

Concurrent Session 2: Hereditary Cancer Syndromes

CN02-02 Hereditary colorectal cancer. S. M. Lipkin. University of California, Irvine, CA.

Colorectal cancer in developed countries has an incidence worldwide of more than a million cases annually. It is the second leading cause of cancer death in the United States. An anticipated ~155,000 people will be diagnosed with colorectal cancer, and an estimated 55,000 individuals will die in the U.S. this year. Genetics has made important contributions to the identification of individuals who are at increased risk for colorectal cancer. This session will review advances in the field of colorectal cancer prevention. These advances include new risk assessment models for Lynch Syndrome cancer risk, improved approaches to understanding the role of missense mutations in colorectal cancer susceptibility, and the discovery of novel susceptibility loci from genetic association studies.

Concurrent Session 4: Xenobiotic Metabolism and Cancer

CN04-01 The UDP-glucuronosyltransferases and cancer susceptibility. P. Lazarus. Penn State University College of Medicine, Hershey, PA.

The UDP-glucuronosyltransferases (UGTs) are a superfamily of enzymes that detoxify a diverse range of compounds by their conjugation to glucuronic acid in a reaction with a hydrophilic co-substrate, UDP-glucuronic acid (UDPGA). The attached sugar alters the biological properties of the compound and enhances its excretion in the urine or bile, usually converting substrates into less pharmacologically active, more water soluble products and facilitating their removal from the body via the urine or bile. In addition to a variety of endogenous compounds like steroids and bilirubin, the UGTs are highly active against a variety of xenobiotic substrates including many pharmacologic agents and drugs as well as carcinogens.

Glucuronidation is a major mode of metabolism for an important group of carcinogens in tobacco and tobacco smoke, the tobacco-specific nitrosamines (TSNAs), and the structurally-related agents, nicotine and its metabolite cotinine. This presentation will focus on recent studies examining the UGTs involved in the N-glucuronidation of TSNAs, nicotine and cotinine, and the identification of a UGT2B10 haplotype that is associated with significantly decreased N-glucuronidation capacity in human liver microsomes. Utilizing microsomes from HEK293 cells over-expressing individual UGTs, only UGTs 1A4 and 2B10 exhibited N-glucuronidating activity against the TSNAs 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), the major procarcinogenic metabolite of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N'-nitrosornicotine, N'-nitrosoanabasine and N'-nitrosoanatabine. The $K_{M,s}$ for UGT2B10 were 6-22-fold lower than those of UGT1A4 against these TSNAs, and were similar to those observed for human liver microsomes (HLM). The overall activity of UGT2B10 was 3-27-fold higher than UGT1A4 against these TSNAs as determined by V_{max}/K_M after normalization by levels of UGT2B10 versus UGT1A4 mRNA. Similarly, UGT2B10 exhibited high N-glucuronidation activity against both nicotine and cotinine with apparent $K_{M,s}$ that were 37- and 3-fold lower than that observed for UGT1A4 against nicotine and cotinine, respectively. The K_M of microsomes from UGT2B10-overexpressing cells for nicotine and cotinine was similar to that observed for human liver microsomes (HLM) against both substrates. Since real-time PCR analysis demonstrated that UGT2B10 was expressed at a level that, on average, was 26% higher than that observed for UGT1A4 in a screening of normal liver tissue specimens from 20 individual subjects, these data suggested that UGT2B10 is likely the most active UGT isoform in human liver for the N-glucuronidation of TSNAs.

