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Use of *In Vitro* Assays to Predict The Efficacy of Chemopreventive Agents in Whole Animals

Vernon E. Steele, PhD[1], Sheela Sharma, PhD[2], Rajendra Mehta, PhD[3], Eugene Elmore, PhD[4], J. Leslie Redpath, PhD[4], Colette Rudd, PhD[5], Donya Bagheri, MS[6], Caroline C. Sigman, PhD[6], and Gary J. Kelloff, MD[1]

[1]Chemoprevention Branch, Division of Cancer Chemoprevention and Control (DCPC), National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, MD 20892

[2]ManTech Environmental Technology, Inc, Research Triangle Park, NC 27709

[3]Specialized Cancer Center, College of Medicine, University of Illinois, Chicago, IL 60612

[4]University of California at Irvine, Irvine, CA 92715

[5]SRI International, Menlo Park, CA 94025

[6]CCS Associates, Mountain View, CA 94043

Abstract Five *in vitro* assays have been applied to screen the efficacy of potential chemopreventive agents. These assays measure a) inhibition of morphological transformation in rat tracheal epithelial (RTE) cells, b) inhibition of anchorage independence in human lung tumor (A427) cells, c) inhibition of hyperplastic alveolar nodule formation in mouse mammary organ cultures (MMOC), d) inhibition of anchorage independence in mouse JB6 epidermal cells, and e) the inhibition of calcium tolerance in human foreskin epithelial cells. The efficacy of many of these same agents in whole animal studies of lung, colon, mammary gland, skin, and urinary bladder carcinogenesis has also been measured. The aim herein is to estimate the positive and negative predictive values of these *in vitro* assays against whole animal chemopreventive efficacy data using the same chemicals. For three of these assays—using RTE, A427 cells and mouse mammary organ culture (MMOC)—enough data are available to allow the estimate to be made. Such extrapolations of *in vitro* data to the *in vivo* situation are difficult at best. There are many dissimilarities between the two assay systems. The *in vitro* assays use respiratory and mammary epithelial cells, while the *in vivo* assays use respiratory, mammary, colon, bladder and skin cells. The *in vitro* assays use the carcinogens benzo(*a*)pyrene (B(*a*)P) and 7,12-dimethylbenz(*a*)anthracene (DMBA), while the *in vivo* assays use B(*a*)P, DMBA, *N*-methyl-*N*-nitrosourea (MNU), *N,N*'-diethylnitrosamine (DEN), azoxymethane (AOM), and *N*-butyl-*N*-(4-hydroxybutyl)nitrosoamine (OH-BBN). There are vast differences in pharmacodynamics and pharmacokinetics *in vitro* and *in vivo*, yet it is possible to rapidly screen chemicals *in vitro* for efficacy at one-tenth the cost and complete tests in weeks instead of months. A positive *in vitro* assay was defined as a 20% inhibition (compared with control) for the RTE and A427 assays and a 60% inhibition for the MMOC assay at nontoxic concentrations. For *in vivo* assays, the criterion for a positive result was a statistically significant inhibition of incidence, multiplicity or a significant increase in latency (mean time to first tumor). For an agent to be considered negative in animals, it required negative results in at least two different organ systems and no positive results. Using the battery of three *in vitro* tests, the positive predictive value for having one, two, or three positive *in vitro* assays and at least one positive whole animal test was 76%, 80%, and 83% respectively. The negative predictive values for one, two or all three *in vitro* assays was 25%, 27%, and 50%. From these data it is observed that *in vitro* assays give valuable positive predictive values and less valuable negative predictive values. The mechanisms of chemoprevention are not well understood. Seven categories of agents were examined for their cancer preventing activity both *in vitro* and *in vivo*: antiinflammatories, antioxidants, arachadonic acid metabolism inhibitors, GSH inducers, GST inducers, ODC inhibitors, and PKC inhibitors. Three or even five *in vitro* assays cannot be all-inclusive of the many mechanisms of cancer prevention. However, three assays help to predict whole animal efficacy with reasonable positive predictive values. Much work and development remains to be done to rapidly identify new chemopreventive drugs. 1997 Wiley-Liss, Inc.*

Key words: Chemoprevention, carcinogenesis, *in vitro* assays, animal models

Correspondence to: Dr. Vernon E. Steele, PhD, MPH, Chemoprevention Branch, Division of Cancer Prevention and Control (DCPC), National Cancer Institute, 9000 Rockville Pike, Executive Plaza North, Suite 201, Bethesda, MD 20892.

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The Chemoprevention Branch of the National Cancer Institute (NCI), Division of Cancer Prevention and Control (DCPC) drug development program has the goal of identifying safe and effective chemopreventive agents for clinical use. To reach this goal, a number of potential chemopreventive agents are identified and tested in a series of *in vitro* and *in vivo* efficacy studies. Based on the results generated by these studies as well as preclinical toxicology and pharmacokinetics studies, the promising agents progress to clinical trials sponsored by the Chemoprevention Branch [1–5].

In the Chemoprevention Branch, a battery of *in vitro* prescreens or mechanistic assays [6], *in vitro* assays, and animal cancer models [7] are used to evaluate chemopreventive potential and select promising agents for further development. The subject of this paper is the predictive value of *in vitro* assays for whole animal tumorigenicity tests.

This paper describes the *in vitro* efficacy of single chemopreventive agents tested to date; *i.e.*, 720 test results on 261 agents are described (see Table I). A subset of these results was used to determine the predictive value of these assays in relation to *in vivo* tumor models including lung, colon, mammary gland, skin, and urinary bladder. Initial criteria for selecting the *in vitro* assays included 1) efficiency in terms of time and cost, 2) sensitivity and ease of quantitation, 3) controlled test conditions, 4) relevance to organ systems of interest, 5) use of primary epithelial cells, and 6) if possible, use of human cells. The five *in vitro* assays all use epithelial cells; they measure 1) inhibition of morphological transformation in primary rat tracheal epithelial (RTE) cells [8,9], 2) inhibition of anchorage independence in human lung tumor (A427) cells [10], 3) inhibition of hyperplastic alveolar nodule (HAN) formation in mouse mammary organ cultures (MMOC) [11], 4) inhibition of anchorage independence in mouse JB6 epidermal cells [12], and 5) the inhibition of calcium tolerance in human foreskin epithelial (HFE) cells [13]. The RTE cell transformation assay measures inhibition of carcinogen-induced morphological transformation. The human lung tumor A427 cell and mouse epidermal JB6 cell assays primarily detect agents blocking postinitiation stages of carcinogenesis; the mouse mammary organ culture (MMOC) can detect both antiinitiators and antiproliferatives, depending on the treatment condition (*e.g.*, with DMBA alone or with DMBA and TPA). The HFE cell assay measures inhibition of cell phenotypes associated with transformation by measuring the inhibition of calcium tolerance.

The purpose of this paper is to evaluate the promise of *in vitro* test assays to provide information useful in developing cancer chemopreventive agents for eventual human use.

