Efficacy of Targretin on methylnitrosourea-induced mammary cancers: prevention and therapy dose-response curves and effects on proliferation and apoptosis

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Introduction

A chemically induced model of mammary cancers in rats was developed almost 50 years ago by Huggins and co-workers (1). The resulting tumors were primarily hormonally responsive and showed many of the histopathological changes associated with ER⁺ breast cancer. This model has been employed in the development of new chemopreventive agents and is highly responsive to a number of regimens that are effective in altering human ER⁺ breast cancer, including selective estrogen receptor modulators (SERMS), aromatase inhibitors and pregnancy (2–4). Although many agents have been examined that do not obviously alter the hormonal axis, perhaps the most effective of these non-hormonal agents has been retinoids that are agonists for the retinoid X receptor (RXR) nuclear receptors (2,5,6).

Various aspects of the chemopreventive and chemotherapeutic properties of the RXR receptor agonist Targretin (LGD 1069) were examined in the methylnitrosourea (MNU)-induced model of mammary cancer. The administration of Targretin at dose levels of 60, 20 or 6.7 mg/kg body wt/day by gavage decreased the number of mammary tumors by 96, 85 and 78%, respectively. When Targretin was administered in the diet at 92 and 275 mg/kg diet cancer multiplicities were reduced by 78 and 92%, respectively. A wider range of dietary doses of Targretin at 15, 50 and 150 mg/kg diet reduced the number of mammary tumors by 38, 55 and 70%, respectively. Treatment of rats with different regimens of Targretin (250 mg/kg diet) yielded cancer multiplicities of 4.3 for non-treated rats, 0.5 for rats treated continuously with Targretin, 2.1 for rats treated with Targretin for 8 weeks followed by 10 weeks of the control diet and 1.6 for rats treated with Targretin alternating 3 days on and 4 days off. Targretin was also examined as a therapeutic agent by treating rats with at least one palpable mammary tumor for 5 weeks. A high dose of Targretin (272 mg/kg diet) caused partial or complete regression of ~65% of the cancers over this time period. In contrast, in animals treated with 15 mg Targretin/kg diet only 1 of 12 cancers showed significant regression. Finally, the effect of a limited exposure to Targretin (7 days) on cell proliferation and apoptosis in small mammary tumors was determined. Targretin at 150 mg/kg diet strongly decreased proliferation (75%) and increased apoptosis (300%), while a lower dose of Targretin (15 mg/kg diet, which still prevented 30% of cancers) had no effect on apoptosis but did decrease cell proliferation. Determination of serum IGF1 levels showed that treatment of rats with highly effective doses of Targretin at 272 mg/kg diet or at 60 or 20 mg/kg body wt/day by gavage caused significantly decreased serum IGF1 levels.

Abbreviations: BrdU, bromodeoxyuridine; CAR, constitutive androstenedione receptor; IGF1, insulin-like growth factor 1; LXR, liver X receptors; MNU, methylnitrosourea; PPAR, peroxisome proliferator-activated receptors; RAR, retinoic acid receptor; RXR, retinoid X receptor.

Fig. 1. Chemical structures of 9-cis-retinoic acid and Targretin.
RAR-specific retinoids, including skin toxicity and headaches (11). More recently, compounds (including Targretin) have been synthesized which are relatively specific ligands for the RXR receptors. Tests of these agents in the chemically induced mammary cancer model have shown that they are profoundly effective, acting as both preventive and therapeutic agents in this model (5,6,12). In addition, 9-cis-retinoic acid and the rexinoids (RXR-selective retinoids) have strong synergistic effects when administered with SERMs (2,12).

