Effect of the Retinoid X Receptor-Selective Ligand LGD1069 on Mammary Carcinoma After Tamoxifen Failure

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Background: We have previously shown that a retinoid X receptor (RXR)-selective ligand (a retinoid), called LGD1069, is highly efficacious in both the chemoprevention and chemotherapy for N-nitrosomethurea-induced rat mammary carcinomas. To evaluate a possible role for retinoids in breast cancer therapy further, we have designed and characterized a novel carcinogen-induced model to mimic the clinical situation in which the tumors of patients stop responding to tamoxifen therapy and develop resistance to this drug. Methods: Rats with experimentally induced mammary tumors were treated with tamoxifen to select a population with primary tumors that failed to respond completely to the drug. Once the failure of tamoxifen therapy had been established, LGD1069 was added to the treatment regimen, and the tumors in these animals were compared with tumors in a group of animals that remained on tamoxifen alone. Results: LGD1069 in combination with tamoxifen for up to 20 weeks yielded an overall objective response rate of 94% (95% confidence interval [CI] = 86%–100%) (includes complete and partial responses) in primary tumors compared with a rate of 33% (95% CI = 11%–56%) in primary tumors treated with tamoxifen alone, a statistically significant difference (two-sided P < 0.0001). In addition, the LGD1069 and tamoxifen combination was associated with a statistically significant decrease in total tumor burden (two-sided P = 0.03). In a second study, tumors that failed to respond to tamoxifen therapy exhibited a 51% (95% CI = 34%–71%) objective response rate when treated with LGD1069 alone for 6 weeks after tamoxifen therapy was withdrawn. Conclusion: We have demonstrated that the RXR-selective ligand LGD1069 in combination with tamoxifen is a highly efficacious therapeutic agent for tumors that fail to respond completely to tamoxifen. This finding suggests that retinoid therapy offers a novel approach to the treatment of breast tumors that may have developed resistance to antihormonal therapies such as tamoxifen. [J Natl Cancer Inst 1999;91:2118–23]

Tamoxifen is the most widely prescribed drug for breast cancer in the world. It is highly effective as an adjuvant therapy following removal of the primary tumor and prolongs both the time to recurrence of disease and survival rates in humans. Tamoxifen is also highly efficacious, causing tumor regression in nearly half of the patients with advanced metastatic disease. However, the use of tamoxifen has limits because the majority of patients eventually stop responding to tamoxifen therapy and many develop resistance to tamoxifen after long-term administration, some in as short as 1–2 years (1–3). Although additional second- or third-line endocrine therapies, such as aromatase inhibitors, or novel antiestrogens, such as idoxifene, can partially overcome tamoxifen resistance (3,4), there remains a great need for novel therapies that maintain antitumor efficacy after the failure of tamoxifen.

During the last few years, we have focused our efforts on the design and pharmacologic characterization of both naturally occurring and synthetic compounds that interact with the retinoid X receptors (RXRs). The RXRs are members of the intracellular receptor superfamily that play a pivotal role in multiple endocrine signaling pathways. This activity stems from their ability to heterodimerize with numerous intracellular receptors, including the retinoic acid receptors (RARs), vitamin D receptor, peroxisome proliferator-activated receptors (PPARs), thyroid hormone receptors (TRs), and liver X receptors (LXRs) (5). Thus, RXR-selective ligands (retinoids) control unique signaling pathways and offer multiple opportunities for the development of new therapies for diseases that range from cancer to metabolic disorders such as type II diabetes (6).

We have previously demonstrated that the novel retinoid LGD1069 is highly efficacious in two different models of carcinogen-induced mammary carcinoma. LGD1069 is as potent as tamoxifen in preventing the development of mammary tumors in the N-nitrosomethurea-induced mammary carcinoma model (7). In addition, LGD1069 was much more efficacious than traditional RAR-selective retinoids in this model (8,9). In a model of a more advanced disease state, in which classical retinoids are not active (10), LGD1069 is highly efficacious against established carcinogen-induced rat mammary tumors, causing regression in 72% of primary tumors compared with only 33% of primary tumors for tamoxifen (11). Furthermore, combination therapy with doses of LGD1069 and tamoxifen that are ineffective separately demonstrated a greater than additive antitumor effect over monotherapy. LGD1069 has been shown to be safe and tolerable in an initial clinical trial (12).