METHODS

In Vitro Assays

RTE Cell Transformation Assay: This assay measures the ability of potential chemopreventive agents to inhibit B(*a*)P-induced transformation of primary RTE cells. An agent is considered positive if it reduces the formation of type II and type III foci by 20% at one or more of the concentrations tested. Protocols for this assay have been published previously [8,9,14]. Each agent is tested for solubility in culture media. Insoluble compounds are dissolved in DMSO or ethyl alcohol. Lumenal epithelial cells are isolated from tracheas excised from 8–12 week old rats. The cytotoxicity of each chemopreventive agent is determined by exposing 20,000 cells to the highest soluble concentration and four log dilutions of the test agent for 24 hours. The treated cells are incubated for six more days, then they are fixed, stained and the colony forming efficiency (CFE) is determined. For chemoprevention assays, the highest concentration considered to be evaluable is that at which the CFE is not reduced below 80% of the untreated controls. Four half-log dilutions of this concentration are also tested in the chemoprevention assay. A second cytotoxicity assay is done concomitantly with the transformation assay. This assay is used to determine the number of colony-forming cells per plate for each of the experimental doses of chemopreventive agent. In the chemoprevention assay, 20 dishes per test concentration and control are tested. The controls are medium, solvent, carcinogen, and positive chemopreventive agent (all-*trans*-retinoic acid). The cells (20,000 per plate) are exposed to B(*a*)P plus chemopreventive agent for 24 hours. The cells are then rinsed and replaced in RTE culture medium with the chemopreventive agent and maintained in a 5% CO_2 incubator at 37°C for 30 days. Fresh medium and chemopreventive agents are supplied biweekly. At day 14, the cells are transferred to a modified RTE medium. After 30 days, the number of type II and type III transformed foci per plate are determined. An

agent is considered positive if the inhibitory activity at any non-toxic dose is greater than 20%; results at cytotoxic concentrations are considered inconclusive.

Human Lung Tumor A427 Cell Assay: In this assay, the ability of potential chemopreventive agents to inhibit the expression of tumor phenotype is tested in human A427 tumor cells. Expression of the tumor phenotype is measured by anchorage independent growth in soft agar. For each agent tested, a range-finding cytotoxicity test is carried out to determine non-toxic concentrations for the chemopreventive efficacy assay. A second cytotoxicity test is carried out concomitantly with the efficacy assay to ensure that the agents are non-toxic at the concentrations tested. In the efficacy assay, the highest non-toxic concentration and five half-log dilutions of each agent are tested in triplicate. Anchorage independence is determined by total number of colonies. Test agents are considered positive if one or more of the non-toxic concentrations inhibit anchorage-independent colony formation by 20% or more of the medium and/or solvent controls in a valid test. A test is considered valid if 1) the positive control (13-*cis*-retinoic acid or all-*trans*-*N*-(4-hydroxyphenyl) retinamide (4-HPR)) inhibits anchorage-independent growth by at least 20% or the test agent significantly inhibits anchorage-independent growth compared with solvent controls, 2) colony growth in the solvent control is sufficient to allow determination of statistically significant results, 3) valid data are produced at three or more concentrations of the test agent, including the highest non-toxic concentration, and 4) the agent is not toxic at the valid concentrations in the concomitant cytotoxicity assay.

Mouse Mammary Organ Culture (MMOC), Inhibition of Hyperplastic Alveolar Nodule (HAN)-Like Lesions: This assay measures the ability of potential chemopreventive agents to inhibit carcinogen-induced HAN lesions in mouse mammary glands in organ culture [15–22]. Two protocols are used for this assay, a complete carcinogenesis protocol and an initiation/promotion protocol. For both, female BALB/c mice, 28 days of age, are treated with estradiol and progesterone for nine days prior to sacrifice. At sacrifice, the thoracic mammary glands are excised from the mice and incubated in Waymouth's 752MB medium supplemented with antibiotics and growth hormones. Chemopreventive activity is defined as 60–100% inhibition of lesions at one or more non-toxic doses.

In the complete carcinogenesis protocol, the mammary glands are incubated in Waymouth's 752MB medium for 10 days in the presence of the potential chemopreventive agent. Each agent is tested at five concentrations ranging from 0.001– 10 M. On day three of the incubation, the glands are treated for 24 hours with 2 μg/ml of the carcinogen DMBA. After the 10-day incubation, the glands are transferred to medium containing only insulin and incubated for 14 more days. Incubation in the presence of insulin results in regression of the glands, except for the HAN. At the end of the 24-day experiment, the glands are fixed, stained, and evaluated for the incidence of HAN.

In the initiation-promotion protocol, the same procedure as that for the complete carcinogenesis protocol is used, except that the glands are treated with test agent prior to and during treatment with DMBA (days 0–4) or during treatment with TPA at 25 ng/ml (days 9–24). Agents effective during treatment with DMBA are considered to be antiinitiators, while those active during treatment with TPA are considered to be antipromoters. As will be noted, many compounds are active in both protocols.

Mouse JB6 Epidermal Cell Assay: The JB6 epidermal cell assay examines the ability of potential chemopreventive agents to inhibit anchorage-independent growth induced by the tumor promoter TPA. This assay is based on research reported by Colburn and colleagues [23–26] who demonstrated that the mouse epidermal cell line, JB6 clone 41, specifically detected the inhibition of TPA-induced anchorage independent growth by all-*trans*-retinoic acid (which is used as the positive control in the chemoprevention studies). A preliminary cytotoxicity assay is done to determine appropriate concentrations for the chemoprevention assay. A second cytotoxicity test is carried out concomitantly with the efficacy assay to determine the cloning efficiency at the concentrations used in the chemoprevention assay, and cytostatic activity is determined by counting cells after four days of growth in the presence of various concentrations of the potential chemopreventive agents. An agent is considered positive if it significantly $p<0.05$ inhibits TPA-induced anchorage-independent growth at one or more non-toxic concentrations. Because the assay measures inhibition of effects induced by a tumor promoter, it is expected to detect chemopreventives acting during the promotion and progression phases

of carcinogenesis more reliably than those acting during initiation.

Human Foreskin Epithelial (HFE) Cell Assay: This assay measures the ability of potential chemopreventive agents to inhibit growth induced in human foreskin epithelial cells by the carcinogen propane sultone. For each agent tested, a range-finding toxicity test is carried out to determine appropriate non-toxic doses for the chemopreventive efficacy studies. A second cytotoxicity test is incorporated as part of the efficacy assay. The concentrations of the test agents in the HFE calcium tolerance assay consist of the highest tested non-toxic concentration (determined from the dose-range finding assay), plus four half-log dilutions of that concentration. Multiwell dishes are seeded with normal or early passage HFE cells and cultured for two days to allow cells to begin cell division. All dishes are then exposed to 7.5 μg/ml of propane sultone. The test agents are added to the appropriate groups on the same day and at each media change thereafter. The medium is changed twice weekly during the study. The positive control (all-*trans*-retinoic acid, 0.03 g/ml) is added at each media change. Propane sultone exposure is repeated following the first two subcultures. The multiwell dishes are trypsinized and reseeded until the cells reach passage three. At this time, the medium is changed to Keratinocyte Growth Medium (KGM) and, when the cells are in confluent stage, they are trypsinized and plated in a 96-well dish. After sufficient growth, the cells are stained and relative growth is determined using a plate reader.

