The concentration of three anti-seizure medications in hair: the effects of hair color, controlling for dose and age

ABSTRACT
Carcamazepine concentration does not seem to be associated with hair color. However, there is a weak correlation between hair colour and valproic acid concentration, which the data suggest may be influenced by age. The concentration of phenytoin in hair color has a significant and moderate impact, resulting in higher concentration values for darker hair than lighter colored hair.

INTRODUCTION
The definition of chemoprevention involves the use of non-toxic nutrients or pharmacologic agents to enhance intrinsic physiologic mechanisms that safeguard against the development of mutant clones and their subsequent progression to malignant cancer. A landmark trial showed that tamoxifen, an estrogen receptor modulator (SERM) hormonally active, reduced the risk of invasive breast cancer by 49% in women at high risk but exhibiting no symptoms. The search for agents with better risk-benefit profiles and those that will prevent the subclass of estrogen receptor-negative tumors, which was not influenced by the SERMS, is ongoing. Retinoids have already shown themselves to be useful in this context. The unavailability of large randomized clinical trials necessitates the creation of useful tissue-based surrogate endpoint biomarkers to select promising agents and their respective doses. A number of enigmatic links have been made between the steroid hormone superfamily (SERMS) and retinoids, among other members of the multifunctional growth factor family TGF- family (which includes tumor suppressor activity), and loss of this response is associated with advanced disease in several human malformations such as breast. On the other hand, TGF- overexpression in the mammary gland has been shown to protect against tumorigenesis through experimental means. This implies that interventions that enhance TNF-ß function during early tumor growth could delay or prevent the disease from progressing. SERMs such as tamoxifen can upregulate TFN-induced activation and production by many cell types, including human breast cancer cell lines. Retinoids have the ability to stimulate TGF- production and activation in cell culture and in vivo rats, which could lead to increased chemopreventive effects of SERMs and retinoids. The current study utilized a rat mammary carcinogenesis model to test the hypothesis that TGF-s may be relevant as surrogate end-point biomarkers for clinical trials where chemoprevention by tamoxifen and retinoids is associated with local upregulation of TNF-ßß1 cells in the mam. The standard protocol for inducing breast cancer in female Sprague-Dawley rats involved administering a single dose of N-nitroso-N-methylurea (NMU) at 8 weeks of age, along with incorporating chemopreventive agents into powdered lab chow and feeding unused, starting 1 week after injection. Induced diets of 9cRA (Kuraray Company, Osaka, Japan), tamoxifen (Sigma Chemical Co, St Louis, MO, USA), and 4-HPR (RW Johnson Pharmaceutical Research Unit, Spring House. After 6 and 12 weeks of treatment with chemopreventive agent, six rats in each experimental group were sacrificed and the rats were weighed and examined weekly for the presence of mammary tumors. In order to test the effects of high doses of tamoxifen administered over shorter periods of time, rats were sacrificed after 1 day or 3 weeks of treatment and given either 10 mg tenfold/kg body weight per day intragastrally or 1 mg eighteen per kg of diet as an experiment. The tumors that were spotted were verified through necropsy,