To study the potential association between UGT2B10 genotypes and NNAL-N-glucuronidation activity in HLM, four common UGT2B10 haplotypes (termed A through D) were identified by genotyping three tagSNPs within the UGT2B10 locus. Haplotype C was found to be

significantly ($P < 0.001$) associated with lower NNAL-N-glucuronidation in HLM, with a 12-fold reduction in NNAL-N-glucuronidation levels and an 11-fold reduction in the ratio of NNAL-N-Gluc:NNAL-O-Gluc observed in HLM from subjects with two copies of UGT2B10 haplotype C. A novel polymorphism resulting in an aspartic acid to tyrosine amino acid change at codon 67 of the UGT2B10 cDNA was identified exclusively in subjects with a haplotype C. Unlike the high activity observed in microsomes from HEK293 cells over-expressing the wild-type UGT2B10^{67Asp}, microsomes from HEK293 cells over-expressing the UGT2B10^{67Tyr} variant exhibited minimal glucuronide formation activity against NNAL or other TSNAs tested *in vitro*. Similarly, the level of glucuronidated nicotine or cotinine in HLMs was correlated with UGT2B10 genotype; the levels of nicotine- and cotinine-glucuronide were 5- and 16-fold lower, respectively, in HLM from subjects with the UGT2B10 (Tyr67Tyr) genotype as compared to subjects with the UGT2B10 (Asp67Asp) genotype. In contrast to the high activity observed for cells over-expressing wild-type UGT2B10 *in vitro*, little or no glucuronidation activity was observed for microsomes from cells over-expressing the UGT2B10^{67Tyr} variant against either nicotine or cotinine.

Together, these data suggest that the UGT2B10^{67Tyr} variant, which is in high linkage disequilibrium with UGT2B10 haplotype C, is a functional SNP that may be responsible for inter-individual variation in TSNA-, nicotine- and cotinine-N-glucuronidation activity in people. This variant may therefore play an important role in altering susceptibility to smoking-related cancers or nicotine-related addictive behaviors.

CN04-02 Glutathione S-transferases and S-glutathionylation in cancer. Kenneth D. Tew. Department of Cell and Molecular Pharmacology, Medical University of South Carolina, Charleston, SC.

The glutathione-S-transferase (GST) super family comprises multiple isozymes (Alpha, Mu, Pi, Omega, Theta, and Zeta) with evidence of functional polymorphic variations. Correlative studies have shown that genetic differences of GST isozyme expression may contribute to cancer susceptibility and treatment. Over the last two decades, a body of data has accumulated linking aberrant expression of GST isozymes with the development and expression of resistance to cancer drugs. In particular, GST Pi (GSTP) is over-expressed in a number of different tumors compared to normal tissues (including ovarian, non-small cell lung, breast, colon, liver, pancreas and lymphoma). Moreover, a significant range of anticancer drugs can cause an increased expression of GSTP in drug resistant selected cell lines (and drug treated patients). From a functional viewpoint, not all drugs used to select for resistance are substrates for catalysis by GSTP. In fact, catalytic constants for GSTP conjugation reactions with virtually all standard cancer drugs are poor.

So why is GSTP so frequently over-expressed in these situations? There is recent evidence that GST isozymes, and GSTP in particular, have multiple functions in cells, many unrelated to thioether bond catalysis with chemical moieties. These include: (1) ligand binding and transport of heme, bilirubin, nitric oxide; (2) protein: protein interactions with possible chaperone like functions; (3) regulation of mitogen activated protein kinases, particularly c-jun NH2 terminal kinase (JNK); (4) mediation of the forward reaction of the post-translational process of S-glutathionylation. Of these, aberrant kinase signaling pathways and altered protein S-glutathionylation patterns are both characteristic of the malignant phenotype.

What happens in the absence of GSTP? GSTP null animals have essentially normal development and life spans. Mouse embryo fibroblast (MEF) cells isolated from wild type or GSTP null animals differ in a number of characteristics related to signaling and growth. For example, the doubling time for wild type cells is 33.6 h compared to 26.2 h for GSTP null. Both early passage and immortalized MEF cells from GSTP null animals express significantly elevated activities of extracellular regulated kinases (ERK1/ERK2). Null animals had constitutively elevated c-jun NH2-terminal kinase (JNK) activity compared to wild type and this is correlated with altered regulation of genes downstream of JNK. As a whole, the genetic absence of GSTP influences the capacity of stress kinases to regulate gene expression and this can have an impact on cell proliferation pathways. The non-lethality of the deletion points to possible functional

redundancy and implies that other GST (or other redox proteins) may substitute for the absence of GSTP. This conclusion would seem to be supported by the data suggesting general redundancy of function amongst, and within, this protein cluster.

