A Novel Retinoic Acid Receptor-selective Retinoid, ALRT1550, Has Potent Antitumor Activity against Human Oral Squamous Carcinoma Xenografts in Nude Mice


ABSTRACT

We have identified a novel retinoid, ALRT1550, that potently and selectively activates retinoic acid receptors (RARs). ALRT1550 binds RARs with $K_d$ values of $\approx 1$ nM, and retinoid X receptors with low affinities ($K_d = 270-556$ nM). We studied the effects of ALRT1550 on cellular proliferation in squamous carcinoma cells. ALRT1550 inhibited proliferation of UM3CC-22B cells in a concentration-dependent manner with an IC$_{50}$ value of $0.22 \pm 0.01$ (SE) nM. 9-cis-Retinoic acid (ALRT1057), a pan agonist retinoid that activates RARs and retinoid X receptors, inhibited proliferation with an IC$_{50}$ value of $81 \pm 29$ nM. In vivo, as tumor xenografts in nude mice, UM3CC-22B formed well-differentiated squamous carcinomas, and oral administration (daily, 5 days/week) of ALRT1550, begun 3 days after implanting tumor cells, inhibited tumor growth by up to 89% in a dose-dependent manner over the range of 3-75 µg/kg. ALRT1550 (30 µg/kg) also inhibited growth of established tumors by 72 ± 3% when tumors were allowed to grow to $\approx 100$ mm$^3$ before dosing began. In comparison, 9-cis retinoic acid at 30 µg/kg inhibited growth of established tumors by 73 ± 5%. Interestingly, retinoids did not appear to alter tumor morphologies in UM3CC-22B tumors. Notably, ALRT1550 produced a therapeutic index of $\approx 17$ in this model, indicating a separation between doses that inhibited tumor growth and that induced symptoms of hypervitaminosis A. In summary, ALRT1550 potently inhibits cellular proliferation in vitro and in vivo in this squamous cell carcinoma tumor model. These data support additional study of ALRT1550 for its potential for improving anticancer therapy in human clinical trials.

INTRODUCTION

Retinoids modulate cellular differentiation and growth and have been studied clinically as chemopreventive agents and as anticancer agents for treating certain malignancies (1-3). Preclinically, retinoids have been shown to suppress carcinogenesis in a variety of epithelial tissues, including skin, oral mucosa, trachea, lung, bladder, and mammary gland (1-4). In the clinic, 13-cis RA$^1$ and ATRA have been shown to suppress oral leukoplakia premalignant lesions (5-7). In addition, after surgery or radiation therapy to remove a tumor mass, 13-cis RA enhanced the antitumor efficacy of cisplatin in this differentiation-sensitive tumor model (8). Others have also demonstrated enhanced efficacy with retinoids in combination with biological response modifiers or cytotoxic agents in both preclinical and clinical studies (reviewed in Refs. 18 and 19; 20-22). Additionally, it has been reported that cytokertatin expression can influence cellular sensitivities to cytotoxic agents (23, 24), raising the possibility that retinoids, which are known to alter cytokertatin expression patterns (17, 25), may alter cellular sensitivities to cytotoxic drugs by modulating cytokertatin expression. These preclinical data support the clinical use of retinoids in combination therapies.

We have reported previously that retinoids suppress epidermoid differentiation in HNSCC 1483 tumors in nude mice (17). Phenotypic modulation of 1483 tumor differentiation state by 9-cis RA (ALRT1057) provided a rationale for combination chemotherapy with retinoids and cytotoxic agents. Subsequent studies demonstrated that 9-cis RA enhanced the antitumor efficacy of cisplatin in this differentiation-sensitive tumor model (18). Others have also demonstrated enhanced efficacy with retinoids in combination with biological response modifiers or cytotoxic agents in both preclinical and clinical studies (reviewed in Refs. 18 and 19; 20-22). Additionally, it has been reported that cytokertatin expression can influence cellular sensitivities to cytotoxic agents (23, 24), raising the possibility that retinoids, which are known to alter cytokertatin expression patterns (17, 25), may alter cellular sensitivities to cytotoxic drugs by modulating cytokertatin expression. These preclinical data support the clinical use of retinoids in combination therapies.