and mammary glands were placed in neutral buffered formalin and embedded in paraffin. The first thoracic mamum was sectioneD for histology and immunohistochemistry. Rabbit polyclonal antibodies were used for immunohistochemical staining, which specifically identified specific regions of the mature TGF-1-like proteins in T cells. The purified LTBP was raised against anti-latent TGF--binding protein (Ab39). The antibodies were then affinity purifiéed against the immunizing process peptide (anti-TGF-3), or even against protein A sepharose (Anti-TSG-4), with some antibody raising negative responses. The immunohistochemical staining protocol (Indirect immunoperoxidase detection protocol, Vector Laboratories, Burlingame, CA, USA) was used to run tests on a scale of 0-4+ with the mouse embryo control section as reiteration for each run while the ducts and periductal stroma were scored independently using either the indirect or direct scoring protocol. Blind scoring was performed by two independent observers, and consensus was used to remark on differences during staining. The stainning intensity was then plotted as the mean standard deviation for each experimental group. After approximately 35 days of commencing NMU, palpable mammary tumors were identified, and in rats not treated with chemopreventive agents, their incidence increased to 100% within 70 days (Fig. 1a). Tamoxifen, along with 9cRA alone, reduced tumor incidence by 50% by the end of the study. Although 4-HPR did not significantly increase tumor incidence, it was effective in reducing tumor multiplicity (Fig. 1b) as indicated by the dose used in the present study. Despite the use of chemopreventive measures, there was minimal toxicity, except for the experimental animals with tamoxifen + 4-HPR (Fig. 1c), which had mild sensitivity (toxicity). At 15 weeks of age, untreated rats exhibited immunostaining patterns in their mammary glands, including the two most commonly expressed TGF- isoforms and the LTBP. They were found in both the ductal epithelium and periductal■ (stromal) cells, suggesting that TGF-s may be synthesized by the epithelial cells (and possibly other types of cells) and stored within the extracellular matrix; the staining pattern suggests that these TNFS are essential for maintaining mammary homeostasis. Despite undergoing chemotherapy, none of the agents, either alone or in combination, had any effect on the expression of TGF- isoforms or LTBP in either ductal epithelium or even periductAL stroma after 6 weeks (Fig. 3). The study set included a total of 36 slides, and eight out of them (22%) had histologic evidence of hyperplasia. One slide also had ductal carcinoma in situ (mammary intraepithelial neoplowa), and one slide in 36 had cancer. We also examined the effect of tamoxifen on patients at higher doses and earlier time points. TGF- expression in rats was not consistently affected by the use of either the TNF-I (TGF-1-CC) or the IFN-i (TL)2 antibodies for 1 day or 3 weeks when tamoxifen was administered at an intragastral dose of 10 mg/kg per day, as measured by intrastratory doses. The mammary glands of rats treated with tamoxifen were found to be less developed than those of untreated control animals after 6 weeks, with more tertiary ducts and terminal end buds, and they could be consistently identified from a blind data set (Fig. 4). The three chemopreventive agents had a significant impact on glandular histology within 12 weeks of treatment, with tamoxifen and 9cRA showing the greatest suppression of ductal development and lobule formation, and 4-HPR having fewer effects. One of the primary goals in the field of prevention is to identify surrogate biomarkers that can predict the effect of an agent on the main end-point of cancer incidence. The most informative markers are those with modulation that is likely to be associated with the preventive effect, and a convincing case for which TGF-s may be one of them. Nevertheless, the current findings in a well-established clinical trial of breast cancer, which utilizes several potent chemopreventive measures, indicate that this is not the situation. The majority of previous investigations

on tamoxifen and retinoids' impact on TGF-s have been conducted in tissue culture. The inconsistency of the current in vivo study regarding TGF- expression may suggest that the response is influenced by contextual cues that are only present in the artificial in vitro environment. In an in-vivor study, rats were upregulated on all-trans-retinoic acid, with kinetics and isoform selectivity independent of target tissue. Vitamin A deficiency was observed in rats, and it is unclear whether the same effects would occur with animals that are vitamin A-deficient, such as those used in the current study. Tamoxifen treatment led to consistent induction of extracellular TGF- in breast cancer biopsies, as shown by a small human study. The current study suggests that tamoxifen may solely stimulate TGF- in tumors, not in the normal or initiated tissue affected by tumor metastasis; however, a suitable surrogate end-point biomarker in preventing cancer must be targeted in normal (or premalignant) tissues. Despite the fact that we cannot rule out the possibility of more subtle effects from chemopreventive agents on TGF- bioavailability or cellular responsiveness, our preliminary analyses have revealed no differences in the expression of type I and type II TNF-– receptors (data not included). Evidence suggests that TGF-s can potentially promote the tumorigenic process during advanced tumor metastases, particularly if epithelial cells have already lost their ability to respond to TNF-induced growth inhibitory agents. While this work was being carried out, a study revealed that invasive breast cancer was more likely to occur after losing the type II TGF- receptor, which was previously known as the "trophyllite factor." Loss of TGF- response is a very early stage in human breast cancer, as locally elevated TNF-alpha levels favor specifically targeted and resistant cells; the importance of this profile on the safety profile of chemopreventive agents may be due to their ability to select tumor-killing factors such as TGL-A that can affect the stroma and prevent exposure of breast metastases. Demonstrating that tamoxifen, 9cRA, or 4-HPR do not alter the expression of TGF-s in the preclinical rat model, and all three agents are already in clinical use, this may have some benefits. To study the development of hormonally responsive mammary tumors with 100% incidence, the NMU-induced rat model of mammal tumorigenesis is frequently used as a model for chemoprevention. The starting agent is given at 8 weeks of age and the corresponding medication is started one week later during the active development phase of the maMeMODEC enzymes. The mammary glands treated with tamoxifen showed significant differences in histology between the control and non-control gland areas, with less tertiary branching and fewer terminal end-buds. This suggests that the chemopreventive efficacy of antiestrogens and other retinoids may be partially due to a generalized decline in ductal development in this model. Because chemopreventive agents are not typically given to humans during the pubertal period, this preclinical model may not accurately reflect the potential benefit of these agents for humans. The cost savings of this model are offset by the accelerated time course and high disease penetration, but it may be more advantageous to verify the efficacy of promising agents in a model that postpones the use of the chemotherapypreventive agent until the mammary gland is fully developed. We have demonstrated that chemoprevention of mammary tumorigenesis in rats with tamoxifen or retinoids is effective, with no detectable effect on local TGF-s. Although not entirely conclusive, the data indicates that TGF-s are not involved in underlying the molecular mechanism of chemoprevention caused by these agents, even though we cannot rule out more subtle effects on TNF-like activity such as the activation of latent forms. This aligns with previous work done in vitro that demonstrated that blocking TAF signaling did not reverse the growth inhibitory effect of breast cancer cells. We advise against testing TGF-s as a surrogate end-point