Cysteine residues in proteins provide a nucleophilic site for a number of disparate post-translational modifications. For example, disulfide bonds between vicinal thiols have major implications for three dimensional protein structures. Contingent upon the steric properties and local environment of the cysteine, lipidation of these residues may occur through S-isoprenylation, S-farnesylation, S-geranylgeranylation or S-palmitoylation. The direct addition of GSH to cysteines with low pKa's creates an S-glutathionylated residue, resulting in an increase in both molecular weight (of 605) and negative charge (from the glutamic acid residue). The scheme in the Figure summarizes some components of this reaction. GSTP can facilitate the forward reaction and glutaredoxin, sulfiredoxin or thioredoxin can contribute to the reverse reaction. The reversible nature of this pathway is important in facilitating a sulfur based regulatory pathway that can expedite response to stress conditions. Importantly, a number of phosphatases can be regulated by S-glutathionylation and this provides a link and conduit with phosphate based signaling pathways. Because the structure, function and cell distribution of proteins can be affected by S-glutathionylation, the importance of GSTP in mediating this reaction could have significant consequences and may be a contributory factor in the high expression levels of GSTP in many tumors.

These multiple functionalities contribute to the recent rational efforts to target GSTs with novel small molecule therapeutics. While the ultimate success of these attempts remains to be shown in the clinic, at least three drugs are in late-stage clinical testing. Two of these (NOV-002 and Telintra) are being tested therapeutically as small molecule myeloproliferative agents. As the field progresses, the concept of designing new drugs that might interfere with protein:protein interactions between GSTs and regulatory kinases provides a novel approach to identify new targets in the search for cancer therapeutics.

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CN04-03 Relationship of PhIP metabolism and DNA binding to cancer susceptibility. J. S. Felton,¹ M. A. Malfatti,¹ K. H. Dingley,¹ E. A. Ubick,¹ N. Mulakken,¹ S. Nowell-Kadlubar,² D. Nelson,¹ N. P. Lang,³ K. W. Turteltaub¹. ¹Lawrence Livermore National Laboratory, Livermore, CA, ²University of Arkansas for Medical Sciences, Little Rock, AR, ³University of Arkansas for Medical Sciences, Central Arkansas Veterans Healthcare System, Little Rock, AR.

Epidemiologic evidence from numerous studies indicates that exposure to heterocyclic amines in the diet is an important risk factor for the development of colon cancer. Well-done cooked meats contain significant levels of heterocyclic amines, which have been shown to cause cancer at multiple sites in laboratory animals. To better understand the mechanisms of heterocyclic amine bioactivation in humans, the most mass abundant heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), was used to assess the relationship between PhIP metabolism and DNA adduct formation. Ten human volunteers were administered a dietary relevant dose of [14C]PhIP 48 to 72 hours prior to surgery for removal of colon tumors. Urine was collected for 24 hours after dosing for metabolite analysis, and DNA was extracted from biopsied colon tissue and analyzed by accelerator mass spectrometry for DNA adducts. All 10 subjects were phenotyped for cytochrome P4501A2 (CYP1A2), N-acetyltransferase 2, and sulfotransferase 1A1 enzyme activity. Twelve PhIP metabolites were detected in the urine samples. The most abundant

metabolite in all volunteers was N-hydroxy-PhIP-N²-glucuronide. Not surprisingly, metabolite levels varied significantly between the volunteers. Interindividual differences in colon DNA adducts levels were observed between each individual. The data clearly showed that individuals with a rapid CYP1A2 phenotype and high levels of urinary N-hydroxy-PhIP-N²-glucuronide had the lowest level of colon PhIP-DNA adducts. This suggests that glucuronidation plays a significant role in detoxifying N-hydroxy-PhIP. The levels of urinary N-hydroxy-PhIP-N²-glucuronide were negatively correlated to colon DNA adduct levels. Although it is difficult to make definite conclusions from a small data set, the results from this pilot study have encouraged further investigations using a much larger study group. (*Cancer Res* 2006; 66(21): 10541-7).

