Targeting the RXR Pathway for the Prevention of Triple-Negative Breast Cancer

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ABSTRACT

Prophylactic treatment with selective estrogen receptor (ER) modulators and aromatase inhibitors targeting the nuclear ER can prevent the formation of ER-positive tumors in women at high risk of breast cancer but does not prevent ER-negative and triple-negative subtypes. In this study, we tested whether nuclear retinoid X receptor (RXR) agonists, IRX4204 and 9cUAB30, which have been evaluated in clinical trials, could prevent the development of ER-negative and triple-negative breast cancers. Our study demonstrates that IRX4204 significantly delays the formation of mammary tumors in three ER-negative mouse models: MMTV-ErbB2, C3(1)/SV40-TAg, and Brca1-deficient with modest toxicities. In some of the MMTV-ErbB2 mice, IRX4204 completely prevented mammary tumor formation, and 60% of the IRX4204-treated Brca1-deficient mice remained tumor-free when all vehicle-treated mice had formed tumors. 9cUAB30 treatment also delays tumor formation in Brca1-deficient mice, albeit to a lesser extent. Biomarker analysis revealed that delayed tumors arising after IRX4204 treatment had decreased Ki-67 expression and increased infiltration of cytotoxic T cells. Our preclinical study data support the further evaluation of use of RXR agonists for the prevention of triple-negative breast cancer.

Prevention Relevance: Treatment with the RXR agonist IRX4204 significantly delays tumor formation and increases CD8-positive T-cell infiltration in ER-negative murine breast cancer models. This suggests that immune modulation may be critical for rexinoid-based prevention of ER-negative mammary tumors and supports their use in future breast cancer prevention trials for high-risk individuals.

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Introduction

Despite the advancements in breast cancer treatment, breast cancer incidence is on the rise (1). Women of African descent, women with a strong family history of cancer, and women born with a deleterious variant in risk genes, such as *BRCA1* or *BRCA2*, have an even greater chance of developing breast cancer in their lifetime (2). It is also known that familial breast cancer associated with *BRCA1* mutations is more likely to be triple-negative breast cancer (TNBC; ref. 3). TNBCs are often highly aggressive tumors with a poor disease prognosis, even after treatment with chemotherapy. The ability to identify women at high risk of breast cancer provides an opportunity for early intervention to prevent tumor development.

At present, prophylactic bilateral mastectomy is the most effective strategy for reducing breast cancer risk. However, this invasive procedure is irreversible and associated with potential complications. Studies with selective estrogen receptor (ER) modulators and aromatase inhibitors have shown that breast cancer prevention is feasible (4, 5). However, these drugs do not prevent ER-negative tumors, including TNBC, and their unwanted side effects discourage patients from using them for prevention. We and others have found that PARP inhibitors can delay *Brca1*-mutant tumor formation in mice and could be beneficial for the prevention of breast cancer (6). However, currently available PARP inhibitors are associated with toxicities that may not be acceptable to women without cancer. Thus, there remains a need for the development of effective therapies with minimal toxicity for the prevention of TNBC.

The retinoid X receptor (RXR) heterodimerizes with other nuclear receptors, such as the retinoic acid receptor, to regulate the expression of genes controlling critical signaling pathways and biological functions such as development, metabolism, and inflammation. As such, retinoic acid receptor agonists (retinoids)

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and RXR agonists (rexinoids) have been used for the treatment of cancers, including acute promyelocytic leukemia, hairy cell leukemia, and Kaposi's sarcoma (7, 8). The RXR-selective rexinoid 9cUAB30 has been shown to delay tumor growth and increase survival in preclinical models of breast cancer with fewer toxicities than early-generation retinoids (9). We and others have shown that the highly specific RXR agonist IRX4204 can also inhibit the growth of mammary tumors (10, 11). Rexinoids are well tolerated in both animals and humans and are less toxic than naturally occurring retinoids (8, 11), making them ideal treatments for cancer prevention.

For this study, we evaluated the cancer-preventive efficacy of RXR agonists in three preclinical mouse models representing different genetic and pathologic states of ER-negative breast cancer and TNBC: MMTV-Erbb2 (ER-negative/HER2-overexpressed), C3(1)/SV40-TAg (TNBC), and Brca1-deficient (TNBC). Treatment with IRX4204 or 9cUAB30 prior to tumor development significantly delayed the formation of breast cancer in each of these models. Delayed tumor onset in the Brca1-deficient mice with IRX4204 10 mg/kg treatment was accompanied by increased infiltration of $CD8^+$ T cells and activation of the antitumor immune response. Our findings suggest that RXR agonists can be effective agents for delaying or preventing ER-negative breast cancer and TNBC mammary tumor development, in part by modulating the tumor-immune response. These results support testing RXR agonists in cancer prevention trials of women at high risk of developing breast cancer.