Toward the goal of developing retinoids that will be effective as single agents or in combination regimens, new retinoids that selectively activate RAR subtypes have been synthesized and examined in preclinical anticancer models. In this report, we have identified a novel retinoid, ALRT1550, that selectively activates RARs. Using a human oral squamous carcinoma cell line, UM3CC-22B (26), that has been reported to be sensitive to ATRA in clonogenic assays (27), we studied the effects of ALRT1550 on cellular proliferation. Additionally, retinoid receptor expression patterns before and after retinoid exposure were determined to identify receptors that may mediate antiproliferative effects in UM3CC-22B cells. We report now that ALRT1550 potently inhibits UM3CC-22B growth in vitro and in vivo. Of importance, ALRT1550 produced a positive therapeutic index for inhibiting tumor growth in nude mice. Interestingly, retinoids did not

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4 The abbreviations used are: 9-cis RA, 9-cis retinoic acid; ATRA, all-trans retinoic acid; 13-cis RA, 13-cis retinoic acid; HNSCC, head and neck squamous cell carcinoma; MTD, maximum tolerated dose; RAR, retinoic acid receptor; RXR, retinoid X receptor; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; FACS, fluorescence-activated cell sorting; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
appear to suppress the epidermoid differentiation state of UMSCC-22B tumors, in contrast to prominent suppression of differentiation produced in HNSCC 1483 tumors (17), demonstrating that this new model is sensitive primarily to growth-inhibitory effects of retinoids.

MATERIALS AND METHODS

Reagents and Chemicals. All procedures were carried out under subdued, ambient laboratory light and without application of excessive external heat to minimize isomerization of all compounds and metabolites. ALRT1550 [(2E,4E,6E)-7-(3,5-di-tert-butylphenyl)-3-methylocta-2,4,6-trienoic acid; lot 0005003] and ALRT1057 (9-cis RA; lot 0002018) were synthesized at Ligand Pharmaceuticals, Inc. (San Diego, CA; Ref. 28) and recrystallized to >99% purity. Compound purity was verified by elemental analysis, nuclear magnetic resonance spectra, and high-performance liquid chromatography-UV assay.

Powders were stored at -80°C prior to use.

Cell Line. The UMSCC-22B cell line was derived from a patient with squamous cell cancer of the hypopharynx (26). Histopathology showed that the original tumor from which this cell line was derived was moderately well differentiated. The patient bearing this tumor had not been treated with chemotherapy or radiation prior to isolation of UMSCC-22B. The tumor was aggressive, and the patient died within 9 months of diagnosis of hypopharyngeal cancer. Cells were cultured routinely in DMEM:F12 medium (BioWhittaker, Inc., Walkersville, MD) supplemented with 10% FCS (Hyclone, Logan, UT), 2 mM l-glutamine, and 5 units/ml penicillin-5 μg/ml streptomycin (Life Technologies, Inc., Grand Island, NY) at 37°C in 95% air-5% CO2 (standard culture conditions). Cells were tested and found to be free of Mycoplasma.

Cellular Proliferation Assays. Two thousand cells were plated in 1 ml of culture medium in 24-multiwell dishes (Costar, Lincoln Park, NJ) and incubated overnight under standard culture conditions. The following day, medium was removed and fresh medium containing 0.1% (w/v) ethanol vehicle or retinoids in 0.1% ethanol was added. Cell numbers were assessed using a ZB1 Coulter Counter (Hialeah, FL). Dose-response curves for ALRT1550 and 9-cis RA were determined on day 12. Data were plotted as a percentage of retinoid to vehicle-treated control. Cellular proliferation was also assessed by MTT (Promega Corp., Madison, WI) dye reduction. The same protocol was used as described above, except that 5000 cells were plated in 96-well multiwell dishes (Falcon, Lincoln Park, NJ), and proliferation by MTT reduction was scored by spectrophotometric assessment of dye reduction at 570 nm. Cellular viabilities were also determined by monitoring trypan blue staining and propidium iodide uptake (by FACS) of cellular suspensions.

In Vivo Tumor Xenograft Studies. Cells in log phase were harvested and resuspended in culture medium at a concentration of 1 × 10^7 cells/ml prior to s.c. implantation into mice. Female athymic nu/nu mice (Taconic Labs, Germantown, NY), were quarantined for 1 week after birth until full hair growth, and were then weaned and quarantined for an additional week. Mice weighing 20 ± 2 g were used for experiments. Mice were quarantined for 1 week prior to study and allowed access to food (Purina Chow 5010, St. Louis, MO) and water ad libitum. Mice were implanted with 1 × 10^6 cells bilaterally into axial regions with a 24-gauge needle/1-cm subcutaneous syringe (Becton Dickinson, Rutherford, NJ) as reported previously (17). Animals were randomized 8 h after tumor implantation into treatment groups or after tumors had reached ≥100-mm^3 volumes. Two paradigms were used. Treatment was begun either 3 days after implantation of tumor cells or after established growing tumors had formed. Each group generally consisted of four to six animals bearing two tumors per animal. Retinoids were administered with a 20-gauge intragastric feeding tube (Popper & Sons, New Hyde Park, NY) daily, 5 days/week, in 0.1 ml of super-refined sesame oil (Croda, Inc., Parsippany, NJ). Tumors were also recorded at least weekly.