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Concurrent Session 5: Bioactive Food Components and Cancer Prevention

CN05-01 Whole foods or phytochemicals? Considerations for chemoprevention. J. W. Fahey. Johns Hopkins University School of Medicine, Baltimore, MD.

Most scientists have now come to accept the views of Richard Doll and Richard Peto that a sizable fraction of cancer mortality is influenced by diet. However, acceptance of this epidemiologic reality leads to a most complex issue: Should part of a proactive strategy to address the prevention of cancer and chronic disease encourage a better diet and more exercise, or should it yield to the realities of reduced energy expenditure in the modern world? Should it support the rational development of supplements that might not require, for example, the consumption of at least five servings per day of fruits and vegetables? A large variety of phytochemicals have been identified, and mechanistic work on isolated compounds highlights multiple mechanisms for their cancer preventive activity. Bioavailability and efficacy of these compounds as components of whole foods are clearly impacted by complex interactions that are not yet well understood. There are three questions that are particularly perplexing, and although there is no consensus on any of them, it is proposed that they deserve to be the substrate for creative minds over the next decade: (1) When long-term prophylaxis is indicated, should persons at increased risk for particular cancers be encouraged to take (i) pharmaceuticals, (ii) food products or supplements enriched in potentially protective compounds, or (iii) whole foods? (2) Are there true synergies or matrix effects within the huge variety of concoctions, decoctions, preparations, elixirs, extracts, and tonics reported to have protective activities? There has been much descriptive work on the efficacy of these folk medicines, yet rigorous evaluation of the biological effects of these reported synergies is still an elusive goal. (3) Dogmatic approaches to protection from chronic disease frequently assume that one would need to attain high levels of protection in order for there to be value in this strategy. Whereas this may be the only paradigm that permits us to measure risk reduction using currently available tools, does it not also incorporate the flawed logic that the only good risk reduction is a large risk reduction?

CN05-02 Isolation and evaluation of natural product cancer chemopreventive agents. J. M. Pezzuto,¹ C. Chang,² B. A. Craig,² M. Cushman,² W. Fenical,³ H. H.S. Fong,⁴ A. Mesecar,⁴ R. C. Moon,¹ R. B. van Breemen⁴. ¹University of Hawaii at Hilo, Hilo, HI, ²Purdue University, West Lafayette, IN, ³Scripps Institution of Oceanography, La Jolla, CA, ⁴University of Illinois at Chicago, Chicago, IL.

Throughout history, natural products have played a dominant role in the treatment of human ailments. The association of salicylates with the willow, and quinine with cinchona, are renowned examples. Similarly, the legendary discovery of penicillin transformed global existence. Traditional remedies, largely based on terrestrial plants, still dominate therapeutic

practices throughout the world, and natural products comprise a large portion of current-day pharmaceutical agents, most notably in the areas of antibiotic and cancer therapies (1). Similarly, natural products are of major importance in the field of cancer chemoprevention (2). Prominent examples include sulforaphane and phenethyl isothiocyanate (cruciferous vegetables), epigallocatechin-3-gallate (green tea), curcumin (turmeric), resveratrol (grapes), sulfur-containing compounds and selenium (the genus *Allium*), and lycopene (tomatoes) (3). Clinical trials demonstrate promise (4). Consequently, it is reasonable to search for new natural product cancer chemopreventive agents.

For the discovery of novel natural chemopreventive agents, one approach is to evaluate crude natural products, such as plant extracts, and isolate active principles. Following biological activity, standard methods of fractionation can be employed. If a suitable receptor or target enzyme is available, the more efficient process of ultrafiltration-mass spectrometry can be used (5). Mass spectrometry is also a useful tool for studying the absorption and metabolism of lead compounds (6-7). Resulting information is crucial when considering future development.