The encouraging results with LGD1069, alone or in combination therapy, led us to explore the arena of tamoxifen failure, a major hurdle in breast cancer therapy. We set out to develop a novel carcinogen-induced mammary tumor model, which closely mimics the human clinical situation, to examine the effect of LGD1069 on tumors that had stopped responding to tamoxifen therapy and may have developed resistance.

Materials and Methods

Formulation of Compounds

LGD1069 (Targretin®; Ligand Pharmaceuticals Inc., San Diego, CA) was suspended in an aqueous solution composed of 10% (vol/vol) polyethylene glycol/ Tween 80 (99.5 : 0.5) and 90% of 1% (wt/vol) carboxymethylcellulose (Sigma Chemical Co., St. Louis, MO). LGD1069 (100 mg/kg of body weight) was administered orally to animals in 1 mL by a 16-gauge gavage needle (Popper and Sons, New Hyde Park, NY) based on an average body weight. Tamoxifen (Sigma Chemical Co.) was formulated in purified sesame oil (Croda, Parsippany, NJ) by first dissolving in a small volume of ethanol and then evaporating the ethanol under a stream of purified nitrogen to solidify the tamoxifen into the sesame oil. Animals were dosed with a volume of 0.1 mL (subcutaneously) at a dose of 800 μg of tamoxifen/kg of body weight.

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Development of Tamoxifen-Resistant Tumors, Tumor Induction, Measurement, and Scoring

N-Nitrosomethylurea (Sigma Chemical Co.) was formulated as an aqueous solution of 10 mg/mL by wetting N-nitrosomethylurea powder with 3% acetic acid and dissolving it in sterile saline. Fresh solutions of N-nitrosomethylurea were injected within 30 minutes of preparation. Virgin female Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN), 50 days of age, received 50 mg/kg of body weight (intravenously, in the tail vein) as previously described (7) (Fig. 1, left, point A). Rats were housed in a U.S. Department of Agriculture (Washington, DC)-registered facility in accordance with National Institutes of Health guidelines for the care and use of laboratory animals. All animals received food (Teklad LM485-7012; Harlan Sprague-Dawley, Inc.) and water ad libitum. Beginning 5 weeks after tumor induction, animals were examined for tumors twice a week. Tumors were measured with electronic calipers (Mitutoyo Inc., Utsunomiya, Japan), and cross-sectional areas were determined by multiplying the longest length of the tumor by the greatest perpendicular width of the tumor.

The primary tumor was defined as the first tumor (or tumors, if more than one tumor achieved the predetermined size within 4 days of initiating treatment) to reach 75 mm² in cross-sectional area. Subsequent tumors to reach 75 mm² in cross-sectional area were defined as second primary tumors; i.e., they are not derivatives or metastases of the first tumor but arise as independent tumors following N-nitrosomethylurea induction. Treatment with tamoxifen at 800 μg/kg of body weight (subcutaneously) was initiated once an individual animal had a qualifying primary tumor (Fig. 1, left, point B). Tamoxifen treatment continued for 6 weeks, and tumor responses were scored at the end of the initial tamoxifen treatment (Fig. 1, left, point C). The following categories were used to score the tumor response: progressive disease—the tumor grew over the course of treatment, and its final area was at least 40% greater than its initial area; stable disease—the tumor did not fluctuate more than 40% from its initial area throughout the course of treatment; partial response—the tumor regressed more than 40% from the initial area or showed at least two consecutive decreases in area that totaled more than 40% from the maximum tumor area; and complete response—the tumor was no longer measurable or no longer palpable if the tumor was palpable but not measurable, it was recorded as having a cross-sectional area of 4 mm². These criteria are similar to the methods of Rose and Mountjoy (13) and the methods that we published earlier (11).