Materials and Methods

Drugs

IRX4204 was obtained from Io Therapeutics, Inc. and dissolved in sesame oil. The synthesis and characterization of IRX4204 have been described previously (12). 9cUAB30 was obtained from the NCI Division of Cancer Prevention Repository and dissolved in sesame oil.

Mice

MMTV-erbb2 (RRID: IMSR_JAX:002376) mice were purchased from The Jackson Laboratory. C3(1)/SV40-TAg mice (13) were generated by breeding FVB wild-type females with heterozygous C3(1)/SV40-TAg males. To generate the conditional Brca1-deficient female mice, we mated 129S1 $BRCA1^{co/co}$; MMTV-$Cre^{+/+}$;$p53^{+/-}$ males with $BRCA1^{co/co}$;MMTV-$Cre^{+/+}$; $p53^{+/+}$ females. The characteristics of each animal model are detailed in Supplementary Table S1 with a representative PCR confirmation of genetic status in Supplementary Fig. S1. Virgin animals were used to avoid confounding effects of hormonal surges during pregnancy in all mouse models. All animal experiments were performed in accordance with M.D. Anderson Institutional Animal Care and Use Committee–approved protocols.

Animal studies

IRX4204 (1, 10 or 20 mg/kg), 9cUAB30 (5 mg/kg), and sesame oil (vehicle) were administered via oral gavage for all preclinical experiments based on comparable human doses, and scheduling was chosen to achieve maximum preventive efficacy without inducing any severe toxicities. The primary endpoint for all prevention experiments was the time to tumor formation (TTF). Palpable tumors were defined as tumors having volume equal to or greater than 100 mm^3, with tumor volume calculated by $V = (\text{width}^2 \times \text{length})/2$. A secondary endpoint of toxicity was determined by weight loss, hair loss, and moribund status. For the MMTV-ErbB2 model, we used 15 mice in each group (vehicle and IRX 10 mg/kg) and began treatment at 12 weeks of age for five times a week. At 44 weeks of age, treatment frequency was reduced to three times a week. At 55 weeks of age, treatment frequency was reduced to once a week until treatment was dropped completely at 60 weeks. For the first study in the Brca1-deficient model, we used 11 mice per group (vehicle, IRX 1 mg/kg, IRX 10 mg/kg, and 9cUAB30) and began treatment at 12 weeks of age (5 times a week by oral gavage). Treatments in the first Brca1-deficient study were disrupted because of laboratory shutdowns associated with the COVID-19 pandemic. Treatment was initially started 5× per week. Upon the COVID-19 shutdown, the mice were treated 2× per week and then later 1× per week. A detailed description of the treatment schedule can be found in Supplementary Table S2. For the second Brca1-deficient study, we used 10 mice per group (vehicle, IRX 10 mg/kg, IRX 20 mg/kg, and 9cUAB30), and treatment was consistently administered three times a week until experiment endpoints were reached. For C3(1)/SV40-TAg studies, we used 8 mice in the vehicle group and 9 mice in the treatment group. Because C3(1)/SV40-TAg mice develop aggressive mammary tumors more quickly than other models, treatment of C3(1)/SV40-TAg mice was started at 7 weeks of age for 5 days a week.

Hematoxylin–eosin and IHC analyses

Tumor samples were processed by fixation in a 10% formalin solution, followed by embedding in paraffin. Hematoxylin–eosin staining and IHC staining of tumor tissue slides was performed at the Baylor College of Medicine Breast Center Pathology Core with Ki-67 primary antibody (Lab Vision, cat. #RM-9106, RRID: AB_2341197), cleaved caspase-3 [CC3; Cell Signaling Technology, cat. #9664 (also 9664P), RRID: AB_2070042], cyclin D1 (Cell Signaling Technology, #2978, RRID: AB_2259616), and CD8a (Abcam, Ab217344, RRID: AB_2890649. 4). Images were acquired with Aperio ImageScope (Leica Biosystems) and processed with Aperio ImageScope Pathology Slide Viewing Software (Leica Biosystems; RRID: SCR_020993).

nanoString

Total RNA was isolated from formalin-fixed, paraffin-embedded tissue sections using the RNeasy FFPE Kit (Qiagen) according to the manufacturer's instructions. RNA quantity was assessed on Qubit, and RNA integrity was assessed using the TapeStation system (Agilent). Total RNA

(100 ng) was used as input for the NanoString PanCancer IO360 Panel and run on the nCounter MAX/FLEX system by the Advanced Technology Genomics Core at MD Anderson Cancer Center. Analysis was performed with the Advanced Analysis program in nSolver 4.0 software. All genes expressing less than 20 counts were removed from analysis, and expression was normalized to the following housekeeping genes: *MRPL19*, *OAZ1*, *PSMC4*, *SDHA*, *SF3A1*, and *TMUB2*.