Many intermediate end-point biomarkers have been examined as indicators that treatment with a given agent is likely to be effective in cancer prevention. Two such biomarkers are cell proliferation and apoptosis. Their appeal is that: (i) these relatively generalized phenomena should indirectly reflect agents that have a wide variety of primary targets; (ii) these biomarkers have been employed clinically because there is a relationship between proliferative index and prognosis in many cancers, including breast cancer (13). We have previously employed these end-points in the MNU mammary cancer model to examine agents that modulate the hormonal axis, e.g. aromatase inhibitors and SERMS (14,15). In the present studies these two parameters were evaluated in mammary tumors treated with varying doses of Targretin.

Insulin-like growth factor 1 (IGF1) is a polypeptide of 70 amino acids that affects cell proliferation, differentiation and apoptosis in breast cancer cells (16,17). Epidemiological studies have shown that high systemic levels of IGF1 are associated with an increased risk for breast cancer in premenopausal women (18). In addition, there is evidence suggesting that circulating IGF1 levels may play a role in the chemopreventive/chemotherapeutic activities of SERMS (18).

A variety of questions relative to Targretin as a chemopreventive/chemotherapeutic agent were examined: (i) what is the minimal dose needed for preventive efficacy; (ii) does limited treatment have preventive efficacy; (iii) what are the therapeutic dose-response characteristics and are the doses of Targretin necessary for prevention and therapy similar; (iv) do the effects of short-term Targretin exposure on proliferation and apoptosis in mammary cancers parallel the anticancer effects of this agent?

Materials and methods

Supplies
Chemicals and other materials were obtained as follows: trioctanoin and corn oil, Sigma Chemical Co. (St Louis, MO); MNU, NCI Chemical Repository (Bethesda, MD); Targretin, NCI Chemoprevention Repository (Rockville MD); Teklad mash (4%) diet and Sprague-Dawley rats, Harlan Sprague-Dawley Inc. (Indianapolis, IN), Targretin was made by custom synthesis and confirmed by various analytical techniques.

HPLC analyses of Targretin
Targretin levels and its homogeneity in each diet or gavage mixture were analyzed by HPLC. Targretin-containing diet samples (0.5 g) were homogenized in 2 ml of an aqueous solution containing 0.5 mg/ml each EDTA and ascorbic acid. These preparations were extracted with 23 ml of methanol/acetone (1:1 v/v). HPLC analyses of the methanol/acetone extracts were performed using Spherisorb ODS-1 5 μm columns (4.6 × 250 mm) (Phase Separations, Norwalk, CT). The eluant was 90% methanol:10% water (retention time 6.12 min). The flow rate was 1 ml/min, detection was by UV absorbance at 260 nm and quantification was by peak area integration and external standardization.

Chemoprevention studies with Targretin
Diets were prepared by mixing Targretin with Teklad (4%) mash diet using a liquid-solid blender (Patterson-Kelly Co., East Stroudsburg, PA). The retinoid was mixed directly into Teklad (4%) mash diet (i.e. no vehicle was required). The vehicle for Targretin in the gavage solutions was ethanol (20%), trioctanoin (40%) and corn oil (40%). The gavage volume was 0.5 ml/rat. For the three experiments listed below female Sprague-Dawley rats were obtained at 28 days of age and housed in polycarbonate cages (5 rats/cage). At 50 days of age the rats received one injection of MNU (50 mg/kg body wt) via the jugular vein.

Experiment I: gavage versus diet. Treatment with Targretin was initiated 5 days after administration of MNU to the rats. Targretin was given either in the diet or by gavage. The groups (15 rats/group) were given 60, 20 or 6.7 mg/kg body wt/day Targretin by gavage or 275 or 92 mg/kg diet Targretin in the feed. The administration of Targretin by gavage at 20 mg/kg body wt was roughly equivalent to the 275 mg Targretin/kg diet dose level (assuming a rat weighing 225 g consumes 16 g food/day). Similarly, the 6.7 mg/kg body wt/day dose level by gavage was equivalent to the 92 mg/kg diet dose level. The study was terminated 120 days after MNU treatment.

