A New Rexinoid, NRX194204, Prevents Carcinogenesis in Both the Lung and Mammary Gland

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Abstract

Purpose: We evaluated the anti-inflammatory and growth-inhibitory properties of the novel rexinoid NRX194204 (4204) *in vitro* and then tested its ability to prevent and/or treat experimental lung and estrogen receptor (ER) – negative breast cancer *in vivo*.

Experimental Design: In cell culture studies, we measured the ability of 4204 to block the effects of lipopolysaccharide and induce apoptosis. For the lung cancer prevention studies, A/J mice were injected with the carcinogen vinyl carbamate and then fed 4204 (30-60 mg/kg diet) for 15 weeks, beginning 1 week after the administration of the carcinogen. For breast cancer prevention studies, mouse mammary tumor virus-neu mice were fed control diet or 4204 (20 mg/kg diet) for 50 weeks; for treatment, tumors at least 32 mm^3 in size were allowed to form, and then mice were fed control diet or 4204 (60 mg/kg diet) for 4 weeks.

Results: Low nanomolar concentrations of 4204 blocked the ability of lipopolysaccharide and tumor necrosis factor-α to induce the release of nitric oxide and interleukin 6 and the degradation of IKBα in RAW264.7 macrophage-like cells. In the A/J mouse model of lung cancer, 4204 significantly ($P < 0.05$) reduced the number and size of tumors on the surface of the lungs and reduced the total tumor volume per slide by 64% to 81% compared with the control group. In mouse mammary tumor virus-neu mice, 4204 not only delayed the development of ER-negative mammary tumors in the prevention studies but also caused marked tumor regression (92%) or growth arrest (8%) in all of the mammary tumors when used therapeutically.

Conclusions: The combined anti-inflammatory and anticarcinogenic actions of 4204 suggest that it is a promising new rexinoid that should be considered for future clinical trials.

Lung cancer and breast cancer are the two leading causes of cancer deaths in the United States, accounting for, respectively, >160,000 and 40,000 deaths every year (1). Prevention will ultimately be the most effective strategy for significantly reducing these mortality rates, but better drugs and drug combinations are needed for both the prevention and treatment of these devastating diseases (2, 3).

Rexinoids, selective ligands for the retinoid X receptors (RXRα, RXRβ, and RXRγ), are multifunctional drugs that are useful for both the prevention and treatment of lung and breast cancer in preclinical animal models (reviewed in ref. 4). Because retinoid X receptors heterodimerize with other receptors in the nuclear receptor superfamily, they modulate the activities of numerous steroid-like molecules and thus regulate cell proliferation, differentiation, and apoptosis pathways. Bexarotene (LGD1069), currently the only rexinoid available for clinical use, is approved by the Food and Drug Administration for the treatment of cutaneous T-cell lymphomas, and clinical trials evaluating its effectiveness for the treatment of breast and lung cancer are ongoing (5 – 8). However, because it also binds to the retinoic acid receptors, bexarotene still retains some undesirable toxicity.

In contrast, the newer rexinoids LG100268 (268; ref. 9) and 4204 (originally synthesized as AGN194204, and now called NRX194204; ref. 10) are more potent and more selective than bexarotene, with essentially no affinity for retinoic acid receptors. These newer agents are isosteric with 9-*cis*-retinoic acid; both 268 and 4204 are locked into the 9-*cis*-oid conformation (and hence do not bind to retinoic acid receptors) by the cyclopropyl group in their side chain (see Fig. 1). Although bexarotene effectively prevents and treats experimental estrogen receptor – negative (ER-) breast (11 – 13) and lung (14 – 17) cancers, 268 is clearly more potent than bexarotene for the prevention and treatment of ER- breast cancer (18 – 20). 4204 enhances the ability of ligands for peroxisome proliferator – activated receptors or cytotoxic drugs, including cisplatin and 5-flurouracil, to inhibit proliferation and induce apoptosis in breast and pancreatic cancer cell lines (21, 22), but its efficacy *in vivo* has not yet been reported.

