

The rexinoid LG100268 and the synthetic triterpenoid CDDO-methyl amide are more potent than erlotinib for prevention of mouse lung carcinogenesis

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Abstract

Female A/J mice injected with the carcinogen vinyl carbamate develop atypical adenomatous hyperplasias in lungs 4 weeks after injection with the carcinogen. The number and severity of tumors then increase over time, making these mice a useful model for evaluating potential chemopreventive agents. The rexinoid LG100268 (LG268), a selective ligand for the retinoid X receptor, and the methyl amide of the synthetic triterpenoid 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) both significantly reduced the number, size, and severity of the histopathology of lung tumors in female A/J mice when fed in diet for 14 to 20 weeks. The total tumor burden was 85% to 87% lower in mice fed LG268 and CDDO-MA than in controls, and the percentage of high-grade tumors decreased from 59% in the controls to 25% or 30% with CDDO-MA and LG268. Erlotinib, which is used to treat lung cancer patients and is an inhibitor of the epidermal growth factor receptor, was less effective in this model. Immunohistochemical staining of geminin, a marker of cell cycle progression, was higher in lung sections from control mice than in mice treated with LG268. Because rexinoids and triterpenoids signal through

different biological pathways, they should be tested in combination for the prevention of lung cancer. [Mol Cancer Ther 2008;7(5):1251–7]

Introduction

Lung cancer is the primary cause of cancer-related deaths, accounting for 1.3 million deaths worldwide and with a 5-year survival rate of only 16% (1). The prevalence of smoking, the most important risk factor for lung cancer, is ~20% in the population in the United States, underscoring the need to develop more effective drugs for both the treatment and the prevention of lung cancer. Three distinct classes of drugs that have been tested in preclinical or clinical studies for the treatment of lung cancer include inhibitors of the epidermal growth factor receptor (EGFR) family, rexinoids (selective ligands for retinoid X receptors), and synthetic triterpenoids. Drugs from all three of these families trigger proteasome-dependent degradation of cyclin D1, which is a useful biomarker of clinical efficacy for cancers in the aerodigestive tract (2–6).

The EGFR network controls growth and survival of epithelial cells, but overexpression or constitutive activation of this network promotes carcinogenesis. Erlotinib, a tyrosine kinase inhibitor of the EGFR, significantly extends survival in non-small cell lung cancer patients (7). Despite its usefulness for treatment of lung cancer, the ability of erlotinib to prevent experimental lung cancer has not been examined. Bexarotene, a synthetic retinoid X receptor agonist that is Food and Drug Administration approved for treating cutaneous T-cell lymphoma, is currently being evaluated in the clinic for the treatment of non-small cell lung cancer (8). Because retinoid X receptors heterodimerize with other nuclear hormone receptors, rexinoids are key physiologic modulators that regulate cell proliferation, differentiation, and apoptosis (9). Bexarotene can prevent lung cancer in mice (10, 11), but because this drug also binds to retinoic acid receptors, it retains some of the toxicities associated with classic retinoids. In contrast, LG100268 (LG268) is a potent and selective retinoid X receptor agonist with essentially no affinity for retinoic acid receptors (12); LG268 is effective for prevention of experimental breast cancer (9) but has not been tested against lung cancer. Synthetic triterpenoids are multifunctional agents that block inflammation, inhibit proliferation of cancer cells, and activate apoptotic pathways *in vitro* and *in vivo* (9). Both the methyl ester (CDDO-ME) and the ethyl amide (CDDO-EA) of the parent triterpenoid 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) are potent inhibitors of lung carcinogenesis (13), and phase 1 and 2 clinical trials with CDDO-ME in cancer and inflammatory

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diseases are ongoing. The CDDO-methyl amide (CDDO-MA) is also highly active in cell culture assays and pharmacodynamically in the lung (14) but has not been evaluated for prevention of lung cancer.

Our objectives were to characterize the use of female A/J mice treated with vinyl carbamate as a relevant autochthonous model of lung cancer, to test three distinct classes of drugs for preventing lung cancer, and to evaluate changes in biomarkers in this model. Here, we report that the retinoid LG268 and the triterpenoid CDDO-MA are highly effective for preventing lung carcinogenesis as measured by significant reductions in the number, size, and severity of lung tumors, whereas erlotinib is only marginally effective in A/J mice.

Materials and Methods

Drugs

The synthesis and structure of LG268 (12) and CDDO-MA (9, 15) have been described. The CDDO-MA used in these experiments was synthesized by Ash Stevens. Erlotinib (16, 17) was provided by OSI Pharmaceuticals. Purity was >95% for all compounds as determined by high-performance liquid chromatography.