Experiment II: low doses. Diets were supplemented with Targretin beginning 5 days after administration of MNU to rats at 50 days of age. Groups (15 rats/group) were given 150, 50 or 15 mg/kg diet or control diet. The study was terminated 126 days after MNU treatment.

Experiment III: limited or intermittent treatment. Targretin (250 mg/kg diet) administration was initiated 5 days after female Sprague-Dawley rats received MNU at 50 days of age. Targretin was given either continually in the diet, intermittently in the diet or for a limited period. The groups (15 rats/group) were given control diet only, Targretin throughout the experiment ( continual treatment), Targretin for an initial period of 8 weeks (limited treatment) or Targretin for 3 days followed by 4 days of control diet, repeated for the duration of the experiment. The study was terminated 126 days after MNU administration.

Rats were palpated for mammary tumors twice each week and weighed once each week. Mammary tumors were excised, weighed and processed for histological classification at termination of the studies. Statistical analyses of tumor incidence and latency were determined using log rank analysis and differences in cancer multiplicity were determined by the Armitage test.

Chemotherapeutic experiments with Targretin

Experiment IV: effect on cancer growth. Rats were treated with MNU as described above and palpated for mammary cancers twice per week. When a rat developed a palpable mammary tumor (~1.0 cm in diameter), the rat was randomized to treatment with a diet containing Targretin (272, 95 or 15 mg/kg diet) or control diet. Cancer size was measured twice a week with calipers. The largest diameter of the cancer was measured and this value multiplied by the perpendicular diameter (size expressed in mm²). Each cancer was measured for 5 weeks to determine its growth pattern.

Experiment V: effect on cancer cell proliferation and apoptosis. Animals (n = 10–12/group) bearing mammary tumors (~1.0 cm in diameter) were treated with Targretin (15 or 150 mg/kg diet) for a period of 7 days. Rats were i.p. injected with bromodeoxyuridine (BrdU) (100 mg/kg body wt) in saline 2 h prior to the time of killing (14). After killing the animals by CO₂ asphyxiation, tumors were removed and fixed in 10% formalin for assessment of histomorphology, BrdU labeling and apoptosis.

Cell proliferation. Proliferating cells in mammary cancers were labeled in vivo with BrdU as previously described (14,15). The nuclei labeled with BrdU were identified employing an anti-BrdU monoclonal antibody (Becton Dickinson, Palo Alto, CA) and ABC kit. More than 1000 cells were randomly scored from each tumor

IGF1 levels
Blood was collected at the time of death of the rats. Following centrifugation, the serum was frozen at ~85 °C until analyzed. IGF1 levels in the serum were determined using a radioimmunoassay kit from DSL (catalog no. DSL-2900; Webster, TX) as previously described (20). Briefly, samples were extracted with acid/methanol, added to the assay tubes with 0.1 ml of guinea pig antiserum directed against IGF1 and incubated overnight. Then 1 ml of goat anti-guinea pig immunoglobulin/polyethylene glycol was added to each tube
in order to precipitate the bound IGF1. The tubes were incubated at room temperature for 30 min and centrifuged at 3000 r.p.m. for 30 min. The supernatant was decanted and the bound IGF1 was counted in an automatic gamma counter. Statistical analysis was performed using Student’s t-test.

Results

Effect of Targretin administered by gavage or in the diet on prevention of MNU-induced mammary cancers (experiment I)

Rats were administered Targretin (60, 20 or 6.7 mg/kg body wt/day) by gavage or in the diet beginning 5 days after MNU administration (Figure 2A). There were no major signs of toxicity in any of the treatment groups. However, animals given the highest doses of Targretin exhibited low grade alopecia (hair loss). At the end of the study the body weights of the rats receiving Targretin by gavage were: 60 mg/kg body wt/day, 261 g; 20 mg/kg body wt/day, 258 g; 6.7 mg/kg body wt/day, 258 g; controls, 241 g. For the rats receiving Targretin in the diet the final body weights were: 275 mg/kg diet, 259 g; 92 mg/kg diet, 253 g; controls, 252 g. The average number of mammary tumors/rat in the MNU-treated only group (control diet) was 3.6. Treatment of rats with Targretin (either 60, 20 or 6.7 mg/kg body wt/day) by gavage reduced the number of tumors by 96, 85 and 78%, respectively. Supplementation of the diet with Targretin at 92 or 275 mg/kg diet decreased mammary cancer multiplicity by 82 and 100%, respectively (Figure 2B).