Test agents are considered positive if they inhibit propane sultone-induced calcium tolerance by 20% compared with propane sultone-treated controls. A test is considered valid if 1) the positive control (all-*trans*-retinoic acid) or the test agent inhibits calcium tolerance by at least 20% at two consecutive non-toxic concentrations, 2) propane sultone induced a significant level of growth (at least 50% above that observed in solvent controls), and 3) three or more non-toxic concentrations of the test agent are evaluated.

Animal Efficacy Screens and Models

The animal efficacy data have been generated by testing the agents in animal cancer models: DEN- and MNU-induced cancers in hamster lung and trachea; AOM-induced tumors in rat colon; MAM-induced tumors in mouse colon; DMBA- and MNU-induced cancers in rat mammary gland, DMBA-induced/TPA-promoted tumors in mouse skin; and OH-BBN-induced tumors in mouse urinary bladder. These models and the test results were described recently [7].

RESULTS

Table I is a summary of the results for five major *in vitro* assays obtained by January 15, 1996, com pared with *in vivo* tests. Of the 268 agents which were tested in these *in vitro* assays, 101 were tested both *in vitro* and in at least one *in vivo* model. The results in this paper will focus on the agents for which both *in vitro* and *in vivo* data exist. The last column of Table I is a summarized version of the relevant animal data. Positive animal data are indicated by an abbreviation of the assay used which represents at least one positive animal result (*i.e.*, significant inhibition of tumor incidence or multiplicity, or significant increase in tumor latency in any organ site). Negative results are shown ([...]) where there were at least two animal negative results and no positive results. A detailed summary of the animal data was previously presented [7]. Because there are many possible reasons for inactivity (*e.g.*, ineffective dose, insolubility, poor uptake by the cells, insensitivity of the system to a particular chemopreventive mechanism), more emphasis is put on positive results than negative results in decision making for agent development.

Correlation of Animal Efficacy and *In Vitro* Results

The comparison of *in vitro* results with animal efficacy was evaluated in three ways. The first method was a determination of concordance (sensitivity, selectivity, and accuracy) of *in vitro* results with animal data. The second method involved scoring each *in vitro* assay against specific targets examined in the animal cancer models. The third method measured the predictive value of a positive or negative *in vitro* result with that for animal chemoprevention test results.

The concordance between single *in vitro* assays and the animal efficacy studies is shown in Table II. Sensitivity is defined as the fraction of agents positive in animal efficacy studies which were positive in the *in vitro* assay. Selectivity is defined as the fraction of agents negative in animal efficacy studies which were

also negative in the *in vitro* assays. Accuracy is defined as the fraction of correct positive and negative comparisons divided by the total number of comparisons. The RTE assay had the highest sensitivity at 75%, followed by the A427 and MMOC assays at 67 and 60%, respectively. The RTE also had one of the highest selectivities at 37% compared to 29% and 38% for the A427 and the MMOC assays, respectively. The overall accuracy of the RTE assay was highest at approximately 65%.

The predictive value of an assay is the ratio of true test results to the total number of results. Therefore, the predictive value of a positive *in vitro* result would be the number of results which were positive both *in vitro* and in animals divided by the total number of positive *in vitro* results (*i.e.*, true positives plus false positives). For a negative *in vitro* result, the value would be the number of true negatives divided by true plus false negative results. Table II shows the positive and negative predictive values of the three *in vitro* assays. For a positive animal test result, a single positive result in any of the five animal models was sufficient, while for a negative animal result, at least two animal tests must have been negative with no positive results. The predictive value for a positive RTE assay was the highest at 76%. This means that, if an agent was positive in this assay, then the probability of obtaining a positive animal result in at least one model would be about 76%. The negative predictive values were very low with the RTE assay having a 23%, the A427, 23%, and the MMOC, 24%.

The overall accuracy of an assay is defined as the total correct responses in that assay compared with *in vivo* data divided by the total agents considered. Again the RTE had the highest accuracy at 65%. The A427 and the MMOC assays were slightly lower at 57 and 54%, respectively.

Using *In Vitro* Assays to Predict Animal Efficacy Test Results by Target Tissue

Tables III and IV summarize the predictive values and concordance of each *in vitro* assay for each of five specific animal target organs. Since many of the agents tested *in vitro* will be evaluated in only one or a few animal models, these values may be useful in guiding further testing.

Hamster Lung Studies (DEN- and MNU-Induced Cancers): The RTE assay appears to be the best prediction of positive and negative results in the lung models; for positives its predictive value was 60% and for negative results, 100%, as shown in Table III. The A427 assay had 36% predictive value for positive results in the lung model, while that for the MMOC assay was 40%. The sensitivities (Table IV) in the lung model for the RTE, A427 and MMOC assays were 100, 63, and 67%, respectively. The RTE assay had the highest overall accuracy at 71%, while the accuracies of the A427 and MMOC assays were less than 50%.

Mouse and Rat Colon Models: The highest positive predictive value for an *in vitro* assay in relation to a colon model was 66% (RTE assay), followed by 56% (A427 assay). The highest negative predictive value in this model was 59% (RTE assay), followed by 56% (A427 assay). The sensitivity in the colon model was highest for the RTE assay (78%), followed by the A427 assay (66%). The specificity values were all quite low; the highest was for the RTE assay at 44%. These results gave the RTE assay the highest accuracy at 63% for *in vivo* colon data. Therefore, based on these test results, it appears the RTE assay may be a good predictor of chemopreventive activity in the colon models. A possible explanation for the correlation of RTE results to results in animal colon may involve the activation of *ras* oncogene in both models [9].

Mammary Models: In the MNU mammary model, the MMOC assay had the highest positive predictive value (46%), followed by the A427 (43%), and the RTE (39%). The MMOC also had the highest negative predictive value (74%). The sensitivity was high for all three *in vitro* assays; the highest was the MMOC assay (74%), followed by the RTE assay (72%), then the A427 (69%). Specificity was markedly lower with the MMOC yielding 46%, and both the RTE and A427 predicting 36% of the negative in vivo test results. The overall accuracy was greatest for the MMOC assay at 57%. Thus, based on the currently available data, it does not appear that the MMOC assay or any of the other *in vitro* systems are useful for predicting positive efficacy of chemopreventive agents in mammary gland *in vivo*; however, the high negative predictive values may be of some help.

Urinary Bladder Model: As shown in Tables II and III, the positive predictive values for this target range from 25–36%; these values are the lowest among the five target tissues. The highest positive and negative predictive value was for the MMOC assay (36% and 70%, respectively). The highest sen-

sitivity was also for the MMOC assay (75%), followed by RTE and A427 assays (54% and 45%, respectively). The accuracy values were all below 46%. Because of the low positive predictive values, none of the *in vitro* assays appears to be useful for identifying agents likely to be active in bladder. The high negative predictive value of the MMOC assay may be of some utility in ruling out agents.