Serial Sacrifice Study in A/J Mice

Seven- to 8-week-old female A/J mice (The Jackson Laboratory) were fed powdered AIN-93G diet (Harlan-Teklad) and injected i.p. with two doses (0.32 mg/mouse), 1 week apart, of vinyl carbamate (Toronto Research Chemicals) dissolved in acidified isotonic saline (pH 4) to prevent the possible degradation of the compound at an alkaline pH. Three mice per time point were sacrificed 1, 2, 4, 6, 8, 12, 16, 20, 28, and 36 weeks after the final injection of vinyl carbamate. Lungs were removed and inflated with neutral buffered formalin, and the number and size of grossly visible tumors on the lungs as well as the number, size, and histopathology of tumors on 5 μ m sagittal H&E-stained lung sections were evaluated as described previously (13).

Prevention of Lung Cancer

Mice were fed chemopreventive agents in the diet for 14 to 20 weeks beginning 1 week after the final injection of vinyl carbamate and thus 2 weeks after the initial carcinogenic insult. Drugs were dissolved in a vehicle of 1:3 ethanol/Neobee oil and then blended into powdered AIN-93G diet with a commercial food blender to assure drug homogeneity in the diet. Vehicle alone was included in the control diet. Diet was stored at 4°C and diet was

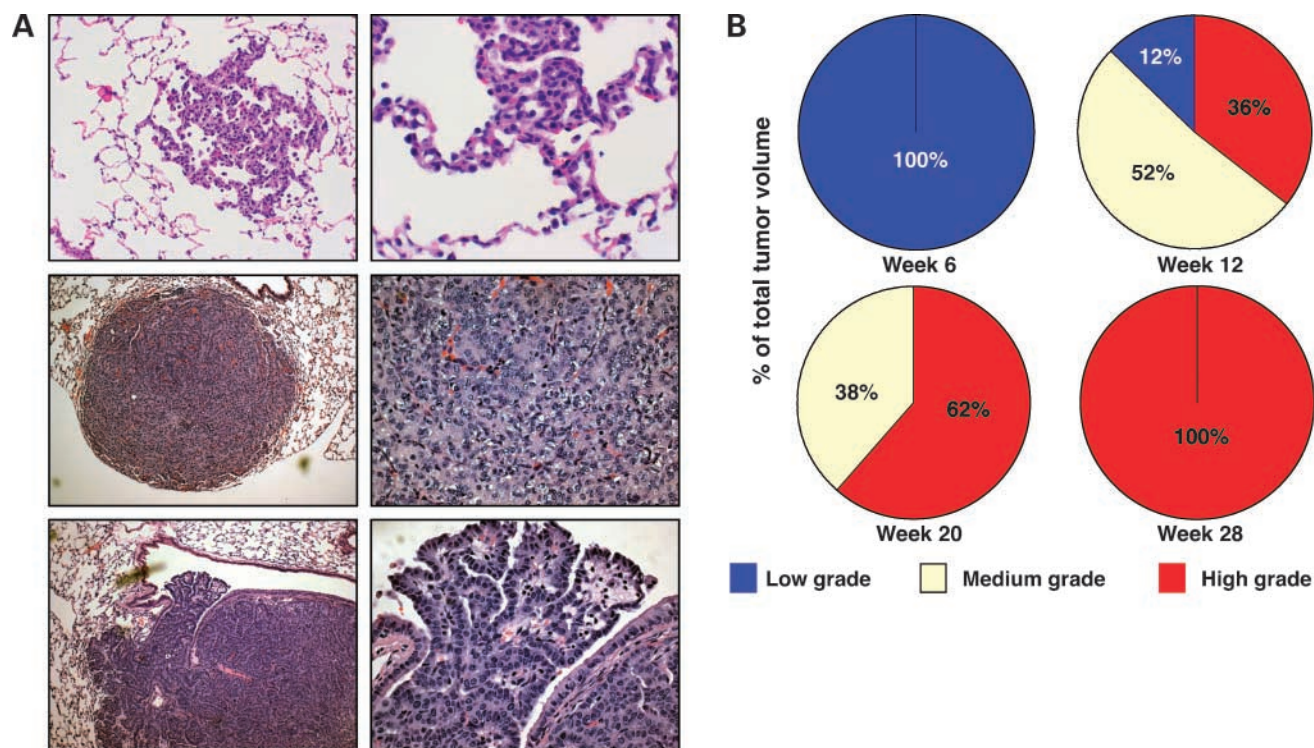


Figure 1. Histopathology of preinvasive and advanced lesions and quantification of the histopathology of a serial sacrifice study. Female A/J mice were injected i.p. with two doses of vinyl carbamate (0.32 mg/mouse), 1 week apart. **A**, representative pictures ($\times 100$ and $\times 400$) of a preinvasive lesion, that is, atypical adenomatous hyperplasia, in the lung of a mouse 4 wk after the final injection of carcinogen (*top*), a high-grade tumor (*middle*), and a tumor invading into a bronchus (*bottom*). **B**, diameter of tumors on slides (six slides per time point) was measured, and the tumors were scored for both histologic and nuclear morphology as described previously (13). Tumors were assigned low grade (low histologic and nuclear grade), medium grade (low histologic and high nuclear grade), or high grade (high histologic and nuclear grade), and the percentages of TTV per grade are shown for weeks 6 to 28.