Effects of low doses of Targretin in the diet on mammary cancer prevention (experiment II)

Based on the significant chemopreventive effects of high doses of Targretin, a second experiment was done in which Targretin was given at 150, 50 and 15 mg/kg diet. The body weights of the rats at termination of the study were 261, 261, 253 and 250 g for the groups receiving Targretin at 150, 50 and 15 and 0 mg/kg diet, respectively. There were no clinical signs of toxicity observed during the study. Mammary cancer multiplicities were reduced from 5.3 in the control diet group to 1.4, 2.4 and 3.5 in the groups receiving 150, 50 and 15 mg Targretin/kg diet, respectively (Figure 3).

Chemopreventive effects of limited or intermittent treatment with Targretin (experiment III)

Rats were treated with a highly effective dose of Targretin (250 mg/kg diet) either continually (weeks 1–18), for a limited time period (weeks 1–8) or intermittently (3 days of Targretin followed by 4 days of control diet) throughout the study. Treatment continually with Targretin increased the final body weights of the rats by 4%, while no differences were observed in rats treated for a limited period or intermittently with the rexinoid. MNU-treated only rats developed an average of 4.3 cancers/rat, while animals given continual, limited or intermittent treatment with Targretin developed 0.5, 2.1 and 1.6 cancers/rat, respectively (Figure 4).

Effects of Targretin as a cancer therapeutic agent (experiment IV)

Rats bearing small palpable tumors (~1.0 cm in diameter) were treated with Targretin and growth of the tumors was monitored daily through 140 days after MNU treatment.
Effects on the growth of individual cancers are shown in Figure 5A–D and graphically in Figure 6. Growth rates of control tumors are often highly variable in this chemically induced model (Figure 5A). Following 5 weeks of treatment, the dose of 272 mg Targretin/kg diet had strong therapeutic effects, the 92 mg/kg diet dose had some therapeutic effects and the dose of 15 mg/kg diet had virtually no effect. To further illustrate this, growth during treatment was split into five categories based on tumor areas determined by caliper measurements: I, complete response (regression from 65-100%); II, partial response (35-65% regression); III, stable disease (35% regression to 35% growth); IV, slow growth (35-150% growth); V, rapid growth (>150% growth). Using these categories, treatment with Targretin at 272 mg/kg diet resulted in 6 of 11 cancers in category I and only 2 of 11 in categories IV and V. Treatment with Targretin at 92 mg/kg diet resulted in 4 of 12 in categories I or II. However, 6 of 12 tumors administered this middle dose of Targretin demonstrated slow or rapid growth. Finally, cancers treated with 15 mg/kg diet showed minimal effects on tumor growth (8 of 11 showing rapid growth).

Fig. 4. Effect of various Targretin regimens on MNU-induced mammary cancers. Diet supplementation with Targretin (250 mg/kg diet) initiated 5 days after MNU. Groups were: ●, Targretin (continual treatment); □, Targretin (limited treatment, 8 weeks); ▲, Targretin (alternating 3 days on, 4 days off); ◆, control diet.