Previously, we published that, following 6 weeks of tamoxifen therapy at 800 μg/kg of body weight (subcutaneously) (Fig. 1, left, point C), approximately 33% of the tumors will completely regress in response to tamoxifen therapy (11). The response in this study (n = 70 tumors) was similar, with 28.6% of the tumors completely regressing. Animals with primary tumors that completely regressed ("Responders" in Fig. 1) were considered to be tamoxifen responsive and thus were removed from the study. The remaining 71.4% of the cases were scored as progressive disease (40%), stable disease (21.4%), and partial response (10%). These groups were designated as having failures of tamoxifen therapy and thus were randomly assigned into two new treatment groups (Fig. 1, left, point C, "Tamoxifen Failures"). The animals with tumors that initially stopped responding to tamoxifen therapy either remained on tamoxifen at 800 μg/kg of body weight daily (Fig. 1, left, points C to D) as a single agent or had LGD1069 daily at 100 mg/kg of body weight (Fig. 1, left, points C to E) added to the tamoxifen therapy. The compounds were administered for a period of up to 20 additional weeks of therapy. In a similar study, animals that had failed to respond to 6 weeks of tamoxifen therapy had tamoxifen withdrawn from their therapeutic regimen before the initiation of daily LGD1069 therapy at 100 mg/kg of body weight.

At the completion of the therapy, based on the intent-to-treat principle, tumors were re-evaluated and again scored as progressive or stable disease or partial or complete response on the basis of their individual therapeutic response following combined LGD1069 and tamoxifen therapy (Fig. 1, left, point E) or continued tamoxifen therapy alone (Fig. 1, left, point D). Following randomized assignment into one of the two treatment groups, there was no statistically significant difference (Table 1; two-sided P = 0.33) between the two treatment groups in their response to the initial 6 weeks of tamoxifen therapy (Fig. 1, left, points B to C). Second primary tumors, as defined above, did not receive the entire initial 6-week course of tamoxifen therapy and thus are not included in the evaluation of these data as primary lesions but are included in the total tumor burden analysis.

### Statistical Methods

Primary tumor response rates and initial animal allocation were compared with the use of Fisher’s exact test (two-tailed), with the null hypothesis of no difference between the two treatment groups. Analysis of tumor burden was performed by use of a Shapiro–Wilk test to test for normality and was followed by a t-test. All statistics and quality control of

![Fig. 1](image-url)

**Fig. 1.** Left: the development and ruxioid treatment of tamoxifen (TAM)-resistant tumors in the rat. Animals are administered N-nitrosomethylurea (NMU) at a dose of 50 mg/kg of body weight (point A). Tumors are allowed to develop, and when at least one tumor on an animal has achieved 75 mm² in cross-sectional surface area (a primary tumor), the animal is then put on tamoxifen therapy, 800 μg/kg per day of body weight (subcutaneously) (point B). Following 6 weeks of tamoxifen therapy (point C), animals are evaluated for primary tumor response. Animals in which the primary tumors regressed were removed from the study because, by definition, their tumors were tamoxifen responsive. The remaining animals had primary tumors that were classified as progressive disease, stable disease, or partial regression and thus as failures of tamoxifen therapy. These animals were randomly assigned either to remain on tamoxifen therapy alone (to evaluate resistance) or to add a retinoid X receptor ligand (called LGD1069) at a dose of 100 mg/kg per day of body weight (orally) in combination with tamoxifen to their therapeutic regimen. Animals were treated for an additional amount of time (see "Materials and Methods" section), and tumors were evaluated for their response to therapy over time and on termination (points D or E). Right: Response of primary tumors to tamoxifen after the initial 6 weeks of therapy. Animals with primary tumors that had completely regressed at the end of the initial therapy were removed from the study. Results reflect only primary tumors and are displayed as percent of progressive disease (tumors that grew during the treatment period), percent of stable disease (tumors that fluctuated <40% from the initial tumor area during the treatment period), percent of partial regressions (tumors that regressed at least 40% from their maximum size during the course of the treatment period), or percent of complete regression (tumors that regressed until they were no longer palpable) (n = 70 primary tumors).
data analysis were performed by Synteract, Inc., Encinitas, CA. All P values are two-sided, and values of less than .05 were considered to be statistically significant.