Table 1. LG268 and erlotinib prevent lung carcinogenesis in A/J mice injected with vinyl carbamate, 14 wk on diet

	Control	LG268, 60 mg/kg diet	LG268, 30 mg/kg diet	Erlotinib, 200 mg/kg diet	Erlotinib, 100 mg/kg diet
Analysis of inflated lungs					
No. mice/group	16	8	8	8	8
No. tumors/group	252	59	70	88	104
No. tumors/mouse (% control)	15.8 ± 1.0 (100)	7.4 ± 1.3* (47)	8.8 ± 1.1* (56)	11.0 ± 1.8 [†] (70)	13.0 ± 1.5 (82)
No. tumors ≤0.5 mm (% of total tumors)	7 (3)	13 (22) [†]	10 (14)	16 (18) [†]	10 (10)
No. tumors >1 mm (% of total tumors)	49 (19)	4 (7) [†]	2 (3) [†]	2 (2) [†]	11 (10)
Analysis of histopathology					
Total no. tumors/group	120	43	43	53	55
Average no. tumors/slide (% control)	3.9 ± 0.3 (100)	2.9 ± 0.5 [†] (74)	2.7 ± 0.3 [†] (69)	3.3 ± 0.4 (85)	3.4 ± 0.3 (89)
TTV, mm ³	250	37.5	38.0	44.5	61.6
Average tumor size, mm ³ (% control)	2.1 ± 0.3 (100)	0.9 ± 0.2 [†] (42)	0.9 ± 0.2 [†] (42)	0.8 ± 0.1 [†] (40)	1.1 ± 0.1 (54)
Average tumor burden, mm ³ /slide (% control)	8.1 ± 1.5 (100)	2.5 ± 0.6 [†] (31)	2.4 ± 0.5 [†] (29)	2.8 ± 0.6 [†] (34)	3.9 ± 0.6 (48)
Total no. low-grade tumors (%)	20 (17)	22 (51)*	20 (46)*	17 (32) [†]	13 (23)
Total no. medium-grade tumors (%)	25 (21)	9 (21)	12 (28)	16 (30)	24 (44) [†]
Total no. high-grade tumors (%)	75 (62)	12 (28)*	11 (26)*	20 (38) [†]	18 (33)*

NOTE: Female A/J mice were injected i.p. with two doses of vinyl carbamate (0.32 mg/mouse), 1 wk apart. One week after the final injection with the carcinogen, mice were fed compounds in diet for 14 wk. Mean ± SE.

* $P < 0.001$ versus control.

[†] $P < 0.05$ versus control.

changed in the feeders twice a week. The drugs are stable in the diet as determined by high-performance liquid chromatography-mass spectrometry analysis and by previous long-term feeding studies with these compounds *in vivo*. Lungs were coded and then evaluated in a blinded fashion as described (13) by two investigators. Data were analyzed by one-way ANOVA and the Tukey test or by one-way ANOVA on ranks followed by Dunn's test (SigmaStat3.5).

Immunohistochemistry

Samples were processed with an anti-geminin antibody (Santa Cruz Biotechnology). Samples were blinded and two separate fields of 100 cells per slide (3 slides per group) were scored and the percentage of geminin-positive nuclei was averaged.

Results

Number, Size, and Histopathology of Lung Tumors in Female A/J Mice Injected with Vinyl Carbamate Increase Over Time

Because of their susceptibility to develop lung tumors when exposed to cigarette smoke or other carcinogens including urethane (ethyl carbamate) and vinyl carbamate (18, 19), A/J mice are commonly used to evaluate potential chemopreventive agents (20–22). Although the progressive nature of lung tumors that develop in A/J mice challenged with carcinogens has been described (23), the high number of highly invasive adenocarcinomas we observed 16 weeks after these mice were injected with two doses of vinyl carbamate (13) led us to examine tumor burden and histopathology at earlier time points than had been reported previously.

Grossly visible tumors were first seen on the surface of the lungs 4 weeks after the final dose of carcinogen, and both the number and the size of the surface lesions increased over time. By 8 weeks after the vinyl carbamate injections, an average of eight surface tumors were observed; 56% of these tumors were <0.5 mm in diameter and none were >1 mm in diameter. By 36 weeks after injection with vinyl carbamate, the average number of surface lesions increased to 20. None of these tumors were <0.5 mm in diameter, but 36% were >1 mm. Preinvasive lesions (Fig. 1A, *top*), atypical alveolar hyperplasias or atypical adenomatous hyperplasias, were seen on lung sections by week 4. These lesions were ~0.6 mm in the greatest dimension and featured a proliferation of neoplastic alveolar pneumocytes with low-grade cytologic features along otherwise intact alveolar septa. There was no septal collapse to indicate progression to invasion or high-grade cytologic atypia. The sectioned tumors were also assigned histologic and nuclear grades (13). Low-grade tumors contain aerated alveoli, nonfused trabecula, and generally uniform nuclei. In contrast, high-grade tumors display solid growth that obliterates the normal architecture of the lung and large, pleomorphic nuclei with distinct nucleoli and mitoses (Fig. 1A, *middle*). The tumors induced by vinyl carbamate are also highly invasive as shown by a tumor in the bronchus (Fig. 1A, *bottom*). As shown in Fig. 1B, all of the tumors were low grade (both histologic and nuclear) at week 6. However, by week 12, only 12% of the total tumor volume (TTV) was low grade, 52% of TTV was medium grade (high histologic or nuclear grade), and 36% of TTV was high grade (histologic and nuclear grade). By week 28, 100% of the TTV was high grade. Notably, the number, size, and severity of tumors in A/J mice injected