Skin Model: The A427 assay has the highest positive (60%) and negative (75%) predictive values for this model. The highest sensitivity of *in vitro* assays compared with the skin model was shared by the A427 and the MMOC (86%) assays. The specificity values were all below 50%. The overall accuracy was highest for the MMOC assay at 57%. In general, the A427 assay may give the best indication of activity in whole animal models.

Combinations of Assays

Since the transformation endpoint of each *in vitro* assay is likely to be modulated by different sets of mechanisms, it has been a practice to use the data generated by the *in vitro* screens not only as single tests but as a battery of tests. Using this strategy, agents positive in all tests were given a higher priority than agents negative in all assays. Therefore the combined data should be of greater significance in predicting animal results than single assay results. To test this strategy, *in vitro* tests were combined as follows: all three tests positive, two or three tests positive, and at least one test positive. Only the results for the A427, RTE and MMOC assays were evaluated against whole animal tumor data. The analyses are shown in Table V for the positive predictive values and in Table VI for the negative predictive values.

If all three *in vitro* assays are positive, then 83% of the time we should see a positive result in the animal assays. There were 24 agents for which all three *in vitro* assays were positive and animal efficacy was shown in 20 cases, leaving four agents thus far negative in animal studies. It appears of little value if all three tests are negative, since the negative predictive value was about that of a coin toss. However very few agents have been negative in all three *in vitro* assays, so these data are not conclusive. If one, two or three tests are positive, then the positive predictive value is only reduced to the 72–76% range. The negative predictive value for two or three negative results was very low at 25–27%.

In summary, the predictive value of the *in vitro* assays for the animal models is based on the results obtained to date, and these are likely to change over time. Overall, the RTE assay appears to be the best single assay overall to predict any whole animal efficacy test and several organ-specific whole animal tests. Several factors are likely to contribute to the predictive values of *in vitro* assays. First is the small number of chemicals tested in all of the *in vitro* and *in vivo* screens. Second and more important is the range of mechanisms and pharmacology represented by the chemicals tested. For example, flavonoids are generally positive in most of the *in vitro* screens, but are negative in most of the *in vivo* screens. This is probably because they are taken up by the cells *in vitro* or are able to affect the carcinogens directly, but are poorly absorbed and unable to reach target tissues or site of carcinogen activation *in vivo* [7]. The exception is colon, where the flavonoids quercetin and rutin were positive *in vivo*. Likewise, the predictive values by target described above do not consider mechanisms of action. Aromatase inhibitors, such as vorozole, which inhibit estrogen biosynthesis, are not likely to be active *in vitro* where this mechanism is not available. A useful adjunct to determining predictive values is to take the pharmacology and mechanisms into consideration. The following section of the paper does this by comparing the results of various structural and pharmacological classes of compounds *in vitro* and *in vivo*.

Correlation of Results with Chemical and Pharmacological Activities

A number of biochemical (*e.g.*, arachidonic acid metabolism, PKC inhibition, ODC inhibition, and GSH/GST enhancement) and pharmacological activities (*e.g.*, antiinflammatory, antioxidant, and anti estrogenic activity) have been associated with chemoprevention [*e.g.*, 1,4]. It is useful to examine and understand how these and other biological activities of agents are associated with chemopreventive activity as measured in the Chemoprevention Branch testing program. Based on the available test results *in vivo*, six biological categories were selected and reviewed. To be selected as a category, both *in vitro* and *in vivo* test results on five agents should have been available. These categories are antiinflammatory (particularly, NSAIDs), antioxidants, arachidonic acid metabolism inhibitors, GSH/GST enhancers, ODC inhibitors, and PKC inhibitors.

1. Antiinflammatories (NSAIDs)

The antiinflammatory agents, particularly NSAIDs, have been tested extensively by the Chemoprevention Branch. These agents include, aspirin, ibuprofen, indomethacin, ketoprofen, piroxicam, and sulindac. In animal models, these agents were most active in the rat colon (80%), and urinary bladder (83%) models. A majority were positive in the A427 and the HFE assay and most were negative in the RTE assay.

2. Antioxidants

One hundred agents classified as antioxidants have been tested in the Chemoprevention Branch testing program. Among these are ascorbyl palmitate, *N*-acetyl-*l*-cysteine (NAC), carbenoxolone, β-carotene, (+)-catechin, ellagic acid, ethylvanillin, 18β-glycyrrhetinic acid, and vitamins such as A and E. A number of these agents were active in all five *in vivo* screens and models; the highest positive number was demonstrated in the rat colon (83%). Therefore, based on these limited data, it appears that antioxidants are primarily positive in the MMOC assay, followed by RTE and A427. The JB6 and HFE assays do not appear to be good screening assays for this class of agents.

3. Arachidonic Acid Metabolism Inhibitors

Eighty-one chemopreventive agents categorized as arachidonic acid metabolism inhibitors (including the antiinflammatories and many of the antioxidants cited above) were tested by the NCI, Chemoprevention Branch. Because of lack of evaluable test results (*e.g.*, at least five for each study), it is not possible to evaluate the subclasses (*e.g.*, cyclooxygenase inhibitors, lipoxygenase inhibitors, phospholipase A_2 inhibitors) individually. In general, as for NSAIDS, the highest activity of this class of agents was observed in the rat colon model (89%). As discussed previously, the chemopreventive mechanism of action of NSAIDs is *via* inhibition of arachidonic acid metabolism, specifically cyclooxygenase inhibition. Therefore, it is expected that the highest chemopreventive activity is observed in the colon model. The lowest chemopreventive activity has been observed in the lung models (16%), followed by urinary bladder (35%) and skin (50%).

4. GSH/GST Enhancers

A total of 45 GSH/GST enhancers were tested in the Chemoprevention Branch drug development program. A number of them were positive in both *in vitro* and *in vivo* studies. Among these were NAC, benzylisothiocyanate, BHA, diallyldisulfide, and oltipraz. These agents were highly active in the *in vivo* screens and models, particularly in the rat colon (71%), followed by DMBA mammary (66%) and lung models (50%). The lowest activity was demonstrated in the urinary bladder (83%), followed by MNU mammary (71%), and mouse colon (71%).

NAC demonstrated chemopreventive activity in the RTE and MMOC assays as well as MNU lung, AOM rat colon, MNU mammary, and OH-BBN mouse bladder studies. Benzylisothiocyanate demonstrated chemopreventive activity in the RTE assay and skin model. BHA was positive in the aberrant crypts assay and mouse colon; this agent was not tested *in vitro*. Diallyldisulfide demonstrated chemopreventive activity in the RTE and HFE assays as well as in the MNU lung and rat colon models. Except for JB6, oltipraz was active in all of the *in vitro* assays and *in vivo* studies.