Largely using terrestrial plants as starting materials, we have isolated and identified a large number of promising cancer chemopreventive agents (8). A notable example is resveratrol (9), a constituent of grapes and grape products, that now has been the subject of nearly 2,000 manuscripts and clinical trials (10). Resveratrol is readily absorbed, but only small quantities of the parent compound are found in the serum of humans. This illustrates the importance of metabolism and the corresponding activity of metabolites. Through crystallographic analysis, we have observed resveratrol interaction at the arachidonic acid binding site of cyclooxygenase, but perhaps more importantly, the 4'-sulfate metabolite is capable of a similar interaction. It logically follows that synthetic organic chemistry is an integral component of cancer chemopreventive drug development, from the perspective of creating derivatives and metabolites (11), as well as scale-up synthesis to provide adequate quantities of lead compounds for testing (12).

Although edible and nonedible terrestrial plants have yielded interesting leads, a new and exciting area of research involves exploring the biodiversity provided by microbes of the marine environment (13). Already, promising leads have been discovered for cancer therapy (13). Recently, as chemopreventive agents, two unusual bicyclic polyketides obtained from the marine actinomycete *Salinispora arenicola* were found to inhibit ornithine decarboxylase induction in cell culture (14), similar to the mode of action of rotenoids (15). Clearly, cancer chemopreventive potential still requires further exploration. We have now established a novel battery of assays, such as interaction with RxR or Keap1, and inhibition of quinone reductase 2 or NF- κ B, and many active leads have been identified. As a result, we are confident that a variety of novel substances of marine origin will be discovered as a result of this work. It is now well established that cancer chemoprevention is a viable strategy in the fight against cancer. The current armamentarium of agents has resulted largely from epidemiological observations, off-shoots of cancer therapeutic agents, or agents that were used for other therapeutic indications. With concerted effort involving a range of expertise, it is clear that new natural product chemopreventive agents with clinical potential can be uncovered using a systematic approach of drug discovery. The authors are grateful to the National Cancer Institute for support provided under the auspices of program project P01 CA48112 entitled "Natural Inhibitors of Carcinogenesis."

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CN05-03 A food-based approach to the prevention of gastrointestinal tract cancers.

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Our laboratories have been evaluating the ability of freeze-dried black raspberries to prevent cancer in the esophagus and colon. Black raspberries contain multiple known chemopreventive agents including vitamins A, C, E and folic acid; calcium and selenium; β -carotene, α -carotene and lutein; polyphenols such as ellagic acid, ferulic acid, p-coumaric acid, quercetin and several anthocyanins; and, phytoesters such as β -sitosterol. The freeze-drying process concentrates these agents approximately 10-fold. In initial studies, lyophilized black raspberry powder was mixed into AIN-76A synthetic diet at concentrations of 5% and 10% and fed to Fischer 344 rats before, during and after treatment with the esophageal carcinogen, N-nitrosomethylbenzylamine (NMBA) (1). The berries were found to inhibit the number of esophageal tumors in NMBA-treated animals by 40-60%. This inhibition correlated with reductions in the formation of NMBA-induced O6-methylguanine adducts in esophageal DNA and the progression of dysplastic lesions into tumors (1). Berries reduced the growth rate of premalignant esophageal cells and inflammation, in part, through down-regulation of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and activator protein-1 (AP-1) (2). Berries also reduced microvessel density (angiogenesis) in NMBA-treated esophagus, in part, by down-regulating the expression of vascular endothelial growth factor (VEGF) (3). Black raspberry diets also inhibited azoxymethane-induced colon adenocarcinomas in rats by up-to 80% (4). The mechanism(s) by which berries prevent colon cancer in rodents are under investigation.

Using biodirected fractionation techniques, studies are being conducted to identify the active inhibitory components in black raspberries. Both organo- and water-soluble extracts of berries were shown to selectively inhibit the growth and stimulate apoptosis of tumorigenic rat esophagus cells in vitro and to down-regulate the transcription activator proteins, AP-1 and nuclear factor-kappa B (NF- κ B), and their associated kinases in JB-6 mouse epidermal cells (5). They also down-regulate VEGF via inhibition of the P13K/Akt pathway (6). Preliminary studies suggest that the anthocyanins in berries are amongst the most active inhibitory components (7).