Signs such as overall health status and potential mucocutaneous irritations were also determined by monitoring trypan blue staining and propidium iodide uptake (by FACS) of cellular suspensions.

Retinoid Receptor Expression Patterns. RNase protection assays utilizing human probes for RAR-α, RAR-β, RAR-γ, RXR-α, RXR-β, and RXR-γ were conducted on RNA isolated from UMSCC-22B cells after 24- or 48-h exposure to ALRT1550 (10^-6 M). Total cytoplasmic RNA was isolated and RNA protection analyses were performed as described previously (17). Briefly, hybridization of cRNA probes was carried out at 45°C overnight, followed by the addition of 300 μl of RNase digestion buffer containing 40 μg/ml of RNase A and 700 units/ml of RNase T1. RNase digestion was performed at 25°C for 1 h. RNase-resistant fragments were resolved by electrophoresis on 6% urea-polyacrylamide sequencing gels. To control for RNA loading, a GAPDH probe was included in all samples. As approximate size markers, 32P-labeledMspI-digested fragments of pBR322 were run on all gels.

Plasmid Constructs. RNase protection probes for RARs and RXRs were constructed by PCR amplification of the cloned sequences as reported previously (3). Fragments were cloned into pGEM4Z (Promega), and identity was confirmed by nucleotide sequencing. Specificity of the probe for human sequences was verified by testing it against both mouse and human RNAs known to contain the corresponding mRNA. A cRNA probe was produced by linearizing the plasmid with EcoRI followed by in vitro transcription utilizing T7 polymerase. A probe corresponding to human GAPDH was purchased from Ambion, Inc. (Austin, TX).

Binding and Cotransfection Studies. Receptor binding assays for RARs and RXRs were performed using 3H-labeled 9-cis RA as the radioligand for RXRs and 3H-labeled ATRA for the RARs as reported previously (28, 29). Cotransfection assays were also performed as described previously (28, 29). K values are mean of four to five experiments with triplicate determinations in baculovirus extracts. EC50 values were determined from four to five experiments determining full dose-response curves ranging from 10^-1 to 10^-5 M in CV-1 cells (see Ref. 28). SEs are ±15% of mean values in both assays.

**Table 1 Competitive binding (K) and Cotransfection (EC50) data for ALRT1550 and 9-cis RA**

<table>
<thead>
<tr>
<th>Retinoid*</th>
<th>RAR</th>
<th>RXR</th>
</tr>
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<tbody>
<tr>
<td>ALRT1550</td>
<td>α</td>
<td>β</td>
</tr>
<tr>
<td>K</td>
<td>1.1</td>
<td>0.7</td>
</tr>
<tr>
<td>EC50</td>
<td>4.0</td>
<td>2.2</td>
</tr>
<tr>
<td>9-cis RA</td>
<td>K</td>
<td>22</td>
</tr>
<tr>
<td>EC50</td>
<td>304</td>
<td>52</td>
</tr>
</tbody>
</table>

*Binding and cotransfection values are in nm. K values are mean of four to five experiments with triplicate determinations in baculovirus extracts.
<table>
<thead>
<tr>
<th>Retinoid (m)</th>
<th>MTT Reduction (% Decrease)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>ALRT1550 (10^{-9})</td>
<td>77</td>
</tr>
<tr>
<td>AGN193109 (10^{-6})</td>
<td>2</td>
</tr>
<tr>
<td>ALRT1550 (10^{-8}) + AGN193109 (10^{-6})</td>
<td>35</td>
</tr>
</tbody>
</table>

* Mean percentage of change in MTT dye reduction after 9 days. Absorbance in control wells was 0.9 ± 0.1 absorbance units at λ 570. Mean values are from one experiment with eight wells per point (SD ± 15%).

m decreased optical absorbances at 570 nm by 49 and 65%, respectively (data not shown). To explore the activity of ALRT1550 on the growth of these cells, we examined the cellular proliferation of these cells treated with ALRT1550 in the presence or absence of a RAR antagonist, AGN193109 (30). AGN193109 (10^{-6} M) had no effect on the growth of the cells but attenuated the growth-inhibitory activity of ALRT1550 (Table 2), suggesting that the antiproliferative activity of ALRT1550 is mediated by RARs. Cellular viability by trypan blue staining was also assessed and exceeded 89% in all groups after 12 days of exposure (data not shown), indicating that retinoids were not toxic to cells at concentrations that markedly inhibited growth.