FACS surface and intracellular staining

Spleens were harvested from mice treated with vehicle ($n = 3$) and IRX4204 10 mg/kg ($n = 6$) at experimental endpoints, and splenocytes were immediately collected for surface and intracellular marker staining. Red blood cells were lysed with RBC Lysis Buffer (Tonbo, cat. #TNB-4300). All steps were performed on ice in FACS buffer containing PBS, 3 mmol/L EDTA, and 2% FCS. For surface markers, 1×10^6 cells per sample were blocked with anti-mouse CD16/32 (Fc Block, 1 μg/30 μL) before staining with Fixability Viability Dye (1:300 final dilution) and surface marker antibodies (1:200 final dilution). For intracellular markers, cells were fixed and permeabilized using Intracellular Fixation and Permeabilization buffer set (eBioscience, cat. #88-8824-00) before staining with intracellular marker antibodies (1:100 final dilution) in 1× Permeabilization Buffer containing Fc Block (0.5 μg/sample). Cells were washed and resuspended in FACS buffer for analysis by flow cytometry. The list of antibodies used for these experiments can be found in Supplementary Table S3.

Blood analysis

Uncoagulated whole blood was used to measure blood concentration of IRX4204 using high-performance liquid chromatography–mass spectrometry analysis. Complete blood count or other blood parameters was measured using an automated hematology analyzer at MD Anderson core facility.

Statistical analysis

TTF analyses were determined by the Kaplan–Meier method and statistically evaluated using the generalized Wilcoxon test. IHC slides were analyzed by comparing percent positivity or Allred scores between treatment groups using Student *t* test. For immune biomarker analysis of splenocytes, percent positive of total cells was compared between treatment groups using Student *t* test with Welch correction. For immune changes measured by nanoString, gene expression was first normalized to total tumor-infiltrating lymphocytes, and relative gene expression was calculated between vehicle ($n = 3$) and IRX4204 10 mg/kg treatment ($n = 3$). Significant differences in immune marker expression between treatment groups were determined by Student *t* test. *P* value less than 0.05 is considered statistically significant.

Results

RXR agonists significantly delay the formation of ER-negative and triple-negative mammary tumors

To determine the preventative effects of RXR agonists on the development of ER-negative breast cancer and TNBC, we measured the TTF in three preclinical models of breast cancer after pretreatment with IRX4204 and/or 9cUAB30. In the HER2-positive, ER-negative MMTV-erbb2 model, vehicle-treated mice had a median TTF of 289 days, with 100% of mice developing tumors by 430 days. Mice treated with IRX4204 (10 mg/kg) starting at 12 weeks of age had a significantly improved median TTF of 417 days, with 20% of the mice remaining tumor-free at 500 days (**Fig. 1A**). These findings directly agree with a previously published study demonstrating a significant delay in tumor formation in MMTV-*neu* mice fed with IRX4204 diet (also named NRX194204) starting at 10 weeks of age (11). In the highly aggressive, triple-negative, C3(1)/SV40-TAg model, vehicle-treated mice showed a median TTF of 117 days compared with 130.5 days in mice treated with IRX4204 (10 mg/kg) starting at 7 weeks of age (**Fig. 1B**). Although IRX4204 treatment is unable to prevent tumor formation in the C3(1)/SV40-TAg model, the delay in tumor formation remains significant ($P = 0.0031$). Notably, once tumors arise, there is no difference in the tumor growth rate or size between vehicle- and IRX4204-treated mice (Supplementary Fig. S2).

Due to laboratory restrictions during the COVID-19 pandemic, dosing schedules were significantly interrupted in the Brca1-deficient mouse model study. Therefore, we performed two similar experiments in the Brca1-deficient model to investigate the preventative effect of IRX4204 and 9cUAB30 with different dosing schedules. In the first experiment, vehicle-treated Brca1-deficient mice had a median TTF of 202 days, with 100% of mice developing tumors by 254 days. Mice treated with 9cUAB30 5 mg/kg and IRX4204 1 mg/kg had an improved median TTF of 228 and 217 days, respectively, but the delay in tumor formation did not reach significance. However, mice treated with IRX4204 10 mg/kg demonstrated a significant delay in tumor formation with a median TTF of 268 days [**Fig. 1C** (left)]. Details on the exact dosing schedule for the first study can be found in Supplementary Table S2.

In the second study with more consistent dosing, vehicle-treated Brca1-deficient mice had a median TTF of 211 days, with 100% developing tumors by 327 days. Mice treated with 9cUAB30 5 mg/kg had an improved median TTF of 260 days, whereas mice treated with IRX4204 10 or 20 mg/kg had a median TTF of 372 and 304 days, respectively [**Fig. 1C** (right)]. Notably, 60% of mice treated with IRX4204 10 mg/kg remained tumor-free at 330 days (when 100% of vehicle-treated mice had developed tumors).