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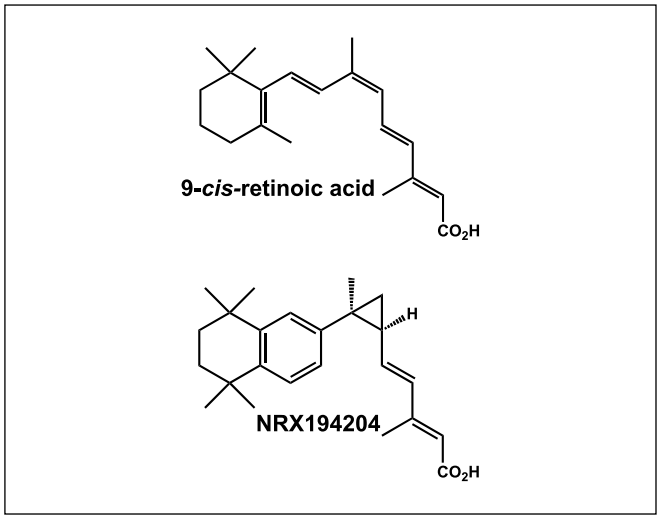

Fig. 1. Structures of 9-*cis*-retinoic acid and NRX194204.

In the present studies, we evaluated the anti-inflammatory effects of the rexinoid 4204 on a macrophage-like cell line and measured its ability to suppress proliferation and induce apoptosis in human lung and breast cancer cell lines. We then tested its ability to prevent cancer in the A/J mouse model of lung cancer (23) and the mouse mammary tumor virus (MMTV)-neu model of ER- breast cancer (24). We report here, for the first time, that 4204 not only reduces the number and size of adenocarcinomas in the lung but also prevents the development of mammary tumors and induces striking tumor regression in established mammary tumors.

Materials and Methods

Reagents and* in vitro *assays. All cell lines were obtained from American Type Culture Collection, except for the E18-14C-27 cells (12), which were kindly provided by Powel Brown (Baylor College of Medicine, Houston, TX). Cells were grown in either DMEM/F12 (A427, H358, and SK-BR-3 cells) or DMEM (E18 and RAW cells) containing 10% fetal bovine serum. Triterpenoids (25, 26) and 4204 (10) were dissolved in DMSO, and controls containing equal concentrations of DMSO (≤0.1%) were included in all cell culture experiments. To measure interleukin 6 release, medium from RAW264.7 cells were analyzed using an interleukin 6–specific Quantikine ELISA kit (R&D Systems). Details of cell culture conditions and all other assays are described in the figure legends or in published methods (27–29).

In vivo *assays.* For the prevention of lung cancer, female A/J mice (Jackson Laboratory) were injected i.p. with two doses (0.32 mg/mouse/dose in saline) of vinyl carbamate (Toronto Research Chemicals), 1 week apart. Beginning 1 week after the final dose of carcinogen, mice were fed 4204 in diet for 15 weeks. For all feeding experiments, 4204 was dissolved in one part ethanol and three parts Neobee oil (Stepan Company) and mixed in powdered 5002 rodent chow (PMI Feeds); the same vehicle was included in all control diets. The evaluation of the lungs has been previously described (30), and the data were pooled from two independent *in vivo* experiments. For the prevention of ER- breast cancer, female MMTV-neu transgenic mice (Jackson Laboratory) were fed control diet or diet containing 4204, beginning at 10 weeks of age. Mice were palpated for tumors weekly for 50 weeks. For treatment studies, a separate cohort of mice was fed normal rodent chow until tumors developed. When tumors were at least 32 mm^3 in volume ($v = lwh/2$), mice were fed 4204 in the diet for up to 4 weeks. Tumors were measured weekly with calipers, and tumor regression was defined as at least a 50% decrease in tumor volume. Active tumor growth was defined as a >50% increase in tumor volume. Further details of the ER- experiments, including evaluation of transgene expression and terminal nucleotidyl transferase–mediated nick end labeling staining, have been previously reported (20). Animal studies have been done in compliance with standards maintained by

