fenretinide in women with metastatic breast cancer. Breast Cancer Res Treat 1999; 57: 277-83
39. Cobleigh MA, Dowlatsahi K, Deutsch TA, et al. Phase I/II trial of tamoxifen with or without fenretinide, an analog of vitamin A, in women with metastatic breast cancer. J Clin Oncol 1993; 11: 474-7
40. Berni R, Formelli F. In vitro interaction of fenretinide with plasma retinol-binding protein and its functional consequences. FEBS Lett 1992; 308: 43-5
41. Lewis KC, Zech LA, Pheng JM. Effects of chronic administration of N-(4-hydroxyphenyl)retinamide (4-HPR) in rats on vitamin A metabolism in eye. Eur J Cancer 1996; 32A: 1803-8
42. Torrisi R, Parodi S, Fontana V, et al. Factors affecting plasma retinol decline during long-term administration of the syn-

thetic retinoid fenretinide in breast cancer patients. Cancer Epidemiol Biomarkers Prev 1994; 3: 507-10
43. Formelli F, Clerici M, Campa T, et al. Five-year administration of fenretinide of fenretinide: pharmacokinetics and effects on plasma retinol concentrations. J Clin Oncol 1993; 11: 2306-42
44. De Palo G, Camerini T, Marubini E, et al Chemoprevention trial of contralateral breast cancer with fenretinide. Rationale, design, methodology, organization, data management, statistics and accrual. Tumori 1997; 83: 884-94
45. 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
46. De Palo G, Veronesi U, Camerini T, et al. Can fenretinide protect women against ovarian cancer ? J Natl Cancer Inst 1995; 87: 146-7
47. Piantadosi S. Vitamin A analogue for breast cancer prevention: a grade of F or incomplete? [editorial]. J Natl Cancer Inst 1999; 91: 1794-5
48. Torrisi R, Pensa F, Orengo MA, et al. The synthetic retinoid fenretinide lowers plasma insulin-like growth factor-I levels in breast cancer patients. Cancer Res 1993; 53: 4769-71
49. Torrisi R, Parodi S, Pensa F, et al. Effect of fenretinide on plasma IGF-I and IGFBP-3 in early breast cancer patients. Int J Cancer 1998; 76: 787-90
50. Favoni RE, de Cupis A, Bruno S, et al. Modulation of the insulin-like growth factor-I system by N-(4-hydroxyphenyl) retinamide in human breast cancer cell lines. Br J Cancer 1998; 77: 2138-47
51. Hankinson SE, Willett WC, Colditz GA, et al. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 1998; 351: 1393-6
52. Budd TG, Adamson PC, Gupta M, et al. Phase I/II trial of all-trans retinoic acid and tamoxifen in patients with advanced breast cancer. Clin Cancer Res 1998; 4: 635-42
53. Camerini T, Mariani L, De Palo G, et al. Safety of the synthetic retinoid fenretinide: long-term results from a controlled clinical trial for the prevention of contralateral breast cancer. J Clin Oncol 2001; 19: 1664-70
54. Decensi A, Torrisi R, Bruno S, et al. Randomized trial of fenretinide in superficial bladder cancer using DNA flow cytometry as an intermediate endpoint. Cancer Epidemiol Biomarker Prevent. In press
55. Conley B, O'Shaughnessy J, Prindville S, et al. Pilot trial of the safety and tolerability, and retinoid levels of N-(4-hydroxyphenyl)retinamide in combination with tamoxifen in patients at high risk of developing invasive breast cancer. J Clin Oncol 2000 Jan; 18 (2): 275-83
56. Mariani L, Formelli F, De Palo G, et al. Chemoprevention of breast cancer with fenretinide (4-HPR): study of long-term visual and ophthalmologic tolerability. Tumori 1996, 82: 444-9
57. Decensi A, Torrisi R, Polizzi A, et al. Effect of the synthetic retinoid fenretinide on dark adaptation and the ocular surface. J Natl Cancer Inst 1994; 86: 105-10
58. Caruso RC, Zujewski J, Iwata F, et al. Effects of fenretinide (4-HPR) on dark adaptation. Arch Ophthalmol 1998; 116: 759-63
59. Anzano MA, Peer CW, Smith JM, et al. Chemoprevention of mammary carcinogensis in the rat: combined use of raloxifene and 9-cis retinoic acid. J Natl Cancer Inst 1996; 88: 123-5
60. Ratko TA, Detrisac CJ, Dinger NM, et al. Chemopreventive efficacy of combined retinoid and tamoxifen treatment following surgical excision of a primary mammary cancer in female rats. Cancer Res 1989; 49: 4472-6
61. Cobleigh MA, Gray R, Graham M, et al. Fenretinide (FEN) versus placebo in postmenopausal breast cancer patients receiving adjuvant tamoxifen (TAM), an Eastern Cooperative Oncology Group Phase III Intergroup Trial (EB193, INT-0151)[abstract]. Proc ASCO 2000; 19: 328A
62. Costantino JP, Gail MH, Pee D, et al. Validation studies for models projecting the risk of invasive and total breast cancer incidence. J Natl Cancer Inst 1999; 91: 1541-8
63. Bonanni B, Ramazzotto F, Franchi D, et al. A randomised trial of fenretinide in HRT users using IGF-1 as a surrogate biomarker [abstract]. Proceedings of the 2000 San Antonio Breast Cancer Symposium. Breast Cancer Res Treat 2000; 64: A152

Correspondence and offprints: Dr *Andrea Decensi*, Chemoprevention Unit, European Institute of Oncology, via Ripamonti 435, 20141 Milan, Italy.
E-mail: andrea.decensi@ieo.it