Minimal toxicities were observed with IRX4204 and 9cUAB30 treatment. There were no significant differences in body weight, triglyceride levels, or blood cell counts between vehicle- and drug-treated mice. We observed an unexplained statistically significant but moderate magnitude elevation in alkaline phosphatase levels in IRX4204-treated mice but did not

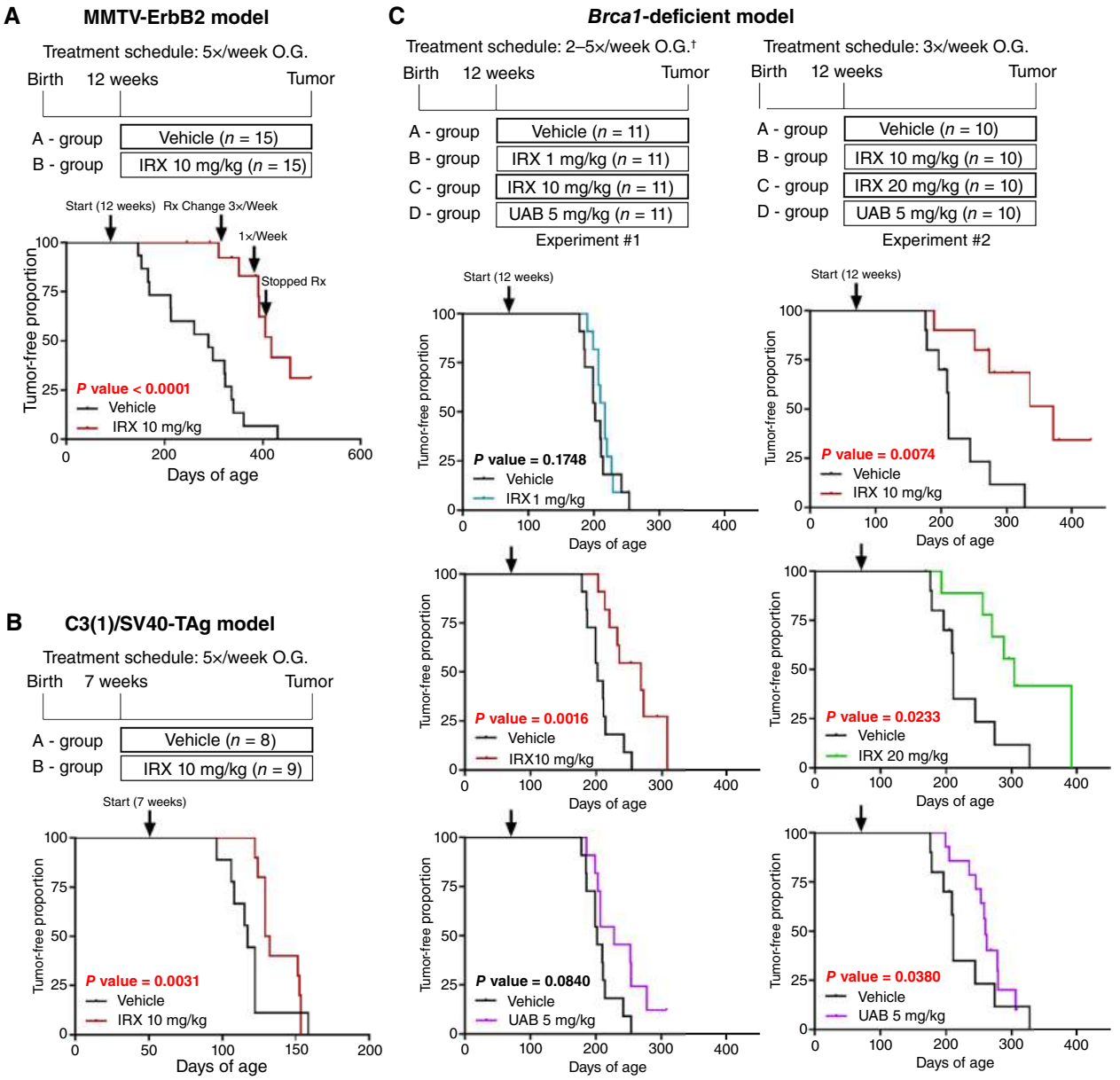

Figure 1.