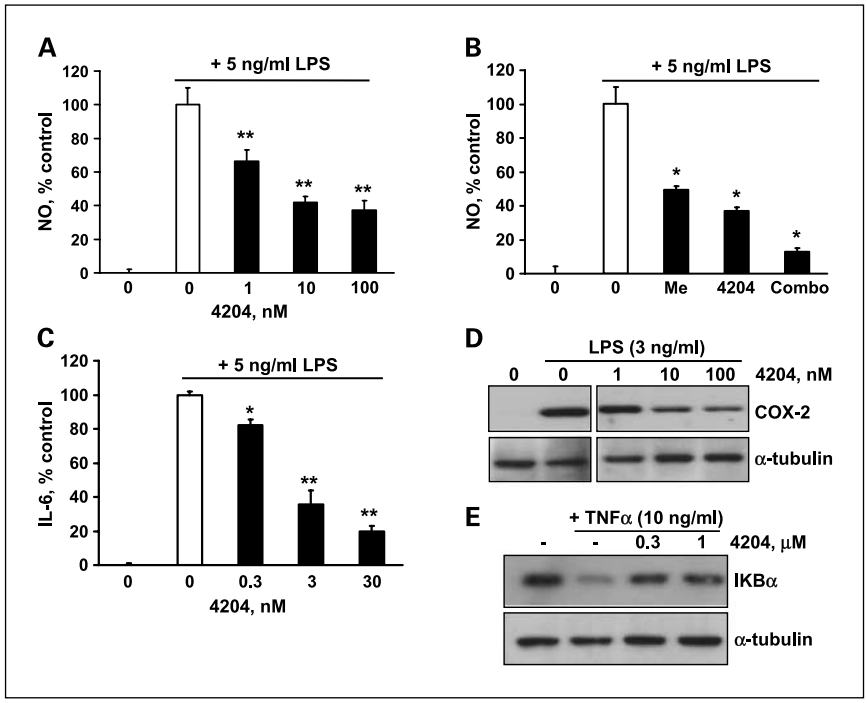

Fig. 2. 4204 inhibits the effects of lipopolysaccharide and tumor necrosis factor-α. RAW264.7 mouse macrophage–like cells were treated either with NRX194204 (*4204*) alone (*A*) or with 4204 (100 nmol/L) alone and in combination (*B*) with CDDO-Me (1 nmol/L) and then stimulated with lipopolysaccharide (*LPS*) for 24 h. The Griess reaction was used to measure nitric oxide in the medium. RAW cells were also treated with 4204 and lipopolysaccharide for 24 h (*C* and *D*), and either the amount of interleukin 6 released into the media was measured using an ELISA (*C*) or cell lysates were immunoblotted with cyclooxygenase-2 antibodies (*D*). *E,* RAW cells were treated with 4204 for 24 h, the cells were then stimulated with tumor necrosis factor-α for 15 min, and cell lysates were immunoblotted using IKBα antibodies. *, $P < 0.05$ versus control; **, $P < 0.001$ versus control.

