Antitumor Action of Physiological Estradiol on Tamoxifen-stimulated Breast Tumors Grown in Athymic Mice

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ABSTRACT

The estrogen receptor (ER)-positive MCF-7 breast cancer cell line can be transplanted into athymic mice and grown into tumors with estradiol (E2) support. Tamoxifen (TAM) blocks E2-stimulated tumor growth; however, continuous TAM treatment results in transplantable tumors within a year that will grow with either E2 or TAM (M. M. Gottardis and V. C. Jordan, Cancer Res., 48: 5183–5187, 1988). Although this model may represent the development of TAM resistance for the treatment of advanced breast cancer, no laboratory model exists to study the exposure of breast cancer to 5 years of adjuvant TAM therapy. We have addressed this issue and report the development and characterization of two tumor lines, MCF-7TAM and MT2, which have been serially transplanted into TAM-treated athymic mice for >5 years. The MCF-7TAM tumor rapidly regresses in response to E2 and then about 50% of tumors regrow in response to E2. Interestingly, tumor regression does not occur if TAM treatment is stopped, probably because E2 levels are too low in ovariectomized athymic mice. The development of the antitumor effect of E2 was documented for MT2 tumors over a 1-year period; TAM-stimulated tumor growth was retained, but E2 caused progressively less of a stimulatory effect. Most importantly, E2-stimulated tumors that regrew after initial tumor regression in both MCF-7TAM and MT2 lines were again responsive to TAM to block E2-stimulated growth. Unlike MCF-7 tumors, the MT2 tumor line contains a single point mutation, Asp351Tyr, in the ER, which was retained after the development of E2-stimulated regrowth. The mutation is associated with increased estrogen-like actions for the TAM-ER complex (A. S. Levenson et al., Br. J. Cancer, 77: 1812–1819, 1998), but we conclude that the mutant ER is not required for TAM resistance. On the basis of the new breast cancer models presented, we propose a cyclic sensitivity to TAM that may have important clinical implications: (a) it is possible that a woman’s own estrogen may produce an antitumor effect on the presensitized micrometastatic disease after 5 years of TAM. Long-term antitumor action occurs because the drug is stopped, but resistance accumulates and tumors start to grow if adjuvant therapy is continued; and (b) although in the clinic TAM-resistant tumors respond to second-line therapies that cause estrogen withdrawal, e.g., pure antiestrogens or aromatase inhibitors, estrogen therapy may also be effective and return the tumor to TAM responsiveness. In this way, a hormone-responsive tumor may be controlled longer in the patient with advanced disease.

INTRODUCTION

Adjuvant TAM has revolutionized breast cancer therapy, but the duration of treatment remains controversial. The recent overview analysis in 1998 (1) demonstrated that increasing adjuvant TAM therapy from 1 to 5 years is more effective in increasing survival, but a recent NSABP trial has shown that 5 years of TAM as adjuvant therapy for node-negative breast cancer carried a better disease-free and distant disease-free survival than >5 years of treatment (2). Large clinical trials will be needed to resolve this question, but it appears that some breast tumors acquire resistance to TAM after 5 years of therapy. Drug resistance to TAM can be manifest in many ways, one of which is TAM-stimulated growth. There is some clinical evidence that TAM-stimulated growth can occur during the treatment of advanced breast cancer (3, 4). In the laboratory, long-term TAM treatment results in TAM-stimulated growth in MCF-7 breast tumors (5, 6). After 1 year of continuous TAM treatment, tumors grow in response to both TAM and E2 (MCF-7TAM, a tamoxifen-stimulated TAM-7 tumor; Ref. 5). However, no laboratory model is available that replicates the clinical use of 5 years of adjuvant TAM. We have addressed the issue and developed two transplantable MCF-7 tumor lines that have been exposed to TAM for >5 years by serially transplanting TAM-stimulated tumors into athymic mice treated with TAM. The two lines MCF-7TAM (7) and MT2 (an MCF-7 tamoxifen-stimulated tumor with an Asp351Tyr mutant estrogen receptor;
MATERIALS AND METHODS