Treatment with RXR agonists IRX4204 and 9cUAB30 delays formation of tumors in ER-negative breast cancer and TNBC models. **A,** IRX4204 and vehicle treatment schema with tumor-free proportion measured using a Kaplan–Meier plot for the MMTV-Erbb2 model ($P < 0.0001$). **B,** IRX4204 and vehicle treatment schema with tumor-free proportion for the C3(1)/SV40-TAg model ($P = 0.0031$). **C,** IRX4204 1 mg/kg, IRX4204 10 mg/kg, 9cUAB30 5 mg/kg, and vehicle treatment schema with tumor-free proportion (left) and IRX4204 10 mg/kg, IRX4204 20 mg/kg, 9cUAB30 5 mg/kg, and vehicle treatment with tumor-free proportion (right) for the two studies performed in the Brca1-deficient model. Treatment was initially started 5× per week. Upon the COVID-19 shutdown, the mice were treated 2× per week and then later 1× per week. †A detailed description of the treatment schedule can be found in Supplementary Table S2. O.G., oral gavage; UAB, 9cUAB30.

observe increases in other liver enzymes including, alanine aminotransferase or aspartate aminotransferase (Supplementary Fig. S3). We also explored the bioavailability and toxicity effects of IRX4204 in 129 wild-type mice (the genetic background for our Brca1-deficient model). After treatment, IRX4204 levels in plasma are highest at 30 minutes and gradually drop below detection by 12 hours (Supplementary Fig. S4). In addition, IRX4204 treatment had no observable effects on normal mammary gland development or the health of other organs, even at the highest doses of IRX4204 with 4 weeks of treatment (Supplementary Fig. S5).

Effect of RXR agonists on biomarker expression

To understand the effects of RXR agonists on normal mammary proliferation, we measured Ki-67 expression in mammary glands of Brca1-deficient mice treated with

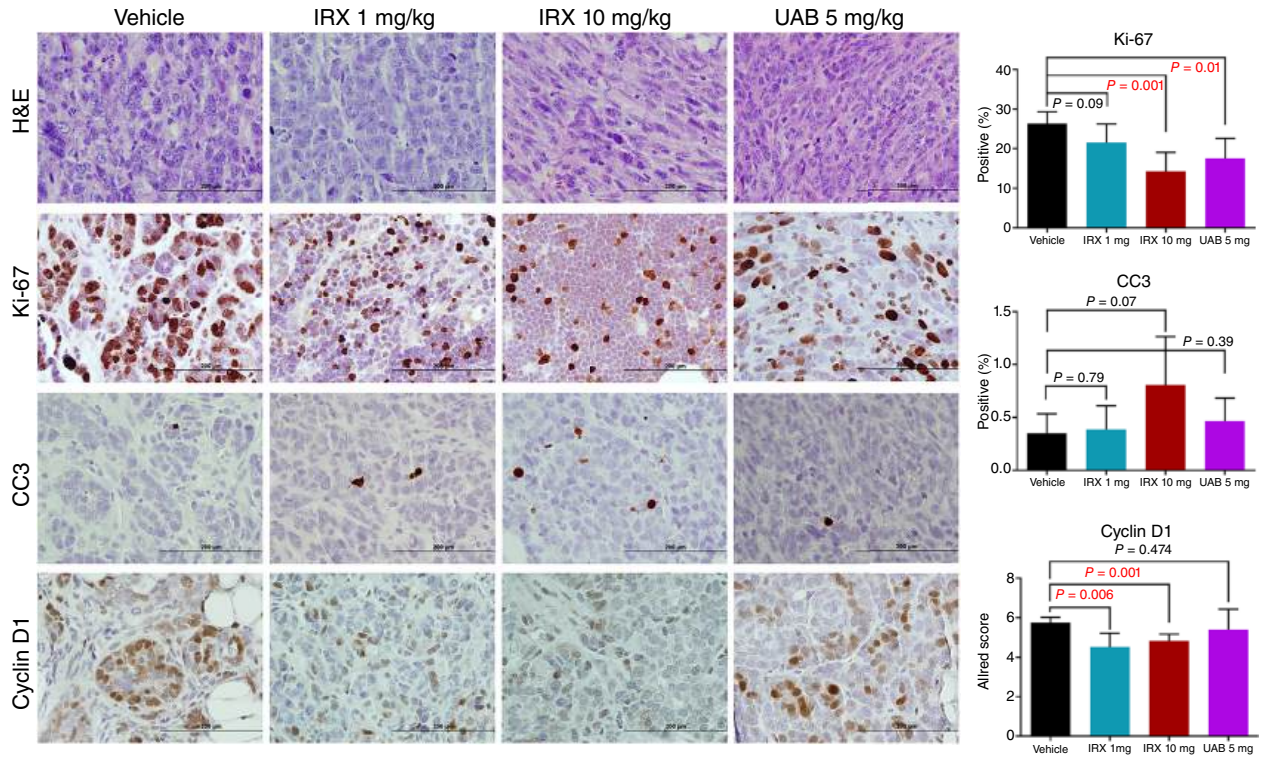

Figure 2.