5. ODC Inhibitors

The chemopreventive activity of 83 ODC inhibitors has been examined in the Chemoprevention Branch drug development program. These agents were highly active in the rat colon model (88%), followed by skin (57%) and DMBA mammary (43%) models. They were most inactive in the lung (91%, MNU; 80%, DEN), followed by urinary bladder (79%) models. NAC, ascorbyl palmitate, aspirin, carbenoxolone, DFMO, ellagic acid, and piroxicam were among the ODC inhibitors positive in the rat colon model. The ODC inhibitors positive in the skin model were 18β-glycyrrhetinic acid, piroxicam, and propyl gallate.

NAC demonstrated chemopreventive activity in the RTE and MMOC assays as well as lung MNU, rat colon, MNU mammary, and mouse urinary bladder studies. Ascorbyl palmitate was positive in the A427, JB6, RTE, and MMOC *in vitro* screens; however *in vivo* it was active only in the rat colon model. Aspirin demonstrated chemopreventive activity in the JB6, RTE, MMOC, and HFE *in vitro* screens as well as the rat colon model. Carbenoxolone was positive in the A427, MMOC, and HFE *in vitro* screens, and in the rat colon and MNU mammary models. DFMO demonstrated chemopreventive activity in the JB6, MMOC, and HFE assays as well as the colon aberrant crypts, colon, mammary, and uri-

nary bladder models. Ellagic acid was positive in the MMOC *in vitro* screen and the rat colon model. Piroxicam demonstrated chemopreventive activity in the A427 and HFE *in vitro* screens and rat colon and mouse urinary bladder models. 18β-Glycyrrhetinic acid was positive in all *in vitro* assays, except for JB6. This agent was also positive in the aberrant crypts assay and in the mouse colon and MNU mammary models. Propyl gallate was positive in the A427, MMOC, and HFE assays as well as the skin model. Based on these limited data, the MMOC (six results) and HFE (six results) appear to be most sensitive to ODC inhibitors; HFE and A427 assays each had five positive results, followed by the JB6 assay with three positive results for these agents.

6. Protein Kinase C Inhibitors

Thirty PKC inhibitors were examined by the NCI, Chemoprevention Branch. Based on the limited data (*e.g.*, five evaluable test results), the highest number of positive test results for this class of agents was found in the mouse colon (60%) and MNU mammary models (37%). None of the agents were active in the urinary bladder model. Positive PKC agents in the mouse colon were 18β-glycyrrhetinic acid, quercetin, and rutin. Those positive in the MNU mammary model included carbenoxolone, tamoxifen, and tamoxifen citrate.

Carbenoxolone was positive in the A427, MMOC, and HFE *in vitro* screens and in the rat colon and MNU mammary models. Tamoxifen and tamoxifen citrate have only been tested in the MNU mammary model. 18β-Glycyrrhetinic acid was positive in all the *in vitro* assays, except for JB6. It was also positive in the aberrant crypts assay and in the mouse colon and MNU mammary models. Quercetin was positive in the RTE, MMOC, and HFE *in vitro* screens and colon aberrant crypts assay and mouse colon model. Rutin demonstrated positive chemopreventive activity in the RTE assay and colon aberrant crypts assay and mouse colon model. It is not possible to draw conclusions on the available data, but it is surprising that more PKC inhibitors were not positive in the JB6 assay, since this assay detects primarily inhibition of tumor promotion which is one of the properties of PKC inhibitors [12].

DISCUSSION AND FUTURE DIRECTIONS

Three or even five assays cannot possibly cover or include all possible mechanisms of cancer chemoprevention. By the same token any single assay is not nearly as inclusive and falls way short in predicting *in vivo* results.

Clearly a battery of *in vitro* tests is the rational strategy, and the data presented support this conclusion. While these three assays are not perfect, positive results in all three do predict positive *in vivo* results at a >80% rate. A positive in any two or even one assay still predicts at >70%. One or more new assays are needed for the battery to attain higher positive predictive values. Negative predictive values for any single assay were not very useful against all animal tests, but may be useful in particular cases. For example the RTE predicted 6/6 negative hamster lung results.

Since it is not possible to test every chemical in every animal assay, a likely explanation of a false positive result is that inappropriate organ systems were tested. Another explanation is that pharmacological or pharmacokinetic problems of absorption, distribution, metabolism or excretion prevented chemopreventive activity in the whole animal. False positives may also be caused by choosing the wrong doses for the whole animal assays. Typically only two doses are chosen for *in vivo* tests while five doses are used for *in vitro* screening assays. Also one could include, as reasons for false positive results, differences such as animal species, carcinogen used, stability, diet interactions and synergies, cell types and interactions, organ site, tissue type, and exposure time.

The major reason for low negative predictive values is false negative *in vitro* results. Such results may be due to the need to metabolize an agent to its active form, repetitive poor solubility in culture media, inactivation by culture components, wrong tissue or cells, wrong carcinogen, wrong species, toxicity, or too short an exposure time.

There remains an urgent need to rapidly and efficiently screen chemical agents for potential human chemopreventive activity. New *in vitro* screens in relevant tissues need to be developed and validated. Screens, utilizing human tissues or primary or immortalized cells, are especially needed, since the goal is to prevent human cancers, and major problems extrapolating animal results to humans still exist. As additional data both *in vitro* and *in vivo* accumulate, a periodic reanalysis for predictive potential should be performed.

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Table I. Chemoprevention Branch Drug Development Program: Summary of Agents Tested in *In Vitro* Assays (as of January 15, 1996)

Agents	A427	JB6	RTE	MMOC	HFE	*In Vivo* Positive/[Negative] Test Result(s)
N-Acetyl-*l*-cysteine	NE	NE	+	+		LM, CR, MM, MB
Ajoene	NE	NE	NE	+		
S-Allylcysteine	NE					CY
Allyl Methyl Disulfide	NE	+	+	NE		
Allyl Methyl Trisulfide	+		NE	NE		
Amiloride	+	NE	NE	NE		
4-Aminobenzoic Acid	NE	+	+	NE		
Amrinone	NE					
Anethole Trithione	+	NE	+	NE	+	CR, CY, MD
Anthranilic Acid	+		+			
Antineoplaston A10	NE		+	NE		
Apigenin	NE	NE	NE	+		
Arachidonic Acid				NE		
d,l-Arginine Hydrochloride	NE	NE	+	NE	+	CY
Ascorbyl Palmitate	+	+	+	NE	+	CR
Aspirin	NE	+	+	+	NE	CR, CY, MB
Astaxanthine	+		+			
BASF-47343	+			+		
BASF-47848	NE	NE	NE	NE		
BASF-47850	NE	+	+	+		
BASF-47851	+	+	+	+		MM
BASF-49011	+					
BASF-49475	+					
BASF-51328	+	NE	+			
Benzyl Isothiocyanate			+			MS
Bestatin	+			+		
Bismuthiol I	NE		+	NE		LD, MB