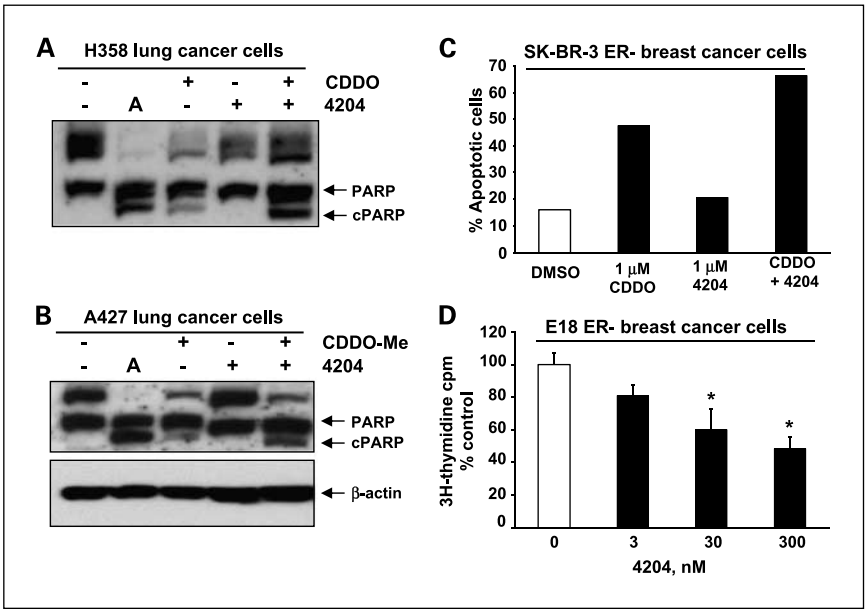

Fig. 3. 4204 enhances apoptosis in lung cancer and ER-negative breast cancer cells. Human lung cancer cells were treated with CDDO and 4204 (*A,* 1 μmol/L each) or with CDDO-Me (*B,* 0.2 μmol/L) and 4204 (*B,* 1 μmol/L) for 48 h and immunoblotted with PARP antibodies. In the PARP gels, A indicates anisomycin (10 μg/mL; positive control for PARP cleavage). *C,* human ER- breast cancer cells were treated with CDDO and 4204 for 72 h, and apoptosis was determined using flow cytometry for annexin V and propidium iodide staining. E18-14C-27 (E18) mouse mammary cancer cells were treated with 4204 for 72 h, and cell proliferation was measured using a [^{3}H]thymidine incorporation assay (*D*). *, $P < 0.05$ versus control.

the Institutional Animal Care and Use Committee of Dartmouth Medical School.

Statistical analysis. Results are mean ± SE and were analyzed by one-way ANOVA followed by a Tukey test (SigmaStat3.5). If the test for normality failed, results were analyzed by one-way ANOVA on ranks (Kruskal-Wallis) and Dunn's test. Terminal nucleotidyl transferase–mediated nick end labeling results were analyzed by the Mann-Whitney rank-sum test. Figure 4 was analyzed by the Wilcoxon signed rank test, and percentages were analyzed using a *Z*-test. All *P* values are two-sided.

Results

4204 blocks the activities of lipopolysaccharide and tumor necrosis factor-α in RAW264.7 macrophage-like cells. Despite the proven efficacy of rexinoids such as bexarotene and 268 for the prevention and treatment of cancer in preclinical animal models (4) and the crucial roles of inflammation (31) and the microenvironment (32) in carcinogenesis, the effects of rexinoids on macrophages have not been widely explored. In RAW264.7 cells stimulated with lipopolysaccharide, low nanomolar concentrations of 4204, both alone (Fig. 2A) and in combination with the triterpenoid, CDDO-methyl ester (CDDO-Me; Fig. 2B), inhibited the production of nitric oxide by 65% to 85% compared with untreated controls. 4204 also blocked the release of interleukin 6 in a dose-dependent manner (Fig. 2C), suppressed the levels of cyclooxygenase-2 protein (Fig. 2D), and at slightly higher concentrations (0.3-1 μmol/L), prevented the degradation of IKBα in cells treated with either lipopolysaccharide (data not shown) or tumor necrosis factor-α (Fig. 2E); nitric oxide, cyclooxygenase-2, I κB kinase, and interleukin 6 are all important targets for chemoprevention (2, 4, 33–37).

The combination of a triterpenoid and 4204 induces apoptosis in lung and breast cancer cells. The effects of 4204 on epithelial cells are more limited, unless used in combination with another agent such as an oleanane triterpenoid. More than 300 derivatives of oleanolic acid have been synthesized, and CDDO-Me and CDDO are currently in phase I clinical trials for the treatment of solid tumors and leukemias (4). 4204 enhanced the ability of CDDO or CDDO-Me to apoptose H358 (Fig. 3A) or A427 (Fig. 3B) human lung cancer cells and SK-BR-3 human ER- breast cancer cells (Fig. 3C), as measured by PARP cleavage or annexin staining. Notably, 4204 only partially inhibited the proliferation of E18-14C-27 cells, derived from a mammary tumor from a MMTV-erbB2 transgenic mouse (ref. 12; Fig. 3D), and did not induce apoptosis of these cells (data not shown).