**RESULTS**

**Therapeutic Efficacy of the LGD1069 and Tamoxifen Combination Therapy on Tumors for Which Tamoxifen Has Failed**

To evaluate the antitumor efficacy of the combination of LGD1069 and tamoxifen in a setting of tamoxifen failure, tumors that have previously failed to respond to tamoxifen therapy were tested for their response to renoxinoid therapy. LGD1069 at a dose of 100 mg/kg per day of body weight (orally) was added to the tamoxifen regimen (Fig. 1, left, points C to E) for one group of animals that were compared with animals that remained on continuous tamoxifen alone (Fig. 1, left, points C to D). This dose of LGD1069 was previously shown to be well tolerated and efficacious in both long-term chemoprevention studies and therapeutic studies on established mammary tumors (7,11).

The addition of LGD1069 to the tamoxifen treatment regimen showed statistically significant antitumor efficacy on tumors for which tamoxifen had failed and had potentially developed tamoxifen resistance when compared with tumors that remained on tamoxifen therapy alone (Table 1). After 6 weeks of additional therapy, only 3% (95% confidence interval [CI] = 0%–9%) of the tumors continued to progress following combination therapy with LGD1069 and tamoxifen, whereas 44% (95% CI = 21%–68%) of the tumors remaining on tamoxifen therapy alone progressed, supporting the premise that these tumors were tamoxifen resistant. LGD1069 in combination with tamoxifen caused a complete response of 56% (95% CI = 39%–74%) of primary tumors compared with 17% (95% CI = 0%–35%) for tamoxifen alone. In addition, a total of 90% (95% CI = 80%–100%) of LGD1069-treated primary tumors showed an objective response to therapy (e.g., complete or partial response) compared with 45% (95% CI = 21%–69%) of the tumors treated with tamoxifen alone (P = .0002). In a second study, animals with tumors that failed to respond to tamoxifen therapy had tamoxifen withdrawn before initiating LGD1069 at a dose of 100 mg/kg per day of body weight (orally) alone (Table 2). This study demonstrated a 51% (95% CI = 34%–71%) objective response rate following an additional 6 weeks of LGD1069 therapy alone. These data suggest that greater response rate is enhanced by the combination of LGD1069 with tamoxifen when compared with tamoxifen therapy alone.

LGD1069 in combination with tamoxifen showed even greater efficacy when therapy was continued beyond 6 weeks (for up to 20 weeks), with a 94% (95% CI = 86%–100%) objective response rate. In this case, 60% (95% CI = 43%–77%) of the LGD1069 and tamoxifen-treated tumors regressed completely and 34% (95% CI = 17%–51%) regressed partially (Fig. 2A). Furthermore, only 18% of the primary tumors on animals treated long-term (up to 20 weeks) with tamoxifen alone showed an objective response, with only 22% (95% CI = 2%–42%) regressing completely. Thus, the addition of LGD1069 to the tamoxifen therapeutic regimen was statistically significantly more efficacious than tamoxifen therapy alone (P < .0001). These data also illustrate that, as tamoxifen therapy is continued (beyond 6 weeks), multiple tumors develop a tamoxifen-resistant phenotype. In contrast, only 6% of the LGD1069-treated tumors progressed, whereas 56% of the primary tumors remaining on tamoxifen treatment alone continued to grow (P < .0001). A more detailed analysis of tumor response reveals that animals with the most aggressive tumors—i.e., those with progressive disease at the initiation of combined LGD1069 and tamoxifen treatment (Fig. 1, left, points C to D)—were much more likely (18 of 20 tumors; Table 1) to manifest an objective response than if they had continued on tamoxifen therapy alone (one of eight tumors; P = .0002). This demonstrates that the addition of LGD1069 to a tamoxifen regimen is an effective therapy against tumors that have failed to respond to tamoxifen therapy or acquired tamoxifen resistance.