The MCF-7 tumors used in these experiments were derived by inoculation of $1 \times 10^7$ MCF-7 cells (originally obtained from Dr. Dean Edwards, University of Texas, San Antonio, TX) into estrogenized athymic mice as described previously (8, 11). MCF-7, MCF-7TAM, and MT2 breast tumors were maintained as serially passaged solid tumors in ovariectomized BALB/c athymic nude mice (Harlan Sprague Dawley, Madison, WI), 4–5 weeks of age, implanted with either E2 or TAM capsules (8, 12). Tumors were routinely passaged by removing a 1.0-cm silastic capsule containing either TAM or E2, depending on the type of experiment. TAM was administered either orally at doses ranging from 500 to 1500 mg as a suspension of 30–40 mg/ml (13) and were used to mimic post- or premenopausal levels of E2. In one experiment, an E2 pellet (1.5 mg) from Innovative Research of America (Toledo, OH) was used to replicate pharmacological doses of E2. Serum levels of 1044 ng/ml were noted (13). The E2 and TAM capsules were replaced every 6–8 weeks (13).

Tumor Measurements. Tumor measurements were performed weekly using Vernier calipers. Tumor cross-sectional area was calculated using the formula: length/2 $\times$ width/2 $\times$ $\pi$. Mean tumor area was plotted against time in weeks to monitor tumor growth.

SSCP. Total RNA was prepared from tumors using the TRIzol reagent (Life Technologies, Inc.). SSCP was performed using the methods originally described by Orita et al. (14, 15) but with minor modifications (16). Tumor total RNA (1 $\mu$g) was reverse transcribed in a 20-$\mu$l reaction containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl2, 0.5 mM each dATP, dCTP, dGTP, and dTTP, 10 mM DTT, 3 $\mu$m oligo(dT)12–14, 100 units of placentat RNase inhibitor, and 200 units of Moloney murine leukemia virus reverse transcriptase. The DNA fragment containing the 351 codon was generated by PCR from 5 $\mu$l of reverse transcription mixture using two primers, 5'-GAGACATGAGAGTCCAA3' and 5'-GGGTGCAGACAATGGTG3'. Control amplification was carried out on 50 pg of double-stranded DNA, coding for either a wild-type ER (HEGO) or a mutant ER (HETO), which contains a single G-to-T point mutation at nucleotide 1559. The PCR cycles and conditions have been described previously (9).

An aliquot of $^{32}$P-labeled DNA was digested with Xbal restriction endonuclease. After restriction digestion, an aliquot of cut and uncut DNA was diluted 1:4 with 0.1% SDS, 10 mM EDTA. Half of each diluted sample was mixed 1:1 with non-denaturating loading buffer (50% glycerol, 0.05% bromphenol blue, and 0.05% xylene cyanol), and the remaining half was mixed 1:1 with denaturing loading buffer (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol). The samples were electrophoresed on a 6% neutral polyacrylamide gel with 14–16 W constant power for 4 h.

Statistical Analysis. Differences in mean tumor area between groups were measured using ANOVA, followed by unpaired Student’s $t$ test.

RESULTS

A MCF-7TAM tumor, which had been passaged in TAM-treated animals for 5 years and then frozen in PEG solution before storage in liquid nitrogen, was thawed and implanted into 4–5-week-old athymic mice and treated with TAM (2-cm capsule) until the mean tumor size reached $\sim 0.4$ cm$^2$. At this point, the TAM capsule was removed, and the animals were randomized into two drug treatment groups. One group was implanted with an E2 (1 cm) capsule that delivers E2 levels of 379 pg/ml serum (13) and the other half a TAM (2 cm) capsule (30–40 ng/ml). The serum levels of E2 and TAM were within the ranges documented in premenopausal women (17) and those taking TAM as adjuvant therapy (17, 18). After 2 weeks, the tumors began to regress in the E2-treated mice, and after 5 weeks, the tumors were $\sim 0.1$ cm$^2$ in size (Fig. 1a). Interestingly, after 6 weeks of E2 treatment, 8 of 18 tumors started to...
grow in the E2 group. We designated this line MCF-7TAME (a newly estrogen-responsive MCF-7TAM tumor).