Effect of RXR agonists on histopathology and IHC of Ki-67, CC3 and cyclin D1 in Brca1-deficient tumors. Left, representative images of Brca1-deficient tumor sections stained with hematoxylin–eosin (H&E; top row), Ki-67 (second row), CC3 (third row), or cyclin D1 (bottom row) and treated with vehicle, IRX4204 1 mg/kg, IRX4204 10 mg/kg, or 9cUAB30 5 mg/kg. Right, quantification of Ki-67 (top), CC3 (middle), and cyclin D1 (bottom) by percent positive cells or Allred scoring. UAB, 9cUAB30.

vehicle or IRX4204 10 mg/kg for 5 days per week. After 4 weeks of treatment, mammary tissues were harvested and analyzed. Treatment with IRX4204 10 mg/kg significantly reduced the Ki-67–positive fraction of cells in normal mammary glands (Supplementary Fig. S6).

To investigate the molecular effects of RXR agonists on tumor inhibition, we evaluated tumor morphology and analyzed the expression of biomarkers for cell proliferation, apoptosis, and the cell cycle. In the MMTV-ErbB2 model, tumors that formed in vehicle-treated mice showed no difference in tumor morphology from tumors that formed after IRX4204 treatment. In addition, we observed insignificant changes in Ki-67 proliferation, CC3 apoptosis marker, and cyclin D1 cell-cycle expression between vehicle- and IRX4204-treated tumors (Supplementary Fig. S7).

In the Brca1-deficient model, tumors arising from vehicle-, IRX4204-, and 9cUAB30-treated mice were all morphologically similar. Tumors treated with IRX4204 (10 mg/kg) showed a significant decrease in Ki-67 and cyclin D1, with an increase in CC3 trending toward significant. Tumors treated with IRX4204 (1 mg/kg) showed a significant decrease in cyclin D1 but no change in Ki-67 or CC3. Treatment with 9cUAB30 showed a significant decrease in Ki-67 expression but no change in CC3 or cyclin D1 (**Fig. 2**; Supplementary Fig. S8).

Effect of RXR agonists on immune modulation

Because retinoids are known to modulate the immune response, we sought to understand the effects of IRX4204 on the tumor-immune response. Total RNA was extracted from vehicle-treated and IRX4204 10 mg/kg–treated Brca1-deficient tumors and analyzed for the expression of 750 cancer-associated genes related to the tumor, immune infiltrate, and microenvironment using the nanoString nCounter PanCancer IO360 panel. With IRX4204 treatment, we observed a significant increase of B cells, mast cells, and $CD8^+$ T cells and a significant decrease in macrophages within the tumor (**Fig. 3A**). IHC of IRX4204-treated tumors in both Brca1-deficient and MMTV-ErbB2 models confirmed the increased infiltration of CD8-positive T cells over vehicle-treated tumors (**Fig. 3B**), indicating that RXR agonists can affect the local tumor-immune response.

We observed similar immune changes in the spleen. Analysis of immune markers from murine splenocytes via flow cytometry revealed that IRX4204 treatment elevates $CD8^+$ T cells and significantly increases B cells while decreasing the population of myeloid-derived suppressor cells (Supplementary Fig. S9). Taken together with the immune findings in the tumor, these data suggest that IRX4204 treatment can delay tumor formation in part through stimulation of the antitumor immune response.