with a single dose of vinyl carbamate were almost identical to the tumor burden in A/J mice injected with two doses of vinyl carbamate (data not shown), but we chose to use two doses of carcinogen in the prevention studies described below to compare the data with our previously published results (13).

LG268 and CDDO-MA Significantly Reduce Tumor Burden in the Lung

To determine whether a drug used clinically for the treatment of lung cancer could also be used for the prevention of experimental lung cancer, female A/J mice were fed erlotinib (200 and 100 mg/kg diet, referred to hereafter as "high dose" and "low dose") beginning 1 week after the final injection of vinyl carbamate. By this time, mutagenic damage has already occurred, and a chemopreventive agent, as used here, does not interfere with metabolism of the carcinogen or tumor initiation. For comparison with erlotinib, in the experiments described below, other groups of mice were dosed with the experimental drugs, LG268 (60 or 30 mg/kg diet, referred to as "high dose" or "low dose") or CDDO-MA (800 mg/kg diet), in a similar chemoprevention design. These doses were well tolerated, as all mice continued to gain weight throughout the experiment. For a first pilot experiment, mice were maintained on LG268 and erlotinib for 14 weeks (Table 1), and the number of grossly visible lung tumors decreased by 44% to 53% with LG268, from an average of 15.8 in the control group to only 7.4 and 8.8 ($P < 0.001$) with LG268 at high and low doses, respectively. The tumors were also smaller, as only 7% and 3% of the tumors in the LG268 groups were >1 mm compared with 19% in the control group ($P < 0.05$) and 22% of the tumors were

<0.5 mm in the high-dose LG268 group versus only 3% in the control group ($P < 0.05$). The high dose of erlotinib significantly ($P < 0.05$ versus control) reduced the total number and the size of grossly visible lung tumors but not the average number of tumors per slide. Overall, the results with erlotinib were not as striking as with both doses of LG268. LG268 also dramatically reduced the average tumor burden per slide by 70%, down from an average of 8.1 mm^3 in the control group to only 2.4 to 2.5 mm^3 ($P < 0.05$) in the LG268 groups. The severity of the tumors was also significantly lower ($P < 0.001$) with LG268, as only 26% to 28% of the tumors in the LG268 groups were high grade compared with 62% in the control group and 46% to 51% of the tumors from mice fed LG268 were low grade versus only 17% in the controls. The high dose of erlotinib also significantly ($P < 0.05$) reduced the average tumor burden per slide by 66% compared with controls (8.1 versus 2.8 mm^3), and both doses of erlotinib significantly ($P < 0.05$) reduced the severity of the tumors. However, LG268 was more effective than erlotinib in this experiment.

To verify these results, we repeated the experiment with the same doses of drugs but extended its duration to 20 weeks on diet (Table 2). These drugs were also well tolerated in this experiment, and the mice in all the groups continued to gain weight. By the end of the experiment, the average weight was 24.2 g in the control group, 25.1 g with low-dose LG268, 22.6 g with high-dose LG268, 21.7 for low-dose erlotinib, 20.5 for high-dose erlotinib, and 21.2 for CDDO-MA or between 94% and 104% of control for LG268 and between 90% and 94% of control for the other drugs ($P < 0.05$ for control versus high-dose erlotinib and CDDO-MA only). Both tumor size and severity were higher than in

Table 2. LG268 and CDDO-MA are more potent than erlotinib for preventing lung carcinogenesis in A/J mice injected with vinyl carbamate, 20 wk on diet