Antiproliferative Effects of ALRT1550 in Vitro. The ability of ALRT1550 to alter cellular proliferation of UMSCC-22B cells was next examined (Fig. 1). Exponential growth was observed over days 6–12 with growth plateauing thereafter. ALRT1550 inhibited cellular proliferation in a concentration-dependent manner, with 83% inhibition observed at 10 nM after 12 days. Inhibitions were detected after 6 days of treatment and became prominent thereafter. Subsequent studies were conducted for 12 days. Both ALRT1550 and 9-cis RA inhibited proliferation to a similar extent, but ALRT1550 was clearly more potent than 9-cis RA (Fig. 2). IC_{50} values for ALRT1550 and 9-cis RA were determined to be 0.22 ± 0.1 and 81 ± 29 nM, respectively.

In addition, cellular proliferation was determined by a MTT assay after 9 days of exposure to ALRT1550. ALRT1550 at 10^{-10} and 10^{-8} to RARs with low nM affinities with ALRT1550 being approximately 10–20-fold more potent at binding, and approximately 30–50-fold more potent at transactivating RARs than 9-cis RA. Additionally, 9-cis RA binds to RXRs with high affinities, whereas ALRT1550 binds with much lower affinities (>200 nM) and does not transactivate the RXRs. These data demonstrate that ALRT1550 is RAR selective as compared to the pan agonist, 9-cis RA, which interacts with all six receptor subtypes.

Antiproliferative Effects of ALRT1550 in Vivo. The ability of ALRT1550 to alter cellular proliferation of UMSCC-22B cells was next examined (Fig. 1). Exponential growth was observed over days 6–12 with growth plateauing thereafter. ALRT1550 inhibited cellular proliferation in a concentration-dependent manner, with 83% inhibition observed at 10 nM after 12 days. Inhibitions were detected after 6 days of treatment and became prominent thereafter. Subsequent studies were conducted for 12 days. Both ALRT1550 and 9-cis RA inhibited proliferation to a similar extent, but ALRT1550 was clearly more potent than 9-cis RA (Fig. 2). IC_{50} values for ALRT1550 and 9-cis RA were determined to be 0.22 ± 0.1 and 81 ± 29 nM, respectively.

In addition, cellular proliferation was determined by a MTT assay after 9 days of exposure to ALRT1550. ALRT1550 at 10^{-10} and 10^{-8}
tumor growth after >3 weeks of dosing in mice bearing established tumors. Values are means (bars, SE) from three experiments.

These data demonstrate that UMSCC-22B cells were sensitive to the antiproliferative effects of retinoids and that ALRT155O was >350-fold more potent than 9-cis RA in inhibiting cellular proliferation in this assay.

Antitumor Effects of ALRT155O in Vivo. A xenograft model in nude mice was implemented to study antitumor effects of ALRT155O in vivo. Prior to initiating tumor efficacy studies, a MTD (the dose that produces <10% weight loss and mild to moderate mucocutaneous irritations) for ALRT155O was determined to be 50 µg/kg (daily, 5 days/week dosing; sesame oil vehicle) in dose-ranging studies. ALRT155O was administered p.o. to mice after implanting tumor cells s.c. as described in “Materials and Methods.” Tumor growth after 40 days was inhibited in a dose-dependent manner by up to 89% over the range of 3–75 µg/kg (P < 0.01 for all doses; Fig. 3A). Animal body weights were monitored simultaneously (Fig. 3B). Control mice gained 16.8% body weight from the start to the end of the study. ALRT155O was well tolerated at doses ≤10 µg/kg. At the lowest dose of 3 µg/kg ALRT155O, mice gained 14.4% body weight, and there was a dose-dependent decrease in body weight changes; at 100 µg/kg ALRT155O, mice rapidly lost ≥25% body weight and were euthanized on day 11 (data not shown). Mild, moderate, and severe mucocutaneous irritation was produced at doses of 30, 50, and 75 µg/kg, respectively.