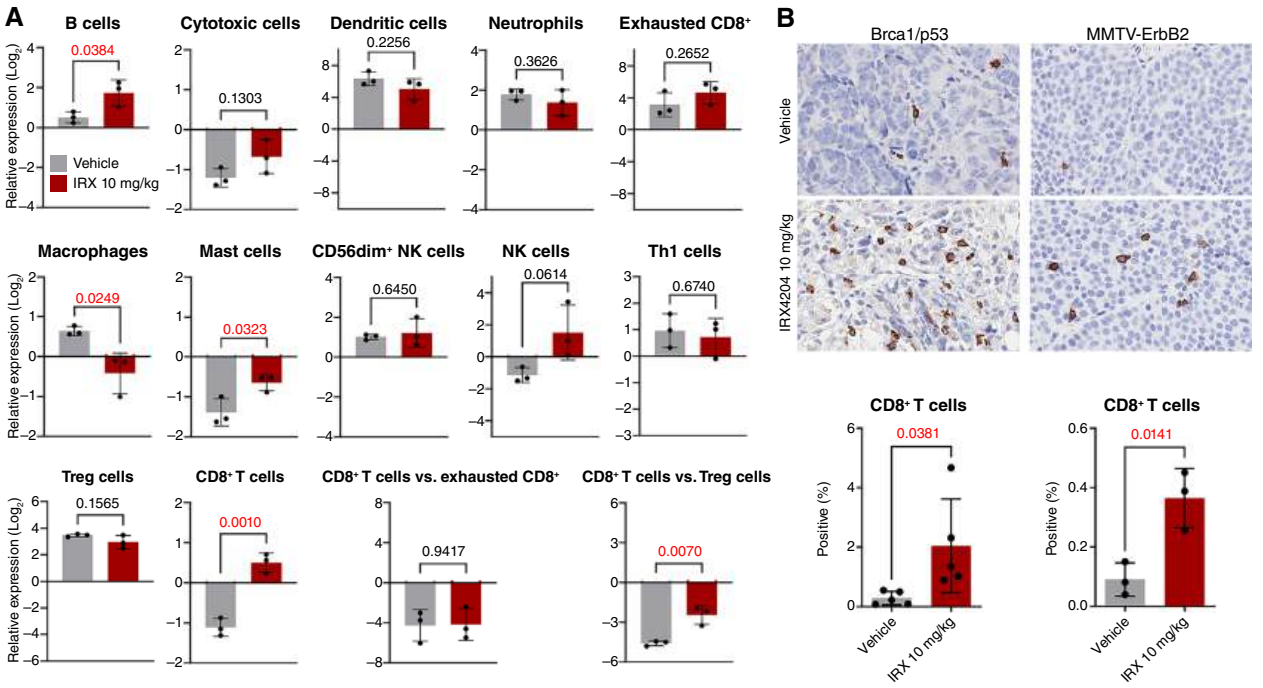

Figure 3.

Effect of IRX4204 on tumor-infiltrating immune cells. **A,** Relative expression of immune cells normalized to total tumor-infiltrating lymphocytes in Brca1-deficient tumors treated with vehicle or IRX4204 10 mg/kg. **B,** Top, representative images of Brca1-deficient tumors (left) and MMTV-Erbb2 tumors (right) treated with vehicle or IRX4204 10 mg/kg and stained for CD8a. Bottom, quantification of CD8a by percent positive cells in Brca1-deficient tumors (left) and MMTV-Erbb2 tumors (right).

Discussion

There are limited preventive therapies for ER-negative and triple-negative breast cancers. For this study, we sought to determine the preventative effects of RXR agonists in preclinical models of ER-negative breast cancer and TNBC. We demonstrated that treatment with the RXR agonist IRX4204 significantly delays the formation of tumors, albeit to varying degrees, in three ER-negative models of breast cancer: MMTV-ErbB2, Brca1-deficient, and C3(1)/SV40-TAg. Biomarker analysis of resulting tumors from the Brca1-deficient model show that IRX4204 treatment can significantly reduce proliferation, induce apoptosis, and inhibit cyclin D1 expression. In addition, IRX4204-treated tumors from both Brca1-deficient and MMTV-ErbB2 mice showed significant increases in $CD8^+$ T-cell infiltration, suggesting that immune modulation may be key to the prevention of ER-negative mammary tumors with rexinoids.

We have demonstrated that the RXR agonist 9cUAB30 can also delay tumor formation in the Brca1-deficient model, although to a lesser degree than IRX4204. Among the ER-negative and triple-negative models tested, IRX4204 treatment was least preventative in the C3(1)/SV40-TAg mouse model and most preventative in the MMTV-ErbB2 model. This may be because of the differences in the TTF, with C3(1)/SV40-TAg developing tumors at an earlier age and MMTV-ErbB2 developing tumors later. Variations in IRX4204 sensitivity may also be because of the oncogenic drivers. We have previously shown that IRX4204 has an inhibitory effect on established HER2-overexpressed tumors but not on established TNBC tumors (10), which could explain the significant preventative effect in the MMTV-ErbB2 model. Because IRX4204 does not inhibit the growth of established Brca1-deficient tumors, we hypothesize that the inhibitory effects of RXR agonists are greatest on premalignant lesions or early-stage tumors. At this time, there is no known direct link between BRCA1 and RXR. However, the RXR pathway controls transcription of many genes involved in metabolism and inflammation, which can indirectly affect how cells respond to DNA damage. Future studies are needed to determine the effect of RXR agonists on tumor initiation, cell differentiation, and DNA damage repair.

The established models used in this study are incredibly valuable for the assessment of retinoids in primary breast cancer prevention. However, like all models used to study human disease, there are limitations to be considered. As fat-soluble molecules, retinoids require lipids to be transported and absorbed effectively. However, lipids themselves can also be ligands for nuclear receptors in the RXR pathway, including heterodimer partners like liver X receptor and peroxisome proliferator–activated receptor. The sesame oil solvent used to deliver IRX4204 and 9cUAB30 in our

experiments is not known to directly bind RXR but theoretically could act as a ligand for RXR partners. Therefore, the vehicle used in these studies (sesame oil) could potentially affect the preventive activity of the rexinoids used in our studies. However, RXR ligands when given as dry powder in the diet of mice also delay mammary tumorigenesis to a similar degree as seen in this study (11). These results suggest that it is the RXR ligand that is predominantly responsible for the cancer-preventive effect seen in our studies. However, further studies will be needed to definitively determine whether lipid-based solvents either attenuate or augment the effects of rexinoids. Another caveat to our studies is that we only determined the effect of the rexinoids on the development of primary mammary tumors; we did not explore the effects of retinoids on secondary tumor formation. This limitation would need to be addressed in future studies for improved translatability to human disease.