To ensure that E2 treatment of MCF-7TAM tumors was not equivalent to stopping TAM alone, we repeated the first experiment but included a no-treatment group (Fig. 1b). Six weeks after stopping TAM treatment or starting treatment with E2, the treatment groups readily displayed a divergence of growth characteristics. The no-treatment group of tumors was significantly larger than the E2-treated tumors at week 11 of the experiment (week 6 of treatment; Fig. 1b). The observation that E2 caused tumor regression rather than tumor growth in the MCF-7TAM tumor raised the question that the animals rather than the tumor had altered over the past 10 years (5). To exclude this possibility, we used a bitransplantation technique we used previously to demonstrate the target site specificity of TAM in breast and endometrial tumors (19). Two groups of 10 mice were bitransplanted with MCF-7TAM and MCF-7TAM2 (a newly TAM-stimulated MCF-7 tumor) and treated with either E2 (1-cm capsule; premenopausal levels) or TAM (1.5 mg/day). Both MCF-7TAM and MCF-7TAM2 tumors grew in response to TAM, but only MCF-7TAM2 grew in response to E2 (P < 0.0001).

To determine the growth characteristics of these newly E2-responsive tumors (MCF-7TAME), experiments were performed to study the effect of E2 and TAM. In the first experiment, athymic mice were implanted with MCF-7TAME tumors and divided into the following four groups: E2 capsule (1 cm; premenopausal levels); E2 capsule plus TAM, 0.5 mg/day p.o.; E2 capsule plus TAM, 1.5 mg/day p.o.; and vehicle treated (Fig. 3a). The TAM doses were based on studies published previously (6, 13). Tumor growth in the E2 group was similar to that seen with the E2 (1-cm) capsule group, and tumor size reached a mean tumor size of 1.3 cm2 at 9 weeks. Similar growth rates as the first experiment were seen in the E2 plus TAM 0.5 mg/day and E2 plus TAM 1.5 mg/day groups, and once again the higher dose of TAM (1.5 mg/day) was more effective in suppressing E2-stimulated growth. It appears that the MCF-7TAME tumors have evolved from exclusive TAM dependence to E2 responsiveness with TAM now acting as an antiestrogen again.

The MT2 tumor line was developed in 1993 and had been serially passaged into TAM-treated animals since that time.
tumor size at a specified time of treatment; bars

In the first experiment, TAM and E2 both stimulated tumor growth, although TAM still maintained its stimulatory effect. By the end of the third experiment, E2 suppressed tumor growth, a phenomenon observed previously only in the MCF-7TAM tumors.

We wanted to establish the level of E2 needed to inhibit MT2 tumors. All previous studies with MT2 tumors had used 2-cm TAM sustained-release capsules that delivered serum levels of ~40 ng/ml to maintain tumor growth (12). MT2 tumors were implanted into athymic mice and divided into the following groups: TAM (2-cm capsule); E2 (0.3-1-cm capsule; post- and premenopausal levels, respectively); or an E2 pellet (pharmacological estrogen), and treated for 11 weeks (Fig. 5).

Two additional experiments were conducted to evaluate the hormonal sensitivity of this newly MT2E (newly estrogen-responsive MT2) tumor using different doses of E2 and one dose of TAM (0.5 mg/day). Our aim was to compare and contrast the actions of the compounds with the previously established MCF-7TAM tumors (Fig. 2). In the first experiment, athymic mice were implanted with MT2E tumors and treated with an E2 1-cm capsule (premenopausal levels), an E2 1-cm capsule plus TAM 0.5 mg/day, and with TAM 0.5 mg/day for 6 weeks (Fig. 6a). Tumor growth was observed in the E2-treated alone group, and TAM 0.5 mg/day blocked E2-stimulated growth at 6 weeks, although this effect did not reach statistical significance (0.7 versus 0.45 cm²; P = 0.11). TAM alone stimulated some tumor growth (0.2 cm²) at 6 weeks, demonstrating that TAM is a weak agonist in this system. To evaluate the effect of a lower dose of E2, athymic mice were implanted with MT2E tumors and divided into the same treatment groups as the previous experiment, but a 0.3-cm E2 capsule was used instead of a 1-cm capsule. The mean tumor sizes at 9 weeks were as follows: E2 0.3-cm capsule, 1.3 cm²; E2 capsule + TAM 0.5 mg/day, 0.58 cm²; and E2 capsule + TAM 1.5 mg/day, 0.23 cm². Symbols, mean tumor size at a specified time of treatment; bars, SE.