Fig. 5. Therapeutic effects of Targretin on growth of palpable MNU-induced mammary cancers. Rats were injected i.v. with MNU (50 mg/kg body wt) at 50 days of age. When a rat developed a small palpable cancer, it was randomized to a protocol with various doses of Targretin in the diet. Tumor sizes were measured with calipers twice each week for a period of 5 weeks. (A) Untreated (control) cancers; (B) cancers treated with Targretin at 15 mg/kg diet; (C) cancers treated with Targretin at 92 mg/kg diet; (D) cancers treated with Targretin at 272 mg/kg diet.
Effects of Targretin on proliferation and apoptosis in mammary cancers (experiment V)

Rats bearing small palpable tumors (~1.0 cm in diameter) were treated with Targretin (15 or 150 mg/kg diet) for a limited period of time (7 days). At killing the cancers were quickly removed and processed to determine proliferation and apoptosis. As shown in Figure 7A, Targretin caused a dose-dependent decrease in proliferation. The high dose of Targretin greatly increased apoptosis, while the lower dose failed to significantly alter apoptosis levels (Figure 7B).

Effects of Targretin on serum IGF1 levels (experiments I and II)

Targretin decreased systemic IGF1 levels in a dose–response manner both when Targretin was administered by gavage and when it was added to the diet (Figure 8). As can be seen, only the higher doses of Targretin (60 or 20 mg/kg body wt/day by gavage or 272 mg/kg diet) caused significant reductions in IGF1 levels, reducing serum IGF1 levels by >35% ($P < 0.05$). Interestingly, doses of Targretin which were relatively effective in reducing MNU-induced mammary carcinogenesis (6.7 mg/kg body wt/day by gavage or dietary doses of 50–100 mg/kg diet) had limited effects on serum IGF1 levels.

Discussion

As discussed in the Introduction, Targretin interacts with the various RXR receptors with high affinity. The interesting aspect of the RXR receptors is that they form heterodimers with a varied group of nuclear receptors including peroxisome proliferator-activated receptors (PPARs), constitutitive androstenedione receptor (CAR), RARs, liver X receptors (LXRs), vitamin D receptors (VDRs) and thyroxine receptor (21). The resulting heterodimers serve as transcriptional regulators in which individual heterodimers (e.g. PPARa–RXRa) can turn on a wide variety of genes. Because RXR agonists can simultaneously activate a wide variety of receptors, this class of agents induces varied pleotropic responses. These pleiotropic responses ensure that a wide range of physiological processes will be altered (22). Thus, RXR agonists have been proposed as potential therapeutic agents to deal with diabetes (23) and cholesterol metabolism (22), as well as cancer (24). At present, the RXR agonist Targretin has been shown to be effective in...
the clinical treatment of cutaneous T cell lymphoma and non-
small cell lung cancer (24).

Prior preclinical data had shown the RXR agonists to be highly effective in certain mammary models of cancer (5,6,12), and the present studies confirm these data. The experiments of Gottardis et al. (5) showed that administration of 100 mg/kg body wt/day Targretin profoundly inhibited tumor multiplicity when administered in a prevention setting. We administered Targretin at doses of 0, 60, 20 and 6.7 mg/kg body wt/day by gavage and obtained cancer multiplicities of 3.6, 0.13, 0.53 and 0.80 cancers/rat, respectively. Reductions in cancer multiplicity due to Targretin were highly significant (P<0.01) at all three doses.

We also examined the efficacy of dietary doses of Targretin since this route of treatment is likely to result in sustained mammary tissue exposure. The doses employed, 275 and 92 mg/kg diet, corresponded to the gavage doses of 20 and 6.7 mg/kg body wt/day. A higher dose of Targretin (873 mg/kg diet) caused a marked decrease in body weight within the first 2 weeks following administration and the group was terminated. As can be seen in Figure 2B, the two doses of Targretin caused a dose-dependent decrease in cancer multiplicity. A subsequent study (experiment II) reduced the dose levels of Targretin (15, 50 and 150 mg/kg diet) to determine the complete dose-response characteristics (Figure 3). Note that the lowest dose (15 mg/kg diet) still caused a 33% decrease in tumor number. A subsequent repeat study (data not shown) at lower doses (25, 5 and 1 mg/kg diet) resulted in an ~35% decrease at 25 mg/kg diet and no effect at the lower doses. Thus, it appears that a dose of ~20 mg/kg diet is required to achieve a statistically significant effect, with the 50% effective chemopreventive dose in the range 40–60 mg/kg diet. In addition to decreasing tumor multiplicity, highly effective doses of Targretin also decreased the size of those cancers which were detected. The data emphasize the shallow nature of the dose-response curve for this agent given either by gavage or by diet (Figure 2A and B).