Retinoids are potent immunomodulators and play a key role in immune homeostasis. A recent study demonstrated that treatment of established mammary tumors with the rexinoid LG100268 increases the ratio of tumor-infiltrating CD8/CD4 T cells and decreases myeloid-derived suppressor cells in preclinical models of breast cancer (14). Our results demonstrate similar findings in the prevention setting using a newer-generation rexinoid, IRX4204, suggesting that the preventative effect of rexinoids is, in part, because of an influence on the tumor-immune response.

In addition to efficacy, limited toxicities are important for effective breast cancer prevention therapies. Newer-generation rexinoids are known to cause fewer side effects than previous-generation agonists. 9cUAB30 has been well tolerated in clinical studies, with common mild toxicities, including headaches, hypertriglyceridemia, and hypercholesterolemia (15). IRX4204 has also been well tolerated in clinical studies, with common side effects, including hypertriglyceridemia and hypothyroidism (16). These side effects are typically mild and manageable but could result in dose reduction or treatment discontinuation. In our preclinical studies, we tested several doses of RXR agonists ranging from 5× less than those used in clinical trials to 5× greater and observed minimal toxicities at the effective doses over long-term use.

Our study has shown that RXR agonists are capable of delaying tumor development in several models of ER-negative breast cancer. Treatment with both IRX4204 and 9cUAB30 was also well tolerated by mice in our study, making them ideal therapies for ER-negative breast cancer prevention trials. Several clinical trials with 9cUAB30 have already been conducted. A phase Ib trial investigating the biologic effects of 9cUAB30 in patients with early-stage breast cancer (NCT02876640) was launched in 2018 but failed to enroll the adequate number of study participants and was terminated. A randomized phase I trial of 9cUAB30 was also conducted in healthy volunteers to assess its cancer-preventative capacity (NCT01935960). The trial was successfully completed, but study results have yet to be published. IRX4204 has not been clinically tested for breast cancer treatment or prevention but was studied in metastatic castration- and taxane-resistant prostate cancers (NCT01540071). The results from this phase I trial demonstrated that IRX4204 is well tolerated in humans, with manageable side effects of decreased thyroid-stimulating hormone and increased triglycerides (16).

Although RXR agonists do not completely prevent cancer development, the results from our studies demonstrate that IRX4204 and 9cUAB30 can significantly delay formation of ER-negative breast cancer/TNBC tumors and support the use of new-generation rexinoids in breast cancer prevention trials for high-risk individuals.

Data Availability

The data generated in this study are available upon request to the corresponding author.

Authors' Disclosures

C.L. Moyer reports a patent for US20210137871A1 issued and a patent for US-11896558B2 issued. A. Contreras reports grants from the NIH/NCI during the conduct of the study. V. Vuligonda is a member of the BOD of Io Therapeutics. M.E. Sanders reports other support from Io Therapeutics, Inc. during the conduct of the study and outside the submitted work; in addition, M.E. Sanders has a patent for "US10,966,950: Use of RXR agonist IRX4204 for the treatment of Her2+ breast cancer with Her2-targeted mAbs" issued to none, a patent for "US-11,224,583: Use of RXR agonist IRX4204 for the treatment of Her2+ breast cancer with Her2-targeted small molecules" issued to none, a patent for "US-11,998,521: Use of RXR agonist IRX4204 for the treatment of Her2-targeted drug-resistant Her2+ breast cancers" issued to none, and a patent for "US-11,896,558: Use of RXR agonist IRX4204 and taxanes for the treatment of Her2+ breast cancer" issued to none. P.H. Brown reports other support from Genetex outside the submitted work. A. Mazumdar reports grants from the NIH/NCI during the conduct of the study; in addition, A. Mazumdar has a patent for IRX-4204 issued to US-11896558-B2 and a patent for IRX-4204 pending to US-20230172890-A1. No disclosures were reported by the other authors.

Authors' Contributions

C.L. Moyer: Data curation, formal analysis, validation, investigation, visualization, writing–original draft. **J.L. Hill:** Data curation, formal analysis. **D. Coleman:** Data curation, formal analysis. **A Lanier:** formal analysis, writing–review and editing. **X. Liu:** Resources, formal analysis. **J. Kawedia:** Resources, formal analysis, writing–review and editing. **A. Contreras:** Resources, data curation, formal analysis. **V. Vuligonda:** Resources, writing–review and editing. **M.E. Sanders:** Resources, writing–review and editing. **S. Sei:** Conceptualization, resources, writing–review and editing. **M.I. Savage:** Project administration, manuscript review and editing. **A. Mohammed:** Conceptualization, resources, writing–review and editing. **P.H. Brown:** Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **A. Mazumdar:** Conceptualization, resources, data curation, software, formal analysis, supervision, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing.

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Note

Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

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