Fig. 3 a and b, dose-response graphs of E2-stimulated MCF-7TAME tumors to TAM and E2 (a mean of 20 tumors/group). a, MCF-7TAME MT2 tumors were retransplanted into athymic mice and mice were divided into four groups: E2 1-cm capsule; E2 capsule + TAM 0.5 mg/day; E2 capsule + TAM 1.5 mg/day; and no treatment. Mice were treated for 8 weeks. The mean tumor sizes at 8 weeks were as follows: E2 capsule, 1.1 cm²; E2 capsule + TAM, 0.5 mg/day, 0.6 cm²; and E2 capsule + TAM, 1.5 mg/day, 0.4 cm². b, the mice were divided into the same groups as the previous experiment, but a 0.3-cm E2 capsule was used instead of a 1-cm capsule. The mean tumor sizes at 9 weeks were as follows: E2 0.3-cm capsule, 1.3 cm²; E2 capsule + TAM 0.5 mg/day, 0.58 cm²; and E2 capsule + TAM 1.5 mg/day, 0.23 cm². Symbols, mean tumor size at a specified time of treatment; bars, SE.

noted previously that the tumors are both E2 and TAM responsive for growth (8). However, after 4 years of serial passage in TAM-treated animals, the growth response to E2 changed during the fifth year. Three consecutive experiments illustrate the development of supersensitivity of the MT2 tumors to the tumoricidal effect of E2. In the first experiment, TAM and E2 both stimulated tumor growth, and no significant difference in tumor size was seen between the E2- and TAM-treated groups (P = 0.97) after 13 weeks (Fig. 4a). TAM-treated MT2 tumors were retransplanted into new athymic mice and once again treated with TAM, E2, or vehicle for 11 weeks (Fig. 4b). Both E2 and TAM stimulated growth; however, there was a difference in mean tumor size at 11 weeks between the TAM- and E2-treated groups (1.02 versus 0.58 cm², respectively; P = 0.08). However, this did not reach statistical significance. In the third experiment, TAM-treated tumors were retransplanted into new athymic mice and treated with the same doses of TAM and E2 for 10 weeks (Fig. 4c). Tumor growth was observed in the TAM-treated group, and at 10 weeks the mean tumor size was 0.35 cm², whereas no tumor growth was observed in the E2-treated group, even at 10 weeks (0.08 cm²; P = 0.0005). The difference between these two groups was statistically significant. It was clear that E2 had gradually lost its ability to stimulate tumor growth, although TAM still maintained its stimulatory effect.

MT2 tumors are known to contain a mutant ER (Asp351Tyr) in contrast to MCF-7TAM tumors, which contain wild-type ER (9). This mutant ER has been shown to enhance...
the estrogenic properties of TAM (9, 10). To determine what role this mutation played in E2 responsiveness or whether it disappeared in E2-responsive tumors, SSCP was carried out on the MCF-7TAM and MT2 tumors. Because MT2E tumors had regained sensitivity to TAM as an antiestrogen, our hypothesis was that a clone of cells grew into tumors that had lost their mutation and thus lost the TAM-stimulated phenotype. SSCP was carried out on the newly E2-responsive MT2 tumors and compared with tumors that were still receiving TAM treatment as well as on MCF-7TAM and MCF-7TAME tumors. Tumor samples from MT2 tumors in the E2 and TAM group produced bands that comigrated with those produced by amplification of the Asp351Tyr mutant ER cDNA (HETO; Fig. 7), demonstrating that the MT2 tumors had retained their mutated ER during clonal regrowth. On the other hand, tumor samples from MCF-7TAM tumors in the E2 and TAM groups produced bands that comigrated exactly with those produced by amplification of the wild-type ER cDNA (HEGO). We conclude that the MT2 tumors retained their ER mutation, despite a phenotypic change in their responsiveness to TAM.

**DISCUSSION**

We have presented data to show that breast tumors in the laboratory progress through different stages of hormonal dependency over 5 years. During the first few years of the acquisition of a TAM-stimulated phenotype, the MCF-7 ER-positive tumors respond to E2 and TAM equally (5). However, we discovered that serially transplanted tumors appear to acquire a paradoxical supersensitivity to physiological E2 administration after stopping TAM (7). E2 causes a dramatic regression of tumors that is more effective than stopping TAM alone. However, tumor growth can be reactivated by E2 in a proportion of...
tumors are implanted into athymic mice and divided into four groups: (a) TAM 0.5 mg/day, 0.46 cm²; (b) E₂ 0.5 mg/day, 0.18 cm²; (c) TAM 0.5 mg/day, 0.46 cm²; and (d) TAM 0.5 mg/day, 0.18 cm².