Toxicology of 4204. The toxicity of 4204 has been investigated in rats and dogs in repeated oral dose (by gavage in rats and capsules in dogs) studies for 4 weeks followed by recovery periods at doses as high as 10 mg/kg/d.[3] There were no severe adverse effects observed in either species even at the highest dose. Slight decreases in food consumption and body weight were observed in dogs during treatment but were reversed during recovery. Similar changes in body weight and food consumption were observed in female rats but males showed slight increases. Hypothyroidism (decreased T3, T4 and TSH levels) was observed in both species but was reversible. Toxicokinetic evaluations showed high dose–dependent systemic exposure to 4204 during these studies. In separate studies in mice and rats, 4204 transiently increased serum triglyceride levels (peaking at 4-5 h), which returned to normal at 24 h. Also, 4204 did not increase hepatic triglyceride output in rats. VTP 194204 showed no evidence of genotoxic activity in a series of assays (Ames test, mouse lymphoma TK assay, *in vivo* micronucleus test).

***4204 reduces the number and size of lung tumors* in vivo.** Bexarotene has been used successfully for both the prevention and treatment of experimental lung cancer (14–17). Because 4204 is a more potent and selective rexinoid (10, 22) and is effective in a number of our standard assays indicative of chemopreventive activity (Figs. 1–3), we tested the ability of 4204 to prevent lung cancer in A/J mice injected with vinyl carbamate. Mice were fed a control diet or a diet containing 4204 (60 and 30 mg/kg diet), beginning 1 week after a second injection of vinyl carbamate. No signs of toxicity were evident, and the mice on the 4204 diet even gained more weight than

[3] R. Chandraratna, unpublished observations.

Table 1. 4204 inhibits lung carcinogenesis

	Control	4204	
		60 mg/kg diet	30 mg/kg diet
Analysis of inflated lungs			
No. of mice/group	51	23	24
No. of tumors/group	791	206	280
No. of tumors/lung (% control)	15.5 ± 0.06 (100)	9.0 ± 0.7* (58)	11.7 ± 0.6* (75)
No. of tumors ≤0.5 mm (% of total tumors)	21 (3)	38 (19) †	49 (17) †
No. of tumors ≤0.5 mm, <1 mm (% of total tumors)	609 (77)	163 (79)	215 (77)
No. of tumors >1 mm (% of total tumors)	157 (20)	5 (2) †	16 (6) †
Analysis of histopathology			
No. of slides/no. of mice per group	102/51	46/23	48/24
Total no. of tumors/group	329	78	103
Average no. of tumors/slide (% control)	3.2 ± 0.2 (100)	1.7 ± 0.2 † (53)	2.1 ± 0.2 † (66)
Total tumor volume on all slides, mm^3	774	67	131
Average tumor volume, mm^3/tumor (% control)	2.4 ± 0.2 (100)	0.9 ± 0.01 † (36)	1.3 ± 0.2 † (54)
Average tumor volume, mm^3/slide (% control)	7.6 ± 0.7 (100)	1.4 ± 0.3 † (19)	2.7 ± 0.5 † (36)
No. of low histologic grade tumors (% of total tumors)	180 (55)	56 (72) †	77 (75) †
No. of high histologic grade tumors (% of total tumors)	149 (45)	22 (28) †	26 (25) †
No. of low nuclear grade tumors (% of total tumors)	150 (46)	36 (46)	51 (50)
No. of high nuclear grade tumors (% of total tumors)	179 (54)	42 (54)	52 (50)
Average tumor volume (mm^3) of low histologic grade tumors (% control)	1.4 ± 0.1 (100)	0.5 ± 0.06 † (36)	0.9 ± 0.2 † (64)
Average tumor volume (mm^3) of high histologic grade tumors (% control)	3.5 ± 0.3 (100)	1.9 ± 0.4 † (54)	2.3 ± 0.5 † (66)
Average tumor volume (mm^3) of low nuclear grade tumors (% control)	1.3 ± 0.1 (100)	0.4 ± 0.06 † (31)	0.7 ± 0.2 † (54)
Average tumor volume (mm^3) of high nuclear grade tumors (% control)	3.2 ± 0.3 (100)	1.3 ± 0.2 † (41)	1.8 ± 0.3 † (56)

NOTE: Female A/J mice were injected i.p. with vinyl carbamate (0.32 mg/mouse), once a week for 2 wk. One week later, the mice were fed 4204 in the diet for 15 wk. Data were pooled from two independent experiments. Values are mean ± SE.
*$P < 0.001$ versus control.
†$P < 0.05$ versus control.