Table I. Chemoprevention Branch Drug Development Program: Summary of Agents Tested in *In Vitro* Assays (as of January 15, 1996) *(continued)*

Agents	A427	JB6	RTE	MMOC	HFE	*In Vivo* Positive/[Negative] Test Result(s)
Brassinin	+		+			
8-Bromoadenosine Cyclic 3':5'-Monophosphate	+		+	NE	+	
2-Bromo-α-ergocryptine	+	+		+		MM
4-Bromophenyl Bromide	+	+	NE	+		
2-n-Butylthiophene	NE					
Butyric Acid, Sodium Salt			NE	NE		CY
Caffeic Acid	+		NE	NE		MM
Caffeic Acid Phenethyl Ester	+					
CAI	+		+	NE	NE	CY, MM
Canventol	NE		+	NE		
Carbenoxolone	+		NE	+	NE	CR, MM
N-(2-Carboxyphenyl)retinamide	+		+	NE		
N-(3-Carboxyphenyl)retinamide	+		+	+		
N-(4-Carboxyphenyl)retinamide	+		+	+		
Carnosine	+	NE	+	NE		[CY, MM]
α-Carotene	+		+	+		
β-Carotene	+	+	+	+	+	MM (injectable
15-*cis*-β-Carotene	+		+	+	+	
trans-β-Carotene (Type I: Crystalline Synthetic, 95% β-Carotene)				+		
β-Carotene (Type III: Crystalline from Carrots, 80–90% β-Carotene, 10–20% α-Carotene, Trace Other Isomers)	+		+			
d-Carvone	+		NE	NE		
(+)-Catechin	+	NE	+	+		MD
Centrophenoxine	+			NE		

Table I. Chemoprevention Branch Drug Development Program: Summary of Agents Tested in *In Vitro* Assays (as of January 15, 1996) *(continued)*

Agents	A427	JB6	RTE	MMOC	HFE	*In Vivo* Positive/[Negative] Test Result(s)
Chelerythrine	+		+	+		
8-Chloroadenosine Cyclic 3':5'-Monophosphate	+			+		
Chlorogenic Acid	+	NE	NE	NE		[CY, MM]
4-Chlorophenyl Acetate	+		+			
Chlorophyllin, Copper Sodium	NE	+	NE	+		[CY, MM]
Chlorpheniramine Maleate	+	+	+			CY
Cimetidine	+			+		
Cineole	+		NE	+		
Clotrimazole	+		+	NE		
Colon Mitosis Inhibitor	+			NE		
Conjugated Linoleic Acids	+			NE		
Cremophor EL	NE		NE	+		
Crocetin	+	+	NE	NE		
Cromolyn Sodium	+	+	+	NE		[CM, CY]
Cryptoporic Acid D				NE		
Cryptoporic Acid E	+		+	NE		
Curcumin	NE	+	+	NE	+	CM, CR, CY, MM
D 609	NE		NE	+		
Daidzein	+					
Deguelin	+		+			
Difluoromethylornithine (DFMO)	NE	+	NE	+	+	CR, CY, MD, MM, MO, MB, MS
Dehydroepiandrosterone (DHEA)	+	NE	+	+	+	CM, MM
DHEA Analog 8354	NE		+	+		CR, CY, MM
Diallyl Disulfide		NE	+	NE		LM, CR, CY
Diallyl Sulfide	+	+	NE	NE		CY

Table I. Chemoprevention Branch Drug Development Program: Summary of Agents Tested in *In Vitro* Assays (as of January 15, 1996) *(continued)*

Agents	A427	JB6	RTE	MMOC	HFE	*In Vivo* Positive/[Negative] Test Result(s)
Diallyl Trisulfide	NE		+			
N-6,*O*-2'-Dibutyryl Adenosine Cyclic 3':5'-Monophosphate	+		+	NE		
Diclofenac	+					CY
Diflunisal	+		+	+		
Dimethylaminobenzalrhodanine	NE					
Dimethyl Fumarate			+			CY
16,16-Dimethylprostaglandin E_2	+					
Diphenhydramine	+	NE	+	NE		[LM, CY]
1,3-Diphenyl-1,3-propanedione	+			NE		
Dipyridamole	+			NE		
Dithiodinicotinic Acid Diethylester	NE					
1,2-Dithiole-3-thione	+		+	+		
2,6-Dithiopurine	NE			+		
Docosahexaenoic Acid	NE	+				
ω-3-Eicosapentaenoic Acid	+	NE		NE		
5,8,11,14-Eicosatetraynoic Acid	NE		+	NE		CY
Ellagic Acid	NE		NE	+		CR
(−)-Epicatechin	+		NE	NE		
(−)-Epicatechin Gallate	+		+	NE		
(−)-Epigallocatechin	+		NE	NE		
Epigallocatechin Gallate	+		+	+		
Ergothioneine		+		+		
Esculetin	NE	NE	NE	+		[CY, MM, MB]
N-(Ethyl)-9-*cis*-retinamide	+		+	NE		
Ethylvanillin	+	+	+	NE		LM, MM
Etoperidone	+	+	+	+		CY

Table I. Chemoprevention Branch Drug Development Program: Summary of Agents Tested in *In Vitro* Assays (as of January 15, 1996) *(continued)*

Agents	A427	JB6	RTE	MMOC	HFE	*In Vivo* Positive/[Negative] Test Result(s)
Farnesol	NE			+		CY
Fenoprofen Calcium Dihydrate	+		+	NE		
Ferulic Acid	NE		+	+		CY
Filipin	NE			+		
Folic Acid	NE	+	+	NE		CY
Forskolin	+			NE		
Fumaric Acid	+	NE	+	+	+	LM, CY, MM
Genistein	+		+			CY
Geranylgeraniol	+		+	+		CY
Gestodene				NE		
Glucaric Acid			+	+		
Glucaric Acid, Calcium Salt	NE	NE	+	+		CY, MM
d-Glucaro-1,4-lactone		NE	+	NE		
Glycerol Monooleate	NE	NE	+	NE		
Glycine	NE	+	NE	+		
18α-Glycyrrhetinic Acid	+	NE	+			
18β-Glycyrrhetinic Acid	+	NE	+	+		CM, CY, MD, MM, MS
HEMF	+		+	+		
Hexadecylphosphocholine	+			NE		
4-HPR	+	NE	+	+	+	LD, MD, MM, MB, MS, LS
HWA 131	NE		+	NE		
Hydrochlorothiazide	+	NE	NE	NE		
Hydrocortisone	+	+		NE		
4-Hydroxyandrost-4-ene-3, 17-dione	+		NE	NE		

Table I. Chemoprevention Branch Drug Development Program: Summary of Agents Tested in *In Vitro* Assays (as of January 15, 1996) *(continued)*