	Control	LG268, 60 mg/kg diet	LG268, 30 mg/kg diet	Erlotinib, 200 mg/kg diet	Erlotinib, 100 mg/kg diet	MA, 800 mg/kg diet
Analysis of inflated lungs						
No. mice/group	23	12	12	12	12	16
No. tumors/group	356	89	144	144	145	146
No. tumors/mouse (% control)	15.5 ± 1.0 (100)	$7.4 \pm 1.0^*$ (48)	12 ± 0.6 (77)	12.0 ± 1.1 (77)	12 ± 0.9 (78)	$9.1 \pm 0.7^*$ (59)
No. tumors ≤ 0.5 mm (% of total tumors)	14 (4)	25 (28)*	18 (13)	19 (13)	7 (5)	63 (43)*
No. tumors >1 mm (% of total tumors)	103 (29)	6 (7)*	19 (13)	10 (7)*	30 (21)	1 (1)*
Analysis of histopathology						
Total no. tumors/group	184	33	78	76	83	73
Average no. tumors/slide (% control)	4.2 ± 0.3 (100)	$1.4 \pm 0.3^*$ (33)	3.3 ± 0.4 (78)	3.2 ± 0.3 (76)	3.5 ± 0.3 (83)	$2.3 \pm 0.3^*$ (55)
TTV, mm^3	559.2	45.3	134.4	131.4	196.0	52.5
Average tumor size, mm^3 (% control)	3.0 ± 0.3 (100)	$1.4 \pm 0.4^*$ (45)	$1.7 \pm 0.4^*$ (57)	$1.7 \pm 0.4^*$ (57)	2.4 ± 0.5 (78)	$0.7 \pm 0.1^*$ (24)
Average tumor burden, mm^3/slide (% control)	12.7 ± 1.5 (100)	$1.9 \pm 0.4^*$ (15)	$5.6 \pm 1.2^*$ (44)	$5.5 \pm 1.0^*$ (43)	8.2 ± 1.0 (64)	$1.6 \pm 0.3^*$ (13)
No. low-grade tumors (%)	25 (13)	3 (9)	18 (23)	23 (30)*	14 (17)	35 (48)*
No. medium-grade tumors (%)	51 (28)	20 (61)*	27 (35)	30 (40)	27 (32)	20 (27)
No. high-grade tumors (%)	108 (59)	10 (30)*	33 (42)*	23 (30)*	42 (51)	18 (25)*

NOTE: Female A/J mice were injected i.p. with two doses of vinyl carbamate (0.32 mg/mouse), 1 wk apart. One week after the final injection with the carcinogen, mice were fed compounds in diet for 20 wk. Mean \pm SE.

* $P < 0.05$ versus control.

the pilot study, as average tumor volume per tumor increased from 2.1 to 3.0 mm³ and average tumor volume per slide increased from 8.1 to 12.7 mm³. The high dose of LG268 again significantly ($P < 0.05$) reduced the average number (7.4 versus 15.5 in control) and size (28% of the tumors in the LG268 group were <0.5 mm versus only 4% of tumors in the controls) of grossly visible lung tumors. Both doses of LG268 and the high dose of erlotinib also significantly ($P < 0.05$) reduced the average tumor burden per slide from 12.7 mm³ in the control group to only 1.9 mm³ with the high dose of LG268, a reduction of 85%, to 5.5 to 5.6 mm³ with the low dose of LG268 or erlotinib. The percentage of high-grade tumors also decreased significantly ($P < 0.05$) from 59% in the control group to 30% to 42% with both doses of LG268 or with the high dose of erlotinib.

We also evaluated the synthetic triterpenoid CDDO-MA in this 20-week study (Table 2). CDDO-MA is similar in potency *in vitro* and *in vivo* to CDDO-EA and CDDO-Me (13); both CDDO-EA and CDDO-ME are potent inhibitors of lung carcinogenesis in A/J mice (13). CDDO-MA also significantly ($P < 0.05$) reduced the average number (9.1 versus 15.5 in the control) and size (43% of the tumors in mice fed CDDO-MA were <0.5 mm versus only 4% in the controls) of grossly visible tumors. Average tumor size and tumor volume were reduced by 76% and 87% compared with control mice, from 4.2 to 2.3 mm³ for volume per tumor, and from 12.7 to 1.6 mm³ for tumor size, respectively. The most striking finding with CDDO-MA, however, was the significant reduction in tumor severity. Forty-eight percent of the tumors in the CDDO-MA group were low-grade tumors in contrast to only 13% in the control group. Moreover, the percentage of high-grade tumors declined from 59% in controls to 25% with CDDO-MA. Photographs of four representative lungs from all six experimental groups are shown in Fig. 2A; these photos clearly show the remarkable efficacy of CDDO-MA and LG268 and the lesser effectiveness of erlotinib for preventing lung tumorigenesis in A/J mice injected with vinyl carbamate.

LG268 Decreases Geminin Staining in the Lung

Although cyclin D1 and Ki-67 protein expression are useful biomarkers of activity in non-small cell lung cancer patients treated with bexarotene and erlotinib (5, 6), no significant changes in the expression of either cyclin D1 or Ki-67 were seen by immunohistochemistry in these studies. Potentially, this finding reflected differences in sensitivity or specificity of the antibodies used for immunohistochemical detection in mouse versus human lung tissues. However, a small number of cells in the nontumor portion of the lungs (average, 0.8%) were positive for staining with the proliferation marker, geminin, in the control group (Fig. 2B), whereas no positive staining was found in the LG268 groups ($P = 0.015$ for low dose of LG268 versus control and $P = 0.007$ for both LG268 groups versus control). Moreover, average geminin staining in tumors was reduced by >40% (3 to 1.7%) in the tumors of mice fed LG268 compared with controls ($P = 0.1$); CDDO-MA

reduced geminin staining in tumors >55% (average, 1.3%) but did not reduce geminin staining in nonmalignant lung (data not shown). In contrast to Ki-67, which is expressed throughout the cell cycle, geminin is only expressed during S and G₂. Elevated geminin expression is a negative prognostic indicator for invasive breast cancer and has been used to measure changes in the cell cycle resulting from DNA damage (24, 25). Our results also suggest it can be used as a biomarker to evaluate the activity of new chemopreventive agents in the lungs of A/J mice.