The effects of limited or intermittent exposure to Targretin (250 mg/kg diet) were also determined. MNU control rats developed 4.3 cancers/rat while rats treated continually with Targretin developed 0.50 cancers/rat (an 88% decrease). Treatment for approximately half of the time of the experiment (weeks 1–8) caused a 50% decrease in tumor multiplicity (P<0.05). However, following the removal of Targretin, cancer multiplicity increased rapidly after a short delay. This would imply limited residual effects of Targretin. These data appear to be consistent with exposure to retinoids in humans; for example, the beneficial effects of 13-cis-retinoic acid and other retinoids are quickly lost once the agents are withdrawn in human trials of squamous cell carcinoma of the skin (25) or leukoplakia (26). In contrast, treatment of patients with acute promyelocytic leukemia with all-trans-retinoic acid appears to have effects beyond the time of treatment (27). These agents were all RAR agonists, however, in contrast to the present RXR agonist. Finally, a discontinuous dosing schedule (3 days on Targretin/4 days off) caused a significant decrease in activity relative to continual treatment (P<0.05).

Bischoff et al. (12) previously showed that Targretin is an effective therapeutic agent in this chemically induced model of mammary cancer. We evaluated various doses of Targretin to determine the dose–response relationship between cancer prevention and cancer therapy. Targretin at 272 mg/kg diet caused complete or partial regression of 8 of 12 tumors over a 5 week treatment period. Targretin at 92 mg/kg diet caused complete or partial regression of 4 of 12 cancers, while the lowest dose (15 mg/kg diet) caused partial regression in only 1 of 12 cancers examined. These results show that the highest dose of Targretin which exhibited significant preventive activity (reducing cancer multiplicity >90%) had therapeutic effects in the majority of cancers. However, the middle dose of Targretin (which decreased tumor multiplicity by 72% in a preventative setting) caused complete or partial regression in 4 of 12 cancers but resulted in stable disease in an additional 5 of 12 tumors. Thus, 75% of these tumors showed stable disease or regression while >85% of control tumors showed moderate or rapid growth. Finally, the lowest dose of Targretin examined (15 mg/kg diet), which decreased tumor multiplicity by 35% in a preventative assay, had no discernible therapeutic effects. These data imply that doses necessary for high preventive activity are themselves therapeutically effective. However, doses which show limited but significant chemopreventive activity (e.g. 15 mg/kg diet) may show minimal therapeutic activity.

The effects of various doses of Targretin given for 7 days on proliferation and apoptosis in small palpable mammary tumors were also examined. We treated for this limited length of time for two reasons. First, previous data from our laboratories had shown that limited treatment with the aromatase inhibitor vorozole resulted in alterations in proliferation and apoptosis with doses which showed chemopreventive activity but limited therapeutic activity (14). Second, longer lengths of exposure (>4 weeks) may reflect resistant cells or, alternatively, cancers which had undergone significant regression. It was found that the dose of Targretin (150 mg/kg diet) which decreased tumor multiplicity >75% resulted in decreased proliferation (P<0.05) and increased apoptosis (P<0.05). In contrast, the dose of 15 mg/kg diet, which had minimal therapeutic efficacy but some preventive efficacy (35% decrease in tumor multiplicity), had limited, albeit significant (P<0.05), effects on proliferation but no effect on apoptosis. These results suggest that one might determine a highly effective dose of Targretin or another RXR agonist by examining for effects on proliferation and apoptosis in this cancer model. In agreement with the decreased proliferative index, we have identified multiple proliferation-related genes whose expression was altered in small cancers by Targretin (150 mg/kg diet) treatment by gene microarray analysis, e.g. cyclin B1 and proliferating cell nuclear antigen (Y.Wang, R.Yao, C.Grubbs, R.Lubet and M.You, unpublished data). The similarities in the doses required for therapy and prevention, the strong effects on proliferation and apoptosis in tumors and the minimal efficacy of limited treatment in the preventative setting all argue that the effects of Targretin are on the lesions themselves and not on normal or recently initiated cells. In preliminary studies employing normal rat mammary epithelia we found that limited treatment (7 days) with Targretin (150 mg/kg diet) decreased cell proliferation by ~35%, implying a more limited effect on normal tissue than on tumors.