The process of carcinogen initiation in this model puts the animals at risk for developing multiple independently arising breast tumors. The above analysis of tumor response is based on the assumption that multiple primary tumors on the same animal will respond independently to treatment. Analysis of the individual tumor response data from animals bearing multiple primary tumors demonstrated that the tumors acted independently and

<table>
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<th>Study No.</th>
<th>Treatment</th>
<th>Complete response</th>
<th>Partial response</th>
<th>Stable disease</th>
<th>Progressive disease</th>
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<tr>
<td>1 (n = 32)</td>
<td>LGD1069 + tamoxifen</td>
<td>(39–74)</td>
<td>(18–51)</td>
<td>(0–15)</td>
<td>(0–9)</td>
</tr>
<tr>
<td>2 (n = 29)</td>
<td>LGD1069 alone</td>
<td>(23–60)</td>
<td>(0–21)</td>
<td>(9–39)</td>
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*Analysis following 6 weeks of additional therapy once tumors had experienced failure of tamoxifen alone. Values in parentheses = 95% confidence intervals.
Fig. 2. The retinoid X receptor ligand (called LGD1069) in combination with tamoxifen causes regression in the majority of established N-nitrosomethylurea-induced mammary tumors. Tumors were allowed to grow to approximately 75 mm$^2$ in cross-sectional surface area, then were treated with tamoxifen at a dose 800 μg/kg per day of body weight (subcutaneously) for 6 weeks to evaluate response to tamoxifen. Animals with tumors that failed to respond to tamoxifen therapy were allocated to one of two groups, either to remain on tamoxifen therapy alone or to add LGD1069 at a dose of 100 mg/kg per day of body weight (orally) to their therapeutic regimen. A) Analysis made after up to 20 weeks of additional therapy, based on intent-to-treat analysis. * = a statistically significant difference between tamoxifen therapy alone and tamoxifen therapy with LGD1069 (two-sided $P = .02$; Fisher’s exact test). + = a statistically significant difference between tamoxifen therapy alone and tamoxifen therapy with LGD1069 (two-sided $P = .0002$; Fisher’s exact test). B) LGD1069 decreases the tumor burden of N-nitrosomethylurea-induced mammary carcinoma-bearing rats. Tumor burden is defined as the mean of the sum of the cross-sectional areas of all tumors. Values indicated are the percent change in mean tumor burden from start of second primary therapy to final analysis, based on an intent-to-treat analysis. * = a statistically significant difference between the percent change in primary tumor burden for LGD1069 + tamoxifen therapy (95% confidence interval [CI] = −86% to −49%) and tamoxifen therapy alone (95% CI = −57% to 87%) (two-sided $P < .0001$; Student’s $t$ test). + = a statistically significant difference between the percent change in total (primary and second primary) tumor burden for LGD1069 + tamoxifen therapy (95% CI = −65% to −3%) and tamoxifen therapy alone (95% CI = −29% to 52%) (two-sided $P = .03$; Student’s $t$ test) (n = 16 animals for tamoxifen alone and n = 22 animals for tamoxifen + LGD1069).