Discussion

In summary, the A/J mouse is a highly reproducible and relevant model for testing drugs for the prevention of lung cancer. Although the EGFR tyrosine kinase inhibitor erlotinib and the retinoid bexarotene are used to treat lung cancer in humans, to our knowledge, neither erlotinib nor the more potent and selective retinoid LG268 has been tested previously for the prevention of experimental lung cancer. Our studies show that LG268, the triterpenoid CDDO-MA and erlotinib, at least in the shorter prevention study, significantly reduce the number, size, and severity of histopathology of lung tumors in female A/J mice. Moreover, this chemopreventive activity occurred after the DNA damage had been initiated, as the drugs were not started until 2 weeks after mice were originally injected with the carcinogen. Erlotinib was not as effective in this model as the retinoid and triterpenoid, although the EGFR inhibitor gefitinib has been shown to significantly reduce lung tumor burden in mice treated with the carcinogen benzo(a)pyrene (26). One explanation why erlotinib is less effective in this model is that vinyl carbamate induces K-ras mutations (27), and lung tumors containing K-ras mutations may be resistant to erlotinib or gefitinib (28–30). Another consideration is that erlotinib specifically targets the EGFR, whereas LG268 and CDDO-MA are multi-functional drugs that target numerous pathways (9), an important consideration in human cancers that are known to contain multiple genetic lesions (31).

Triterpenoids and retinoids target the epithelial cells within the tumor as well as surrounding macrophages, fibroblasts, and endothelial cells (9, 32, 33). These drugs also target multiple cytokines and key signaling molecules, including interleukin-6, tumor necrosis factor- α , nitric oxide, cyclo-oxygenase-2, IKB α , pSTAT3, cyclin D, and Keap1 (9, 34), which are important for the prevention of cancer (35). Thus, defining which signaling pathway is responsible for the potent chemopreventive activity of these drugs *in vivo* is difficult, as no tissue is available for analysis if the compound is effective, and any tumors that develop, by definition, have failed chemoprevention. We are attempting to isolate a cell line from the lung tumors of the mice injected with vinyl carbamate, but these cells will be more useful for elucidating the mechanism of a drug treatment rather than prevention and will still not fully account for the activity of these drugs against the micro-environment of a developing tumor.