We next examined the efficacy of ALRT155O on the growth of established tumors (Fig. 4). In this study, 30 µg/kg ALRT155O were administered to mice bearing 85-mm³ tumors beginning on day 26 after tumor cell implantation. Control tumors achieved volumes of 286 mm³ by day 54 of the study. Tumor growth was inhibited by 22, 45, 68, and 75% versus control after 3, 11, 18, and 28 days of dosing, respectively, with 30 µg/kg ALRT155O (P < 0.03), demonstrating its activity against established tumors. The average percentage of inhibition produced by ALRT155O against established tumors after >3 weeks of dosing was 72 ± 3% (n = 3; Fig. 4, inset). For comparison (also Fig. 4, inset), 30 mg/kg 9-cis RA (ALRT 1057) inhibited the growth of established tumors by 73 ± 5% (P < 0.02, n = 3) without producing mucocutaneous irritations or weight loss.

Therapeutic Index of ALRT155O. To estimate a therapeutic index for ALRT155O, we determined the ratio of the lowest dose necessary to inhibit 50% tumor growth and the MTD. ALRT155O was effective in inhibiting tumor growth at 3 µg/kg, whereas the MTD established in dose-ranging and tumor-efficacy studies was 50 µg/kg based on the criteria of a <10% body weight change and mild to moderate mucocutaneous irritation. On the basis of these data, a therapeutic index of 17 was derived by dividing the MTD of 50 µg/kg by the efficacious dose of 3 µg/kg, which decreased tumor growth by 72%. These data demonstrate that ALRT155O inhibited tumor growth at doses that did not produce adverse effects.

UMSCC-22B Tumors Are Not Sensitive to Differentiation-Suppressive Effects of Retinoids. UMSCC-22B cells formed well-differentiated carcinomas in nude mice with pronounced keratinization (keratin pearls) and cellular stratification as shown by distinct basal and suprabasal cell compartments within nests of carcinoma cells (Fig. 5A). Treatment with ALRT155O for 40 days (study shown in Fig. 3) did not appear to alter epidermoid differentiation (Fig. 5B). Carcinoma nests containing keratin pearls were abundant. In addition, 9-cis RA did not appear to alter tumor morphologies (data not shown), suggesting that UMSCC-22B tumors are not sensitive to differentiation-suppressive effects of retinoids.

Retinoid Receptor Expression in UMSCC-22B Cells. It has been reported that retinoids selective for RAR subtypes are antiproliferative and/or modulate cellular differentiation (14, 31–33). To identify receptors that may mediate antiproliferative effects in UMSCC-22B cells, retinoid receptor mRNA expression patterns were determined both in the presence and absence of ALRT155O. UMSCC-22B cells were observed to express mRNAs for RAR-α, RAR-γ, RXR-α, and RXR-β (Fig. 6). Cells did not have detectable levels of mRNA for RXR-β or RXR-γ. To determine whether treatment of UMSCC-22B with ALRT155O would alter the retinoid receptor expression pattern seen in these cells, UMSCC-22B cells were treated with 10⁻⁷ M ALRT155O for 24 and 48 h (Fig. 6, Lanes 2 and 3, respectively). After exposure to ALRT155O, there was no change in retinoid receptor expression levels (Fig. 6). Specifically, RAR-β expression was not detected either in vehicle-treated or ALRT155O-treated UMSCC-22B cells. In a separate experiment, UMSCC-22B cells were treated with 10⁻⁶ M 9-cis RA; again, there was no change in the retinoid receptor profile after treatment with 9-cis RA (data not shown).

DISCUSSION

This report demonstrates marked antitumor activity of ALRT1550, a RAR-selective retinoid, in a tumor model of head and neck cancer. Of importance, ALRT1550 potently and dose-dependently inhibited tumor growth while producing a positive therapeutic index, supporting additional study of this compound for its potential for improving anticancer therapy in humans.

ALRT1550 was >350-fold more potent than 9-cis RA in inhibiting cellular growth in culture and tumor growth in vivo. Furthermore, these potency differences extend to another squamous carcinoma cell line (cervical carcinoma ME-180), in which ALRT1550 inhibited thymidine incorporation into cells 250-fold more potently than 9-cis RA (28). Comparison of binding and cotransfection affinities of these retinoids for RARs might lead to the expectation that they would have differences in potency of ≥10–50-fold. In culture, 9-cis RA produced

6 D. R. Shalinsky, unpublished observations. Mice gained 2.6% of their total body weight from the start to the end of the study.