Agents	A427	JB6	RTE	MMOC	HFE	*In Vivo* Positive/[Negative] Test Result(s)
3-Hydroxy-2-methyl-4-pyrone				+		
N-(2-Hydroxyphenyl)retinamide	+		+	+		
N-(3-Hydroxyphenyl)retinamide	+		+	+		
N-(4-Hydroxyphenyl)-9-*cis*-retinamide	+		+	+		
Ibuprofen	+	+	NE	+	NE	CR, CY, MM, MB
1*H*-Imidazole-4-thiol	+		NE	+		
Indole-3-carbinol	+		+	+	+	CM, CY, MD, MM
Indomethacin	NE	+	NE	+	NE	CY, MB
Inositol Hexaphosphate, Sodium	NE	+	+	+		[CM, CY, MM]
Ipriflavone	+		NE	+		
Ketoprofen	+		+	NE		CR, CY, MB
Kojic Acid	+			NE		
Labetalol Hydrochloride	+	NE	+	NE		
Lanosterol	NE	NE	NE	+		
Levamisole	NE	+	NE	NE		
Liarozole	+			NE		
d-Limonene	NE	NE	+	+		[CY, MD, MM]
Lovastatin	+	NE	+			
Lycopene	+		NE	+		
Magnesium Carbonate	NE			NE		
Magnesium Chloride	+			NE		
Magnesium Hydroxide	+			NE		
MAK-4			+	NE		
MAK-5	NE		+			
Maltol	+					
d-Mannitol	+	NE	NE	+		[CM, CY, MM]

Table I. Chemoprevention Branch Drug Development Program: Summary of Agents Tested in *In Vitro* Assays (as of January 15, 1996) *(continued)*

Agents	A427	JB6	RTE	MMOC	HFE	*In Vivo* Positive/[Negative] Test Result(s)
Meclizine	+	+	+	NE		CY
Meclofemate Sodium Salt				+		
Melatonin	+	+	+	+		[CY, MM]
Melittin	NE	NE		NE		
Menthol	NE		NE	NE		
2-Mercaptoethanesulfonate, Sodium Salt	NE	NE	+	+		MB
2-Mercaptoethylamine Hydrochloride	+		+	NE		
N-2-Mercaptopropionyl Glycine	+			+		
d,l-Methionine			+	+		CY
4-Methoxyphenol	+	+	NE	NE		[CR, CY, MM]
All-*trans*-*N*-(4-Methoxyphenyl)-retinamide	+		NE			
Methylene Blue	+	+	NE	+		CY
Miconazole	+		NE	+		MD, MM
Misoprostol	NE					
Molybdate, Sodium	+	NE	NE	+	+	MM, MB
Morin	NE	NE	+	NE		
Myricetin	NE	NE	NE			
Nalidixic Acid	+		NE			
Naproxen	+		NE	+		
Naringenin	+		+	+		MD, MM
Nerolidol	+			NE		CY
Nicotinic Acid	NE		NE	NE		
Nifedipine	+		NE	NE		
3-Nitroflavone	+		+	+		
Nordihydroguaiaretic Acid	+	+	NE	+		[CY, MM]

Table I. Chemoprevention Branch Drug Development Program: Summary of Agents Tested in *In Vitro* Assays (as of January 15, 1996) *(continued)*

Agents	A427	JB6	RTE	MMOC	HFE	*In Vivo* Positive/[Negative] Test Result(s)
2-n-Octylthiophene	+		+	+		
18-α-Olean-12-ene-3β,23,28-triol	+		+	NE		
Oltipraz	+	NE	+	+	+	LD, LM, CM, CR, CY, MD, MM, MB, MS
l-2-Oxothiazolidine-4-carboxylate	+	NE	NE	+		[LM, CY, MM]
d,l-Palmitoylcarnitine	+	+	+	NE		[LM, MD, MM]
Pentoxyphylline	+			NE		
Perillic Acid	NE			+		
Perillyl Alcohol	+		+	NE		
Phenethyl Isothiocyanate	+	+	+	+	+	LN, ES
Phenidone	+			NE		
Phenylacetate, Sodium	+			NE	+	
2-Phenylbutyric Acid, Sodium Salt	+				+	
4-Phenylbutyric Acid, Sodium Salt			+			
Phloretin	NE	+	+	+		
Phlorizin	NE		+	NE		MM
Piroxicam	+	NE	NE	NE	+	CR, CY, MB, MS
Polyethylene Glycol 400	NE		NE			
Polyvinylpyrrolidone	+		+			
Praziquantel	NE	+	NE			MM
Prednisone	+	NE				
Promethazine	NE	+	+	+		MD, MM
Propylene Glycol	NE	NE	NE	+		
Propyl Gallate	+	NE	NE	+	+	MM, MS
Pseudoephedrine	NE					
Purpurin	+	NE	+	+		CM, CY

Table I. Chemoprevention Branch Drug Development Program: Summary of Agents Tested in *In Vitro* Assays (as of January 15, 1996) *(continued)*

Agents	A427	JB6	RTE	MMOC	HFE	*In Vivo* Positive/[Negative] Test Result(s)
Quercetin	NE	NE	+	+	+	CM, CY, MD
Quinacrine Hydrochloride	+		+	+		[CY, MM]
Quilizarin	+		+	NE		
Ranitidine	NE	+		NE		
13-*cis*-Retinoic Acid	+					CY
9-*cis*-Retinoic Acid	+		+	+	+	CY
all-*trans*-Retinoic Acid		+	+		+	CY, MO
Retinoyl Glucuronide				NE		
Retinoyl-*d,l*-leucine	NE	+	+	NE		
Retinyl Glucuronide				+		
Rhapontin	+		NE	+		
Rhodamine B	+	+				
Riboflavin 5'-Phosphate	+	+	NE	NE		
Ro 16-9100	+	+	+	+		CY, MM
Ro 19-2968	NE		NE	NE		MM
Ro 21-5535	+		+	+		CY, MM
Ro 23-7553	+		+	+	NE	CY, MM
Ro 24-2637	+		+	+		CY
Ro 24-5531	+		+	+	NE	CY
Ro 25-6760	+		+		NE	
Rutin		NE	+	NE		CM, CY
Sarcophytol A				NE		
Selenite, Sodium	NE		+	+	+	LD, CY, MD
Silymarin	+	+	+	+	+	[CM, CY, MM, MB]
Simethicone	+	+	NE	NE		CY
β-Sitosterol	+	NE	+	+		[CM, CY, MM, MB]

Table I. Chemoprevention Branch Drug Development Program: Summary of Agents Tested in *In Vitro* Assays (as of January 15, 1996) *(continued)*