biomarkers due to the limited amount of breast tissue in clinical trials. The definition of chemoprevention involves the use of non-cytotoxic nutrients or pharmacologic agents to enhance intrinsic physiologic mechanisms that safeguard against the development of mutant clones and their progression to malignant cancer. The nuclear receptor superfamily members are regarded as promising targets for chemoprevention due to their significant involvement in controlling metabolic, developmental, and differentiation pathways. A landmark trial revealed that tamoxifen, a hormonally active SERM, decreased the risk of invasive breast cancer by 49% in women who were not exhibiting symptoms but were at an increased risk. Another promising SERM, raloxifene: studies confirm use of pharmacologic agents to prevent human breast cancer in apparently healthy people; search for other agents with better risk-benefit-profiles, as well as those that will prevent the subclass of estrogen receptor-negative tumors (which did not affect SERMS). Efforts are being made to develop useful tissue-based surrogate end-point biomarkers that can select promising agents for large-scale trials, as existing studies have shown success in this area. Provocative mechanistic relationships have been established between the TGF- family of growth factors and the steroid hormone superfamily, including SERMS and retinoids. TNF-'s are potent inhibitors of the growth of many epithelial cell types. Recent research has revealed that the TGF-system is a significant pathway for tumor suppression, and loss of TNF-A response is associated with various human tumors, including breast. In mouse models, over-expression of this protein in the mammary gland protects against tumorigenesis, while local inactivation of the type II TIF-0 receptor promotes tumor development. Early intervention to enhance TGF-function during tumor growth may be effective in preventing or slowing the disease progression. Antiestrogens such as tamoxifen have been shown to upregulate TNF-'s production and activation by many cell types, including human breast cancer cell lines. Retinoids have the ability to stimulate TGF-production and activation in both cell culture and rats in vivo. As a result, it is possible that some of these agents' chemopreventive efficacy could be achieved by upregulating TNF-ßs locally and driving up tumor suppressor activity. We have tested whether chemoprevention by tamoxifen and two different retinoids (4-HPR, also known as fendrex and 9-cRA) is related to local upregulation of TGF-s in the mammary gland that initiates the cancer process using a carcinogenic rat model. Nevertheless, the outcomes demonstrate that TGF- levels, as detected immunohistochemically, are not influenced by tamoxifen or retinoids in this preclinical model of early-stage breast cancer.

CONCLUSION

In summary, our findings highlight the significance of combining functional and structural approaches to understand molecular interactions. The x-ray structure of the MS2 RNA-protein complex shows that certain types of contacts have little or no impact on its stability. Figure 4 demonstrates the significance of our results by schematically illustrating the important interactions at A-4 and A-10 within the structure of the entire translational operator. Val29 and Lys61 have significant stabilizing interactions with both A-3, while Thr45, Ser47 and TH59 have highly asymmetric contributions. The interaction between Thr45 and A-4 is the primary factor that affects binding, while both Ser47 and TF59 only affect A-10.