Fig. 6  
(a) and (b), dose-response graphs of E₂-stimulated MT2E tumors to TAM and E₂ (a mean of 20 tumors/group). (a) MT2 E₂-stimulated tumors are implanted into athymic mice and divided into four groups: E₂ 1-cm capsule; E₂ capsule + TAM 0.5 mg/day; TAM 0.5 mg/day; and no treatment. The mean tumor sizes at 6 weeks were: E₂ 1-cm capsule, 0.7 cm²; E₂ + TAM 0.5 mg/day, 0.46 cm²; and TAM 0.5 mg/day, 0.18 cm². The mice are divided into the same groups as in the previous experiment, but a 0.3-cm E₂ capsule was used. The mean tumor sizes at 6 weeks were as follows: E₂ capsule, 0.7 cm²; E₂ capsule + TAM 0.5 mg/day, 0.2 cm²; and TAM 0.5 mg/day, 0.19 cm². Symbols, mean tumor size at a specified time of treatment; bars, SE.

Interestingly, before the introduction of TAM, estrogens were considered the hormonal treatment of choice for advanced breast cancer in postmenopausal women (20–25). Estrogens, such as DES and ethinyl estradiol, were found to induce tumor regression (20–25), and this effect was dose dependent, with higher doses producing higher regression rates (23). Doses from 1.5 to 1500 mg/day of DES were used; therefore, the antitumor action of estrogen was pharmacological not physiological. However, with the introduction of TAM, several clinical studies compared TAM to DES for the treatment of advanced breast cancer in postmenopausal women (20, 23, 24), and all found the two agents equal in efficacy. However, patients suffered more side effects with DES, and thus it was felt that TAM was superior to DES for the treatment of advanced breast cancer.

Most laboratory research has focused on the mechanisms of estrogen-stimulated breast tumor growth, and surprisingly, there is little information about the mechanism of estrogen-induced tumoricidal actions. One exception is the T61 breast tumor model established by Brunner et al. (26–29). Unlike the MCF-7 tumor cell line, which was derived from a pleural effusion (30), the T61 tumor was derived from a primary breast cancer. T61 tumor growth in athymic mice is ovarian independent, and both TAM and E₂ inhibit its growth (29). In these studies, tumor inhibition is dose dependent and varies with the specific doses of estrogen ranging from pharmacological to physiological (29). In our study, only physiological doses were needed to induce tumor regression or prevent tumor growth. We suggest that the repeated transplantation of our tumors in TAM-treated animals has resulted in the selection of an MCF-7 tumor that is now supersensitive to the cytotoxic effects of estrogen. It is, however, interesting to note that whatever the mechanisms, our MCF-7TAM tumors regress after ~2 weeks of E₂ treatment, a time course noted by Brunner et al. (26) with the T61 tumors. However, MCF-7TAM and MT2 tumors progress through a cycle of hormonal sensitivity, and the studies reported here have not been done previously with the T61 tumor. In addition, there was no evidence that TAM could stimulate the growth of the T61 tumors.

It is important to point out that the mutant ER found in the MT2 tumor is retained in the E₂-selected tumor subline MT2E (Fig. 7). Mutated ERs have been associated with increased estrogenicity of TAM (9, 10). The MT2 tumors retain the Asp351Tyr mutation, despite their phenotypic change from TAM-stimulated growth (MT2) to E₂-stimulated growth and TAM-inhibited growth (MT2E). We must, therefore, conclude that other cellular factors are essential for the development of the MT2 TAM-stimulated phenotype. The fact that wild-type ER predominates in the MCF-7TAM-stimulated tumor suggests that a mutant receptor is only one of the many potential supporting mechanisms for drug resistance to TAM.