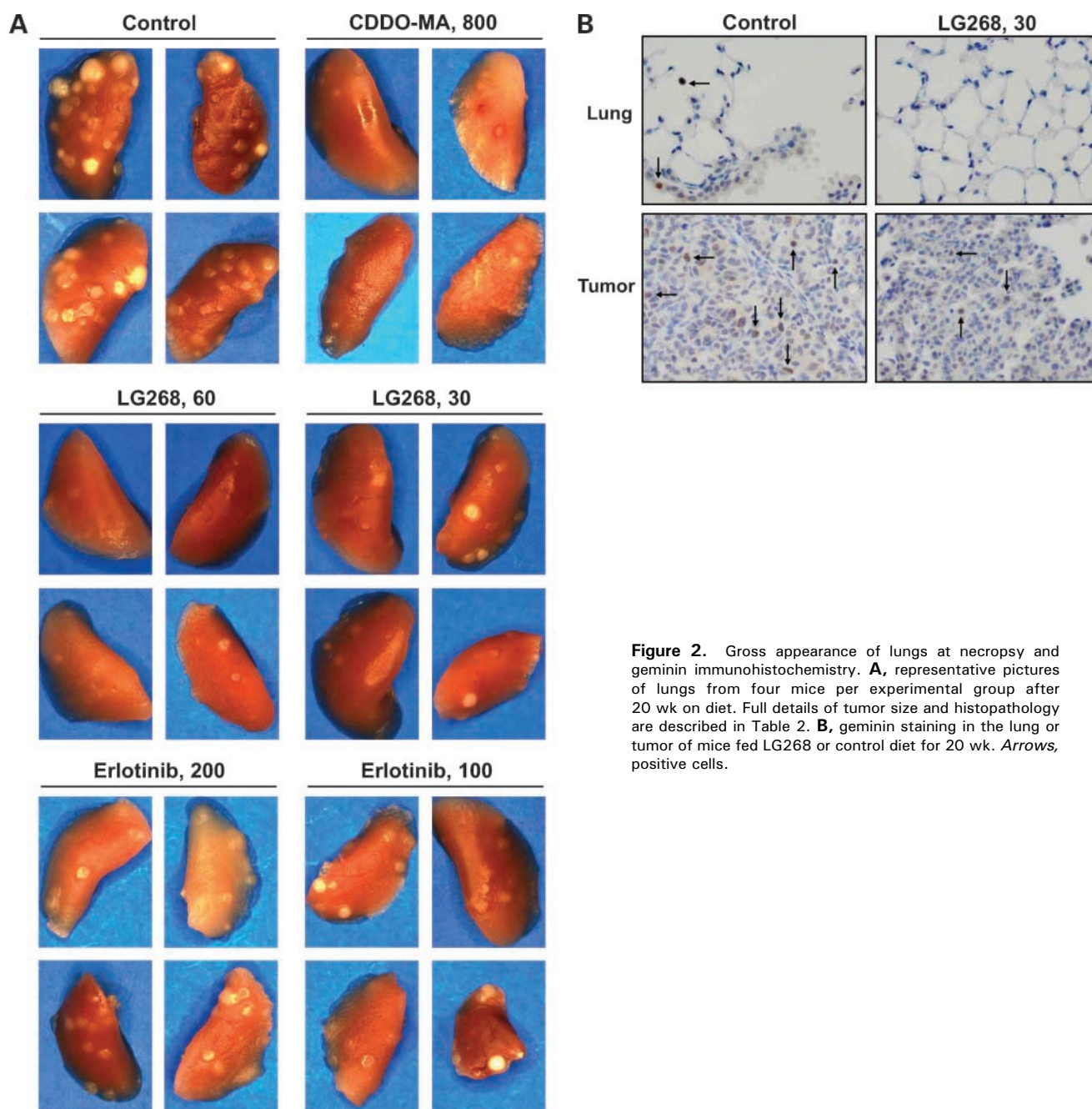


Figure 2. Gross appearance of lungs at necropsy and geminin immunohistochemistry. **A**, representative pictures of lungs from four mice per experimental group after 20 wk on diet. Full details of tumor size and histopathology are described in Table 2. **B**, geminin staining in the lung or tumor of mice fed LG268 or control diet for 20 wk. Arrows, positive cells.

Although CDDO-MA clearly decreases tumor load 20 weeks after dosing with vinyl carbamate (Fig. 2A; Table 2), the methyl amide is not as potent as CDDO-ME or CDDO (13). Recent experiments have revealed that drug levels of CDDO-EA are 2- to 3-fold higher in the lungs than with CDDO-MA (data not shown), suggesting that CDDO-ME or CDDO-EA would be better for clinical use in the lung. The direct comparison of a triterpenoid and rexinoid in these experiments, however, show that both classes of drugs are highly effective and well tolerated, and because they work through different mechanisms (9), the combination of these drugs is being tested for both the

prevention and the treatment of experimental lung cancer in this model and will be described in a future report.

Disclosure of Potential Conflicts of Interest

T. Honda: Triterpenoid patent; G. Gribble: Triterpenoid patent; W. Lamph: stockholder in Ligand Pharmaceuticals; M. Sporn: Reata Pharmaceuticals grant. The other authors disclosed no potential conflicts of interest.