the control animals (data not shown). After 15 weeks on the diet, the number of tumors on the surface of the lungs (Table 1) was reduced by 25% to 42% in the mice fed 4204, with an average of 9.0 and 11.7 tumors, respectively, in the treated groups compared with an average of 15.5 tumors in the lungs of control mice ($P < 0.001$). The size of the tumors was also significantly ($P < 0.05$) smaller in the mice fed 4204, as only 2% to 6% of tumors in either of the treated groups were >1 mm in diameter, whereas 20% of the tumors in the control group were >1 mm. Furthermore, 17% to 19% of the tumors seen in the treated groups were <0.5 mm in diameter versus only 3% in the control lungs ($P < 0.05$).

The highly significant decrease in the number and size ($P < 0.05$) of tumors grossly observed on the surface of the lungs was also seen on histopathologic examination of lung sections. The average number of tumors per slide for the two doses of 4204 were 53% and 66% of control, and most significantly, the average total tumor volume per slide decreased from 7.6 mm^3 in the control group to 1.4 to 2.7 mm^3 in the groups treated with 4204, a decrease of 64% to 81% ($P < 0.05$). As previously described (30), we graded tumors for both histologic and nuclear grade. High histologic grade tumors are tumefactive with fused trabecula, and the normal cellular architecture is obliterated. In contrast, alveoli are visible between septa and the trabecula have not fused in low histologic grade tumors. High nuclear grade tumors contain large pleomorphic nuclei with prominent mitoses and nucleoli; the nuclei are uniform and mitoses and nucleoli less evident in low nuclear grade tumors. The average tumor volumes of low nuclear grade tumors were significantly ($P < 0.05$) lower in the 4204 groups (0.4-0.7 mm^3) than in the control group (1.3 mm^3). Significant ($P < 0.05$) differences between both 4204 groups and the control group in the average tumor volume of high nuclear grade tumors, low histologic grade tumors, and high histologic grade tumors were also observed. In contrast to the triterpenoids (30), the percentage of high nuclear grade tumors was not different in the 4204 groups compared with the control group.

4204 is effective for both the prevention and treatment of ER- mammary tumors. In addition to its chemopreventive activity in the lung, 4204 also suppressed the development of ER- mammary tumors in MMTV-neu mice. In these mice, wild-type neu is expressed in the mammary tissue under the control of a MMTV promoter, and focal ER- mammary tumors begin to appear by 4 months of age (12, 24). For prevention studies, female MMTV-neu mice were fed powdered control diet or diet containing 4204 (20 mg/kg diet), beginning at 10 weeks of age. As shown in Fig. 4, 86% of the control mice had developed tumors before a single mouse fed 4204 developed a tumor, at 32 weeks on diet. By 1 year of age or the 42nd week on diet, 100% of the control mice had developed mammary tumors versus 33% of the mice fed 4204. Even after 50 weeks on diet, tumor incidence in the 4204 group was still only 58% ($P = 0.009$ for 4204 versus control). Tumor multiplicity was also reduced from 1.3 in the control group to 0.7 in mice fed

4204 (P = 0.03 for 4204 versus control). In all of the *in vivo* experiments with 4204, the drug was well-tolerated at the given doses, even after nearly a year of continuous feeding. Indeed, there was even a slight weight gain in the mice fed 4204 compared with controls (data not shown).

Remarkably, 4204 is also extremely effective for treating established ER- tumors (Table 2), despite its limited ability to inhibit proliferation or induce apoptosis *in vitro* in a cell line established from a mammary tumor from these transgenic mice (Fig. 3D). In these studies, mice were maintained on control diet until they had developed tumors with volumes of at least 32 mm^3. The mice were then fed control diet or diet containing 4204 (60 or 30 mg/kg diet); higher concentrations of 4204 were used for the treatment studies, as compared with prevention studies, in an attempt to induce apoptosis. Indeed, 92% of the tumors (n = 25) in mice fed 4204 at 60 mg/kg diet decreased in volume >50% ($P < 0.001$ for 4204 versus control), whereas the other 8% of the tumors were growth-arrested. Notably, 64% of the tumors in the mice fed high-dose 4204 were no longer detectable at autopsy (P = 0.006 for 4204 versus control), after only 2 to 4 weeks on treatment. In contrast, all of the tumors from mice fed control diet continued to grow. Tumors from mice fed a lower dose of 4204 (30 mg/kg diet) also responded to treatment; 67% of the tumors regressed in volume >50% (P = 0.01 versus control), 11% were growth-arrested, and only 22% continued to grow (P = 0.006 versus control). Even with a reduced dose of 4204, 33% of the tumors could not be detected at autopsy.