demonstrated mixed responses to therapy (i.e., an animal bearing two primary tumors had a complete response in one tumor, while the other tumor showed progressive disease) (data not shown). To further ensure that no bias was introduced in the tumor response data by the inclusion of animals bearing multiple primary tumors, the analysis was conducted utilizing tumor response data from animals bearing only one primary tumor. After 20 weeks of additional therapy, animals (n = 14) receiving the LGD1069 plus tamoxifen combination had 79% (95% CI = 49%–95%) of the primary tumors regress completely, whereas only 27% (95% CI = 8%–55%) of the animals (n = 15) remaining on tamoxifen therapy alone had their primary tumors regress completely. Thus, as demonstrated above, the addition of LGD1069 to the tamoxifen regimen was statistically significantly more efficacious at causing complete tumor regression than tamoxifen therapy alone ($P = .009$). In addition, there was a 100% (95% CI = 77%–100%) objective response rate in animals bearing a single primary tumor when LGD1069 was added to the tamoxifen regimen compared with a 40% (95% CI = 16%–68%) objective response rate in animals that remained on tamoxifen therapy alone ($P = .0007$). Therefore, regardless of whether the analysis of tumor response included animals with multiple primary tumors or was performed on animals with only a single primary tumor, the data demonstrate that the addition of LGD1069 to a tamoxifen regimen is effective therapy for tumors for which tamoxifen therapy has previously failed.

Total Tumor Burden Following Combination LGD1069 and Tamoxifen Therapy

The carcinogen N-nitrosomethylurea puts animals at risk for developing multiple mammary tumors. These tumors were not derivatives or metastases of the primary tumor but developed as independent clones as a result of the carcinogen initiation. Tumors were allowed to develop in naive, untreated animals until at least one tumor reached 75 mm$^2$ in the cross-sectional surface area, defined as a primary tumor. Most animals developed additional tumors, defined as second primary tumors, which arose following the initiation of tamoxifen therapy (Fig. 1, point B). This model allows one to study the effect of therapeutic agents on both primary and second primary tumors.

To examine the effect of LGD1069 and tamoxifen combination on all tumors, the total tumor burden was evaluated. Tumor burden was defined as the mean of the sum of the cross-sectional surface area for all tumors, primary and/or second primary, on an animal. This analysis compared the change in total tumor burden from the initiation of LGD1069 and tamoxifen combination therapy with the final analysis (Fig. 1, left, points C to D or C to E). The LGD1069 and tamoxifen combination therapy statistically signifi-
cantly decreased the primary tumor burden by 68% (95% CI = −86% to −50%), from 304 mm$^2$ to 132 mm$^2$ (Fig. 2, B; $P = .03$; Student’s $t$ test). In contrast, the primary tumor burden increased in the tamoxifen-alone group by 15% (95% CI = −57% to 88%), from 341 mm$^2$ to 439 mm$^2$. When both the primary and second primary tumor burdens are combined, LGD1069 decreased the total tumor burden by 34% (95% CI = −65% to −3%) compared with an increase of 12% (95% CI = −29% to 53%) for tamoxifen treatment alone. Therefore, LGD1069 in combination with tamoxifen decreased the growth of all tumors more effectively than did tamoxifen alone.

**DISCUSSION**

During the last 20 years, since the introduction of tamoxifen as a breast cancer therapy, tamoxifen has been proven to be a highly effective therapy for estrogen receptor-positive breast cancer. Breast cancer patients receive tamoxifen as a first-line antihormonal therapy for estrogen receptor-positive tumors. It also is utilized as a therapeutic agent for patients with late-stage or recurrent disease. Whether utilized in an adjuvant setting or as a therapeutic agent for advanced disease, tamoxifen therapy will eventually fail in the majority of patients, and many patients will develop tamoxifen resistance. Although the eventual failure of tamoxifen therapy in patients and the development of tamoxifen resistance are significant setbacks for breast cancer therapeutics, the process is not well understood and only a limited number of models are available to evaluate potential mechanisms and therapeutic agents to overcome or prevent resistance. There are second-line therapies for breast cancer following tamoxifen failure, including other antiestrogenic agents, aromatase inhibitors, progesterone antagonists, and chemotherapy. However, most patients with advanced breast cancer will eventually die of their disease; thus, the need for novel therapies is important.