The therapeutic index was defined as MTD/IC₅₀ for inhibition of tumor growth.
an IC\textsubscript{50} of 81 nm, and the maximum inhibition was observed at 1000 nm. In contrast, ALRT1550 produced an IC\textsubscript{50} of 0.2 nm, and maximum inhibitions were observed at 1–10 nm. Potency differences greater than two orders of magnitude were also observed in vivo, suggesting that ALRT1550 has different pharmacodynamics, different pharmacokinetics, and/or additional mechanisms to regulate tumor cell growth.

Retinoids can repress certain transcription factors, such as AP-1 (15, 16), in addition to inducing transactivation of retinoid receptors (13). It is entirely possible that ALRT1550 exerted its potent effects by both inhibiting AP-1 and/or transactivating RARs, and it is likely that mechanisms underlying the prominent antiproliferative and anti-tumor effects of retinoids demonstrated herein are multifactorial in nature.

A plethora of data in HNSCC models has demonstrated that retinoids suppress squamous cell differentiation \textit{in vitro} (25, 27, 34, 35). We have reported previously that 9-cis RA suppresses epidermoid differentiation in HNSCC 1483 tumor xenografts (17) and postulated that these morphological changes made the tumors more susceptible to combination therapy with cytotoxic agents such as cisplatin (18). Of note in this work is the prominent effect of retinoids on UMSCC-22B tumor growth without an apparent effect on epidermoid differentiation state. The following conclusion can be made from these data: inhibition of growth by retinoids can be dissociated from modulation of tumor differentiation state. Dissociation of effects on differentiation and growth are also supported by our earlier report in which 9-cis RA markedly suppressed epidermoid differentiation while producing small effects on tumor growth (17). These observations suggest that some HNSCC tumors will be more susceptible to the growth-inhibitory effects of retinoids, and others will be more susceptible to their differentiation-suppressive effects. Of importance, both tumor types (or tumors exhibiting various degrees of growth and differentiation sensitivity) may be amenable to retinoid-based therapies, either as single agents or in combination regimens.

Fig. 5. Representative effects of ALRT1550 on morphologies of UMSCC-22B tumors after 40 days of growth in nude mice. A, control: tumors were well differentiated with distinct borders between human carcinoma and host cells. Cellular stratification of basal and suprabasal cells and keratin pearls are evident. B, 30 \mu g/kg ALRT1550: keratin pearls are evident without marked changes in epidermoid differentiation state. Magnification, \times 40.
The loss of RAR-β expression in oral premalignant and malignant squamous cell tissues has been suggested to be associated with HNSCC development (36, 37). On the basis of these observations, investigators have recently addressed this point by genetically altering the levels of expression of retinoid receptors and demonstrating that transfection of certain retinoid receptors (e.g., RAR-α) enhances the antiproliferative effects of retinoids in certain cancer cells (31), and transfection of RAR-β reduces in vitro clonogenicity (38) and tumorigenicity in nude mice (39). On the basis of these observations, we examined retinoid receptor expression patterns in UMSCC-22B cells. UMSCC-22B cells expressed RAR-α, RAR-γ, RXR-α, and RAR-β, and the levels of expression are not significantly altered upon retinoid treatment. Furthermore, RAR-β expression was not detectable nor induced upon retinoid treatment, even in light of the significant antiproliferative response achieved by ALRT1550 treatment. Thus, these data demonstrate that RAR-β did not mediate antiproliferative effects in UMSCC-22B cells, whereas mediation by RAR-α or RAR-γ cannot be excluded. Our data do not address issues related to RAR-β expression and malignant progression; lack of RAR-β expression by UMSCC-22B tumors is entirely consistent with this possibility (34, 36, 37).

In summary, this report identifies a novel RAR-selective retinoid, ALRT1550, that markedly inhibits the growth of HNSCC tumors (line UMSCC-22B). Furthermore, we have developed a new xenograft model of oral cancer that is highly sensitive to growth-inhibitory effects of retinoids. The high potency of ALRT1550 observed in vitro translated into in vivo efficacy with a positive therapeutic index. In this model, RAR-β does not appear to mediate growth inhibition by retinoids, indicating that additional receptors and/or mechanisms are responsible for the antiproliferative effects of retinoids demonstrated herein. These data support additional testing of ALRT1550 for its potential for improving chemotherapy in human clinical trials.

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