***4204 induces apoptosis in mammary tumors but does not inhibit transgene expression* in vivo.** We and others have reported that the rexinoids bexarotene and 268 do not reduce *neu* transgene expression *in vitro* or *in vivo* (12, 20) and 4204 also did not inhibit ErbB2 expression in E18-14C-27 cells (data not shown) or in regressing tumors from mice treated with a 60 mg/kg diet of 4204 for 1 to 2 weeks in the treatment studies (Fig. 4B). Moreover, 4204 significantly ($P < 0.001$) increased the number of terminal nucleotidyl transferase–mediated nick end labeling–positive cells in tumors from mice fed 4204 4-fold compared with control tumors (9.8 $\pm$ 1.0 versus 2.4 $\pm$ 0.6; Fig. 4C).

Discussion

To our knowledge, the study described above is the first to use the novel rexinoid 4204 for the prevention of cancer in relevant preclinical animal models of both lung and ER- breast cancer. Here, we have found that 4204 is more effective at lower doses than previously reported for 268 for both the prevention and treatment of ER- mammary tumors in MMTV-neu mice (20), whereas 268 is more potent than bexarotene (4, 18–20). Interestingly, 4204 was more effective in the MMTV-neu model of ER- breast cancer, with overexpression of only the *neu* gene (24), than in the A/J mouse model of lung cancer, in which the carcinogen vinyl carbamate causes mutations in *K-ras* (38) and undoubtedly also causes other genetic and epigenetic changes (3, 39). 4204 was also strikingly effective for treating ER- mammary tumors (Table 2), and preliminary studies (data not shown) suggest that 4204 is also effective when used for the treatment of lung cancer in the A/J mouse model as well as for prevention. Confirmatory treatment experiments in the lung are ongoing and will be described in a future publication.

Although 4204 is useful as a single agent *in vivo*, the combination of 4204 and a synthetic triterpenoid, such as CDDO-Me, might be even more successful in these models and these experiments are under way. Synthetic oleanane triterpenoids block the activity of inflammatory cytokines, induce phase 2 enzymes that are cytoprotective, and selectively inhibit proliferation and induce the apoptosis of cancer cells (4). Indeed, the combination of 4204 and a triterpenoid was more effective at blocking the release of nitric oxide (Fig. 1B) and activating apoptosis in human lung and ER- breast cancer cells (Fig. 3) than either 4204 or the triterpenoid alone. The combination of 4204 and other ligands for nuclear receptors including selective estrogen receptor modulators, selective peroxisome proliferator–activated receptor-γ modulators, or vitamin D receptors (deltanoids) should also be evaluated (40). Using low doses of two synergistic multifunctional drugs