Epidemiological evidence in humans has indicated that higher IGF1 levels in serum are associated with a variety of human cancers, including breast cancer (16–18). Furthermore, it has previously been shown that tamoxifen and arzoxifene, a highly effective SERM against ER+ breast tumors, reproducibly decreases IGF1 levels (28). However, the aromatase inhibitors, which are also highly effective as therapeutic agents and are active preventive agents, do not alter IGF1 levels in
either animals (3) or women (29). IGF1 levels in serum, which presumably reflect liver production of IGF1, were significantly decreased at the higher preventive doses of Targretin (20 mg/kg body wt/day by gavage or 250 mg/kg diet) but were minimally affected by lower but still relatively active doses of Targretin (e.g. 6.7 mg/kg body wt/day by gavage or 50–100 mg/kg diet). The present results showing modulation of IGF1 levels by RXR analogs is in line with both previously published data on retinoids decreasing IGF1 (31) and studies showing that mice with knockout of RXRs have increased IGF1 levels (32).

The present results obtained in the hormonally responsive mammary cancer model confirm the results previously obtained by Gottardis et al. (5) with Targretin and appear to be in agreement with the findings of Suh et al (6) showing that another RXR agonist LGD 268 is highly effective as a preventive agent in this model. The results also: (i) imply that the preventive effects of Targretin are lost once treatment is stopped; (ii) imply that determining effects on proliferation and apoptosis may provide early data about what will be an effective dose; (iii) imply that serum IGF1 levels are likely to be an inexact surrogate for the effective dose of Targretin. Although IGF1 was decreased by 30–40% by highly effective doses of Targretin, it was minimally affected by doses which were still significantly effective. The data also suggest that the preventive dose is not significantly different from the therapeutic dose. However, there may be a difference as great as double which might be difficult to clearly discriminate. Recent findings by Brown and co-workers (30) have shown that Targretin is highly effective in the treatment of hormonally non-responsive mammary tumors induced in mice by over-expression of the Neu oncogene. Thus, the combination of a SERM or an aromatase inhibitor taken together with a RXR agonist, such as Targretin, is likely to be a highly effective preventive regimen against both ER+ and ER- cancers in humans. These studies do not clearly determine a mechanism for this agent. As mentioned above, RXR agonists form heterodimers with a wide variety of nuclear receptors (PPARs, LXR, RARs, CAR, VDR, etc.) RXRs form active heterodimers (with contributions from both ligands for RXR and orphan receptors) with a more limited number of receptors, including PPARs, LXR, CAR and pregnane X receptor (PXR). Which specific heterodimer or combination of heterodimers contribute to the preventive/therapeutic effects observed in this model is not clear. Although we have observed increased expression of certain PPAR-stimulated genes in these Targretin cancers (e.g. acyl-CoA:lysyl enzyme, A carboxylase and fatty acid hydrolase A ligase long chain 2) (Y. Wang, R. Yao, C. Grubbs, R. Lubet and M. You, unpublished data), this does not prove that it is this receptor that is crucial in achieving the efficacy observed.

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