An examination of the interaction of TAM and E₂ illustrates an important point about the therapeutic effectiveness of TAM as a competitive inhibitor of E₂ action. We have reported the competition between E₂ and TAM in vivo previously (12), and it is important clinically when considering TAM therapy in premenopausal women (17, 31). High doses of E₂ can potentially render the antitumor activity of TAM less effective. In the experiments evaluating TAM action in the second generation of MCF-7TAME tumors, higher doses of TAM (1.5 versus 0.5 mg/day) were more effective in suppressing E₂-stimulated
growth (Fig. 4, a and b). In the experiments evaluating TAM action in MT2E tumors (Fig. 6, a and b), in which the TAM dose was held constant but the E2 dose changed, a 1-cm E2 capsule (premenopausal levels) was more effective in reversing the antitumor effect of TAM than a 0.3-cm E2 capsule (postmenopausal levels). This is powerful evidence that the effectiveness of the antiestrogenic effect of TAM is dependent on the relative concentration of E2 and TAM. A 1-cm E2 capsule produces E2 levels (379 pg/ml) equivalent to that of a premenopausal woman (150–350 pg/ml; Ref. 17), whereas a 0.3-cm E2 capsule produces levels (83.8 pg/ml) equivalent to that of a postmenopausal woman (13). Perhaps TAM would be more effective in a premenopausal woman if estrogen levels were lowered. Some evidence to support this position has been obtained recently by comparing the action of TAM with TAM plus a luteinizing hormone-releasing hormone agonist to produce a medical oophorectomy. The theoretically combined therapy is superior (32), and a preliminary clinical report supports this view (33).

We believe it is appropriate to advance a cyclic model of hormone dependency in breast cancer that is based on our experiences with transplantable MCF-7 breast tumors over the past decade (Fig. 8). The transition from estrogen- to TAM-stimulated tumor growth occurs in some tumors within a year of TAM treatment, but the important observation we now report is the reproducible change from TAM-stimulated growth to E2-inhibited growth. Some breast cancer cells then revert back to estrogen-stimulated growth, and TAM, once again, blocks estrogen-stimulated tumor growth.

Stoll (34) has described previously the regression of tumors during high-dose estrogen therapy, but the tumor eventually regrows, only to regress again when estrogen is removed. Patients can be palliated by intermittent estrogen and withdrawal over many years. Our laboratory results are a variation of this clinical observation, because the physiological estrogen is key to tumor regression after TAM failure. Estrogen treatment is superior to simply withdrawing TAM treatment (Figs. 1b and 5).

Another potentially important point is the return of the resistant tumor to TAM sensitivity. It is possible that patients could be maintained on TAM, with episodic periods of estrogen treatment. However, this hypothesis can only be validated through the clinical trials process.

The question of a mechanism to explain the actions of E2 and TAM as antitumor agents is not a simple issue to answer. The mechanism of TAM-stimulated tumor growth must involve two components: a selection of cells that grow in response to the partial agonist actions of TAM; and a second requirement for estrogen-like angiogenesis to support tumor growth. TAM-stimulated tumors have an increased activation of the VEGF gene
(35), which is an important component for angiogenesis. The mechanism of E₂-induced tumor cell death is also unclear. Indeed, a mechanism for the antitumor action of pharmacological doses of estrogen has never been solved.

The recent discovery of a second ER referred to as ER β (36) introduces a new dimension for the understanding of estrogen action. ER β has a structural organization similar to ER α, the classical ER (36), but there are important differences in the activating functions (37, 38) and the ligand-binding domains (39–42). Most importantly, ER β and ER α are able to activate an AP-1 signal transduction pathway with TAM (43–45), which might be able to amplify the agonist actions of TAM to induce TAM-stimulated growth. However, E₂ inhibits ER β AP-1 pathways (44, 45); therefore, it is plausible that this could be a mechanism for the tumoricidal actions of E₂. Long-term TAM-stimulated tumors, such as MCF-7TAM and MT2, could contain cells selected for the ER β AP-1 pathways. It is difficult to test this hypothesis directly without reliable monoclonal antibodies to quantitate ER β, but it is possible to test the hypothesis experimentally. The pure antiestrogen ICI 182,780 stimulates the ER β AP-1 pathway (43–45); therefore, if the pathway is critical for TAM-stimulated tumors after 5 years of treatment, then ICI 182,780 should stimulate and not block tumor growth. Only about half of the ER α-positive tumors that fail TAM respond to ICI 182,780 with tumor regression (46, 47). Perhaps, tumor growth is supported by an activated AP-1 pathway in the tumors that progress. We are currently testing our hypothesis.

REFERENCES


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