Agents	A427	JB6	RTE	MMOC	HFE	*In Vivo* Positive/[Negative] Test Result(s)
Sobrerol	NE			+		
Sphingosine	+					
Stearic Acid	+			+		
Steviol	+	+	+	NE		
Sulfasalazine	+		NE	NE		[CR, CY]
Sulindac	+		NE	NE		CR, CY, MB
Suramin	+	NE	+	NE		CY
Tamoxifen Citrate				NE		MM
Taurine	+	NE	+	NE		CR, CY
Tea (Black, Extract)			+	+		
Tea (Black, Extract, Decaffeinated)	+		+	+		
Tea (Black, Polyphenols)	+		+	+		
Tea (Green, Extract)	NE		+	NE		
Tea (Green, Extract, Decaffeinated)	NE		+	NE		
Tea (Green, Polyphenols)	+		+	+	+	
Temaroten	+		+	NE		MM
Tempol	+		+	+		
Terfenadine	+		NE	NE		
Tetracycline	NE		+	+		CY
Theaflavin Mixture	●		+	+		
Theophylline		+		+		
Thioctic Acid	NE	NE	NE	+	+	
Thiolutin	+	+	+	+		
Thiopurinol	+					
Thiosulfate, Sodium	+	NE	NE	NE		CY
2-Thioxo-4-thiazolidinone	+		+	NE		

Table I. Chemoprevention Branch Drug Development Program: Summary of Agents Tested in *In Vitro* Assays (as of January 15, 1996) *(continued)*

Agents	A427	JB6	RTE	MMOC	HFE	*In Vivo* Positive/[Negative] Test Result(s)
Tolmetin	+		+			CY
Toremifene						MD, MM
Transforming Growth Factor β	+	+	+	+		
Trifluralin	NE					
Triprolidine	NE	+	+			CY
Uric Acid	NE	NE	+	NE		
Ursolic Acid	+	+	NE	NE		
Vanillin	+	+	NE	NE		
Verapamil	+	+	+	+		CY
Vitamin D_3	+			+		
Vitamin E Acetate		+	NE	+		
Vitamin E Succinate PEG 1000	NE	NE	+	+		[LM, MM, MB]
Vitamin K_3	+	+	+	NE		
(+)-Vorozole				NE		MM
W-7	+		NE	+		

Abbreviations: BASF-47343, BASF-47848, BASF-47850, BASF-47851, BASF-49011, BASF-49475, and BASF-51328, retinoid analogs; CAI, 5-Amino-1-((3,5-dichloro-4-(4-chlorobenzoyl)phenyl)methyl)-1*H*-1,2,3-triazole-4-carboamide; D 609, *O*-(Octahydro-4,7-methano-1*H*-inden-5-yl)carbonodithioic acid ester; Ro 25-6760 and Ro 16-9100, arotenoids; Ro 19-2968, Ro 21-5535, Ro 23-7553, Ro 24-2637, and Ro 24-5531, vitamin D analogs; W-7, *N*-(6-Aminohexyl)-5-chloro-1-naphthalenesulfonamide; LD = Lung DEN, LM = Lung MNU, LN = Lung NNK, LB = Lung B(*a*)P, CM = Mouse Colon, CR = Rat Colon, CY = Colon Crypts, MD = Mammary DMBA, MM = Mammary MNU, MO = Skin Transgenic, MB = Mouse Bladder, MS = Skin DMBA, SB= Skin B(*a*)P, LS = Lymphatic System, PR = Prostate, PA = Pancreas, and ES = Esophagus

Table II. *In Vitro versus In Vivo* Results – Sensitivity, Selectivity, Accuracy and Predictive Value of the A427, RTE and MMOC assays *versus* All Organ Sites in Whole Animals

In Vitro Assay	*In Vitro* Result	*In Vivo* Results Sensitivity	*In Vivo* Results Selectivity	Predictive Value	% Total (Accuracy)
		+	–		
A427	+	48	17	48/65	74
	–	24	7	7/31	23
	Total	48/72 (67%)	7/24 (29%)	55/96	57
RTE	+	55	17	55/72	76
	–	18	10	10/28	36
	Total	55/73 (75%)	10/27 (37%)	65/100	65
MMOC	+	42	15	42/57	74
	–	28	9	9/37	24
	Total	42/70 (60%)	9/24 (38%)	51/940	54

Table III. *In Vitro versus In Vivo* Results – Positive and Negative Predictive Values of the A427, RTE and MMOC Assays *versus* Specific Organ Sites in Whole Animal Studies

In Vitro Assay	Result	Lung		Colon		Mammary Gland		Bladder		Skin	
		No.	%	No.	%	No.	%	No.	%	No.	%
A427	+	5/14	36	35/62	56	24/56	43	5/17	29	6/10	60
	–	4/7	57	14/32	44	18/29	62	6/12	50	3/4	75
RTE	+	9/15	60	42/64	66	23/59	39	7/28	25	4/10	40
	–	6/6	100	17/29	59	20/29	69	5/11	45	1/4	25
MMOC	+	6/15	40	31/59	53	25/55	46	9/25	36	6/11	55
	–	4/7	57	15/35	43	26/35	74	7/10	70	2/3	67

Lung Studies with DEN, NNK and MNU; Colon Studies in Rat and Mouse with AOM and MAM Acetate, Mammary Studies with DMBA and MNU; Bladder Study with OH-BBN, Skin Studies with DMBA and B(*a*)P

Table IV. *In Vitro versus In Vivo* Results – Sensitivity, Specificity, and Accuracy of the A427, RTE, and MMOC Assays *versus* Specific Organ Sites in Whole Animal Studies

In Vitro Assay		Lung		Colon		Mammary		Bladder		Skin	
		No.	%	No.	%	No.	%	No.	%	No.	%
A427	Sensitivity	5/8	63	35/43	66	24/35	69	5/11	45	6/7	86
	Specificity	4/13	31	14/41	34	18/50	36	6/18	33	3/7	43
	Accuracy	9/21	43	49/84	58	42/85	49	11/29	38	9/14	64
RTE	Sensitivity	9/9	100	42/54	78	23/32	72	7/13	54	4/7	57
	Specificity	6/12	50	17/39	44	20/56	36	5/26	19	1/7	17
	Accuracy	15/21	71	59/93	63	43/88	49	12/39	31	5/14	36
MMOC	Sensitivity	6/9	67	31/51	61	25/34	74	9/12	75	6/7	86
	Specificity	4/13	31	15/43	35	26/56	46	7/23	30	2/7	29
	Accuracy	10/22	45	46/94	49	51/90	57	16/35	46	8/14	57

Lung Studies with DEN, NNK and MNU; Colon Studies in Rat and Mouse with AOM and MAM Acetate, Mammary Studies with DMBA and MNU; Bladder Study with OH-BBN, Skin Studies with DMBA or B(*a*)P.

Table V. Positive Predictive Values of Combinations of *In Vitro* Assays *versus In Vivo* Assays in All Organs

In Vitro Assays No. Positive	No. of Agents	*In Vitro* Results		Positive Predictive Value	
		Positive	Negative	Ratio	%
All three	24	20	4	20/24	83
Two or three	50	36	14	36/50	72
One, two, or three	78	59	19	59/78	76

Table VI. Negative Predictive Values of Combinations of *In Vitro* Assays *versus In Vivo* Assays in All Organs

In Vitro Assays No. Negative	No. of Agents	In Vitro Results		Positive Predictive Value	
		Positive	Negative	Ratio	%
All three	2	1	1	1/2	50
Two or three	20	15	5	5/20	25
One, two, or three	55	40	15	15/65	27