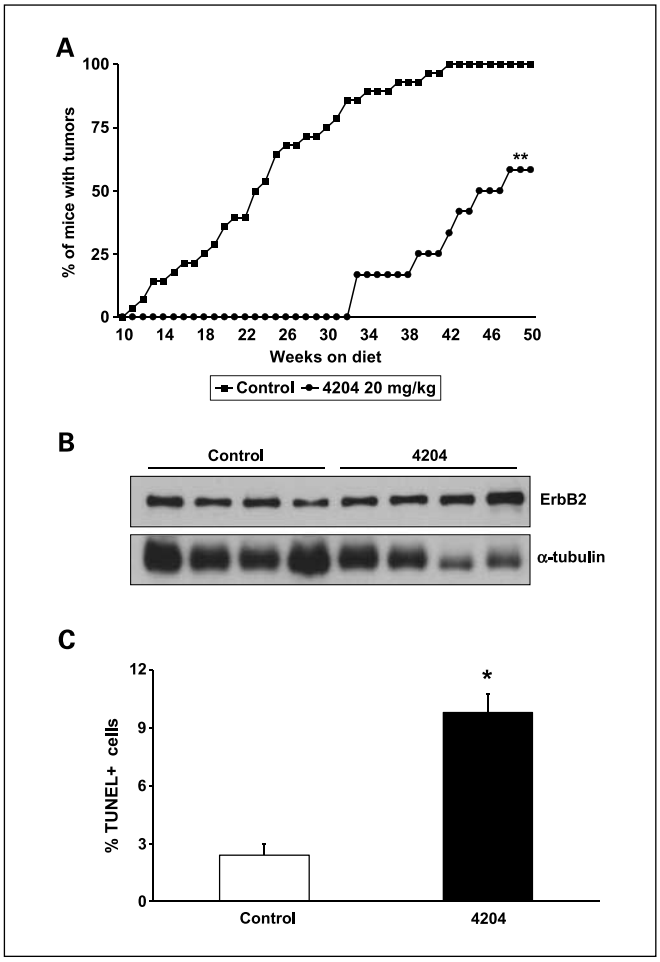

Fig. 4. 4204 delays the development and induces the apoptosis of ER- mammary gland tumors without inhibiting ErbB2 transgene expression. *A,* MMTV-neu transgenic mice were fed powdered control diet or 4204 in the same diet, beginning at 10 wk of age. Mice (n = 28 for control and n = 12 for 4204) were palpated weekly for tumors, and no tumors were evident before 20 wk of age. **, $P < 0.001$ versus control. Tumors were allowed to form, and mice were then treated with control diet or 4204 (60 mg/kg diet) for 1 wk. Tumors then were harvested and analyzed by Western blot for ErbB2 expression (*B*), or tumor sections from MMTV-neu mice (four per group) were analyzed for apoptosis by terminal nucleotidyl transferase–mediated nick end labeling (*TUNEL*) staining (*C*). Columns, mean of >1,000 cells from four tumors per group; bars, SE; *, $P < 0.001$ versus control.

Table 2. 4204 induces regression of ER-negative mammary gland tumors in MMTV-neu transgenic mice

Treatment	Control	4204 (60 mg/kg diet)	4204 (30 mg/kg diet)
No. of mice in treatment protocol	8	20	8
No. of tumors in treatment protocol	8	25	9
No. of tumors with a >50% reduction in tumor volume (%)	0	23 (92)*	6 (67) †
No. of tumors with arrested growth (%)	0	2 (8)	1 (11)
No. of tumors with active growth (%)	8 (100)	0 (0)*	2 (22) †
No. of tumors not detectable at necropsy, complete regression (%)	0	16 (64) †	3 (33)

NOTE: When tumors in female MMTV-neu mice grew to at least 32 mm^3 in volume, the mice were fed a control diet or 4204 for up to 4 wk. Tumors were measured weekly with calipers, and tumor regression was defined as a >50% decrease in tumor volume. An increase in tumor volume of >50% was classified as active tumor growth, whereas growth-arrested tumors did not increase or decrease in size >50% over a 4-wk period.
*$P < 0.001$ versus control.
†$P < 0.05$ versus control.

should reduce the problems of elevated triglyceride levels that hamper the further development of rexinoids (2).

Notably, 4204 is very effective at inducing apoptosis in mouse mammary tumors *in vivo* (Fig. 4C), despite its limited effectiveness at inducing the apoptosis of E18-14C-27 cells derived from these tumors *in vitro*. Because retinoid X receptors heterodimerize with many other receptors in the nuclear receptor superfamily, rexinoids such as 4204 modulate a number of regulatory networks that control cell growth and survival. However, 4204 clearly has important effects on RAW cells (Fig. 2), and tumor-associated macrophages and other stromal cells in the tumor microenvironment are emerging as important targets for chemoprevention (32, 41). Although additional studies such as the identification of biomarkers or oligonucleotide arrays, which have been previously described for bexarotene (42), are clearly needed to help define the molecular pathways targeted in the tumor stroma, our data support testing 4204 in upcoming clinical trials.

Acknowledgments

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