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References

- Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2006. *CA Cancer J Clin* 2006;56:106–30.
- Witschi H, Espiritu I, Suffia M, Pinkerton KE. Expression of cyclin D1/2 in the lungs of strain A/J mice fed chemopreventive agents. *Carcinogenesis* 2002;23:289–94.
- Dragnev KH, Pitha-Rowe I, Ma Y, et al. Specific chemopreventive agents trigger proteasomal degradation of G1 cyclins: implications for combination therapy. *Clin Cancer Res* 2004;10:2570–7.
- Petty WJ, Dragnev KH, Memoli VA, et al. Epidermal growth factor receptor tyrosine kinase inhibition represses cyclin D1 in aerodigestive tract cancers. *Clin Cancer Res* 2004;10:7547–54.
- Dragnev KH, Petty WJ, Shah S, et al. Bexarotene and erlotinib for aerodigestive tract cancer. *J Clin Oncol* 2005;23:8757–64.
- Dragnev KH, Petty WJ, Shah SJ, et al. A proof-of-principle clinical trial of bexarotene in patients with non-small cell lung cancer. *Clin Cancer Res* 2007;13:1794–800.
- Shepherd FA, Rodrigues PJ, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005;353:123–32.
- Khuri FR, Rigas JR, Figlin RA, et al. Multi-institutional phase I/II trial of oral bexarotene in combination with cisplatin and vinorelbine in previously untreated patients with advanced non-small-cell lung cancer. *J Clin Oncol* 2001;19:2626–37.
- Liby KT, Yore MM, Sporn MB. Triterpenoids and retinoids as multifunctional agents for the prevention and treatment of cancer. *Nat Rev Cancer* 2007;7:357–69.
- Wang Y, Zhang Z, Yao R, et al. Prevention of lung cancer progression by bexarotene in mouse models. *Oncogene* 2006;25:1329.
- Pereira MA, Kramer PM, Nines R, et al. Prevention of mouse lung tumors by targetin. *Int J Cancer* 2006;118:2359–62.
- Boehm MF, Zhang L, Zhi L, et al. Design and synthesis of potent retinoid X receptor selective ligands that induce apoptosis in leukemia cells. *J Med Chem* 1995;38:3146–55.
- Liby K, Royce DB, Williams CR, et al. The synthetic triterpenoids, CDDO-methyl ester and CDDO-ethyl amide, prevent lung cancer induced by vinyl carbamate in A/J mice. *Cancer Res* 2007;67:2414–9.
- Yates MS, Tauchi M, Katsuoka F, et al. Pharmacodynamic characterization of chemopreventive triterpenoids as exceptionally potent inducers of Nrf2-regulated genes. *Mol Cancer Ther* 2007;6:154–62.
- Honda T, Honda Y, Favaloro FG, Jr., et al. A novel dicyanotriterpenoid, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-onitrile, active at picomolar concentrations for inhibition of nitric oxide production. *Bioorg Med Chem Lett* 2002;12:1027–30.
- Moyer JD, Barbacci EG, Iwata KK, et al. Induction of apoptosis and cell cycle arrest by CP-358,774, an inhibitor of epidermal growth factor receptor tyrosine kinase. *Cancer Res* 1997;57:4838–48.
- Pollack VA, Savage DM, Baker DA, et al. Inhibition of epidermal growth factor receptor-associated tyrosine phosphorylation in human carcinomas with CP-358,774: dynamics of receptor inhibition in situ and antitumor effects in athymic mice. *J Pharmacol Exp Ther* 1999;291:739–48.
- Witschi H. A/J mouse as a model for lung tumorigenesis caused by tobacco smoke: strengths and weaknesses. *Exp Lung Res* 2005;31:3–18.
- Meuwissen R, Berns A. Mouse models for human lung cancer. *Genes Dev* 2005;19:643–64.
- Gunning WT, Kramer PM, Lubet RA, Steele VE, Pereira MA. Chemoprevention of vinyl carbamate-induced lung tumors in strain A mice. *Exp Lung Res* 2000;26:757–72.
- Gunning WT, Kramer PM, Steele VE, Pereira MA. Chemoprevention by lipoxygenase and leukotriene pathway inhibitors of vinyl carbamate-induced lung tumors in mice. *Cancer Res* 2002;62:4199–201.
- Pereira MA, Li Y, Gunning WT, et al. Prevention of mouse lung tumors by budesonide and its modulation of biomarkers. *Carcinogenesis* 2002;23:1185–92.
- Foley JF, Anderson MW, Stoner GD, Gaul BW, Hardisty JF, Maronpot RR. Proliferative lesions of the mouse lung: progression studies in strain A mice. *Exp Lung Res* 1991;17:157–68.
- Gonzalez MA, Tachibana KE, Chin SF, et al. Geminin predicts adverse clinical outcome in breast cancer by reflecting cell-cycle progression. *J Pathol* 2004;204:121–30.
- Perez RP, Lewis LD, Beelen AP, et al. Modulation of cell cycle progression in human tumors: a pharmacokinetic and tumor molecular pharmacodynamic study of cisplatin plus the Chk1 inhibitor UCN-01 (NSC 638850). *Clin Cancer Res* 2006;12:7079–85.
- Yan Y, Lu Y, Wang M, et al. Effect of an epidermal growth factor receptor inhibitor in mouse models of lung cancer. *Mol Cancer Res* 2006;4:971–81.
- You M, Candrian U, Maronpot RR, Stoner GD, Anderson MW. Activation of the Ki-ras protooncogene in spontaneously occurring and chemically induced lung tumors of the strain A mouse. *Proc Natl Acad Sci U S A* 1989;86:3070–4.
- Pao W, Wang TY, Riely GJ, et al. KRAS mutations and primary resistance of lung adenocarcinomas to Gefitinib or Erlotinib. *PLoS Med* 2005;2:e17.
- Eberhard DA, Johnson BE, Amler LC, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *J Clin Oncol* 2005;23:5900–9.
- Massarelli E, Varella-Garcia M, Tang X, et al. KRAS mutation is an important predictor of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. *Clin Cancer Res* 2007;13:2890–6.
- Sjoberg T, Jones S, Wood LD, et al. The consensus coding sequences of human breast and colorectal cancers. *Science* 2006;314:268–74.
- Albini A, Sporn MB. The tumor microenvironment as a target for chemoprevention. *Nat Rev Cancer* 2007;7:139–47.
- Vannini N, Lorusso G, Cammarota R, et al. The synthetic oleanane triterpenoid, CDDO-methyl ester, is a potent antiangiogenic agent. *Mol Cancer Ther* 2007;6:3139–46.
- Liby K, Royce DB, Risingsong R, et al. A new retinoid, NRX194204, prevents carcinogenesis in both the lung and mammary gland. *Clin Cancer Res* 2007;13:6237–43.
- Sporn MB, Liby KT. Cancer chemoprevention: scientific promise, clinical uncertainty. *Nat Clin Pract Oncol* 2005;2:518–25.