The $N$-nitrosomethylurea-induced mammary carcinoma model in the rat is a hormone-dependent model that has been historically employed to identify tamoxifen and other antihormonal agents or retinoids as potential therapeutic agents for both the prevention and treatment of human breast cancer (8,14,15). Our previous work (7,11) demonstrated that LGD1069, a rexinoid, not only was a highly effective chemopreventive agent in this model but also was more efficacious than tamoxifen at causing complete response of established rat mammary carcinomas. Furthermore, we demonstrated that the combination of ineffective doses of LGD1069 and tamoxifen led to a statistically significant number of complete responses of established mammary tumors. To further explore the activity of retinoids in a carcinogen-induced mammary carcinoma model, we have developed a novel model that escapes antihormonal tamoxifen therapy yet—of interest—still retains sensitivity to rexinoid therapy. The current data clearly demonstrate that LGD1069 can overcome tamoxifen failure and provide a rationale for combining LGD1069 with tamoxifen in the clinic to increase the antitumor efficacy of tamoxifen, as well as providing a rationale to use LGD1069 as a novel therapy in patients for whom tamoxifen therapy has failed. Additionally, in an adjuvant setting, LGD1069, in combination with tamoxifen, may delay the time to disease progression and/or enhance therapeutic efficacy.

We have shown that LGD1069 is an effective chemopreventive and chemotherapeutic agent in the $N$-nitrosomethylurea-induced mammary tumor model (7,11); thus, LGD1069 can influence both the development and the maintenance of the malignant phenotype. The efficacy of tamoxifen therapy is dependent on the expression of estrogen receptors; thus, its utility is limited to tumors that express these receptors. Similarly, the recently approved breast cancer therapeutic agent Herceptin is directed toward tumors that express the HER-2/neu proto-oncogene. RXRα is expressed ubiquitously in adult tissue, which is different than many receptor subtypes. Thus, unlike tamoxifen or Herceptin that are targeted therapies, LGD1069 does not depend on the restricted expression pattern of a sex-steroid hormone receptor or a specific growth factor receptor; therefore, the agent may have effects on a variety of breast tumors as well as other cancers. The potential mechanisms by which LGD1069 functions in these cancer models may include interference with the estrogen signaling pathway, modulation of breast cancer epithelial cell differentiation, or an antiproliferative effect. Although these potential mechanisms for the action of LGD1069 may imply that the compound would have wide-ranging activities in many cells and tissues, LGD1069 has been shown to be safe and tolerable in a phase I clinical trial (12).

To further elucidate the mechanism by which retinoids exert their anticancer activity, we are trying to identify the key heterodimeric partners for RXR in mammary carcinoma models. RXR forms heterodimers with a number of nonsteroid receptors, such asRAR, LXR, PPAR, and TR. Although the heterodimeric partner involved in mediating the action of LGD1069 on mammary carcinoma is not known, one likely family of partners are the PPARs. RXR–ligand activation of the RXR:PPARγ heterodimer has been shown to play a critical role in adipocyte differentiation (16). Consistent with that observation is the preliminary evidence from our laboratory that suggests that the mechanism of action of RXR–ligands in breast cancer involves altering the genetic program of differentiation in mammary epithelial cells by inducing the modification of lipid metabolism leading to terminal cell division (17). This hypothesis is further supported by the observation that activation of the RXR:PPARγ transcriptional pathway can induce terminal differentiation of malignant breast epithelial cells as well as other human tumor cells with diverse origins, including liposarcoma and prostate and colon carcinomas (18–22). Thus, activation by LGD1069 of the RXR:PPAR heterodimer (23) or other receptors (such as the orphan receptor LXR) may play a major role in cellular metabolism or differentiation by reprogramming gene expression. This modification of gene expression may lead to a novel therapy for breast cancer, allowing LGD1069 to interfere with mammary carcinoma at three distinct phases of the disease: 1) during carcinogenesis as a chemopreventive, 2) as a therapeutic agent acting on established disease, and 3) as an effective therapeutic agent in the presence of antiestrogen failure.

**REFERENCES**