Recently, we have initiated prevention trials in humans to determine if berries might exhibit chemopreventive effects in the esophagus and colon. In an initial Phase I clinical trial, freeze-dried black raspberries, administered orally for 7 days at a dose of 45 grams per day, were found to be well tolerated (8). Ellagic acid and the anthocyanins; cyanidin 3-glucoside, cyanidin 3-sambubioside, cyanidin 3-xylosylrutinoside and cyanidin 3-rutinoside, were all absorbed into the blood with peak plasma levels occurring within 2-4 hours of oral berry consumption (8). The absorption of these compounds however, was minimal and represented less than 1% of the administered dose. Several Phase IIa clinical trials of lyophilized black raspberries are underway in subjects with Barrett's esophagus, esophageal dysplasia and rectal polyps to determine if berries will modulate various histological and molecular biomarkers of esophageal and colon tumor development. Current progress in these trials has been summarized (9). Supported by NCI grants CA103180 and CA96130 and the USDA.

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Concurrent Session 6: Clinical Prevention Trials

CN06-05 Innovative Cancer Prevention Clinical Trial Designs.

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The rapidly increasing understanding of the mechanisms of carcinogenesis and the availability of many new experimental agents provide both opportunities and challenges for cancer prevention drug development. The design of clinical prevention trials has to account for different target organ biology and tissue accessibility, leading to a variety of trial designs currently in use for phase I and II trials. Depending on the nature of the endpoint being assessed, trials range from multi-month treatments with the aim of regressing premalignant lesions to short-term treatments with the aim of demonstrating an effect on a pharmacodynamic endpoint. The amount of information gleaned from the various designs differs considerably. For progress to occur in a timely fashion, new and more efficient clinical trial models need to be developed. As cancer treatment strategies become more targeted and less toxic, the same agents may be appropriate for both prevention and treatment. This provides unprecedented opportunities to assess chemopreventive efficacy during the use of agents for cancer treatment or in the presurgical setting. "Prevention-relevant endpoints", such as biomarkers of proliferation or pharmacodynamic effects, can be assessed in short term presurgical settings or longer-term neoadjuvant settings while the patient is awaiting definitive surgical treatment. A major benefit of these approaches is that the acquisition of tissue occurs within the context of "standard of care" and presumably large amounts of tissue become available for analysis. The "prevention-relevant" endpoints can potentially be assessed in cancer, premalignant, and histologically uninvolved tissues, generating a greater understanding of the relevance of the endpoint to carcinogenesis and of the effect of the agent during the various stages of carcinogenesis. This information is critical for the design of subsequent clinical trials, because it targets the testing of the agent to the phases of carcinogenesis that are most likely to be responsive to the intervention.

Assessment of "prevention-relevant" endpoints, such as via bronchoscopies or colonoscopies to assess agent effects on bronchial dysplasia and aberrant crypt foci, respectively, can also be nested in cancer treatment (or adjuvant) trials which use agents that have potential for cancer prevention. If agents have an appropriately benign toxicity profile, this allows simultaneous early development for prevention and treatment indications. When agents are being used for several months, as in the adjuvant setting, their effect on premalignancy can be more appropriately assessed than during shorter presurgical trials. Since individuals with a variety of cancers are more likely to have concurrent premalignant disease (due to field carcinogenesis) than individuals without cancer, the targeting of a cohort with cancer for preventive agent development is particularly efficient.

As we further define the molecular and histologic progression events involved in the genesis of multiple cancers and develop new imaging or monitoring capabilities to follow these events, new opportunities are arising for clinical trial design. For instance, lung imaging by spiral CT allows the visualization of peripheral lung nodules, some of which are precursors to adenocarcinoma, thereby allowing the nesting of chemoprevention trials into spiral CT screening programs. As another example, identification of populations at high risk for hepatocellular carcinoma on the basis of cirrhosis from hepatitis C infection and mild alpha-fetoprotein elevation allows the design of a clinical trial with alpha-fetoprotein as the primary endpoint. The development of clinical trial designs that minimize invasive assessments of efficacy is a high priority.

The past successes and failures have identified several important research areas that require significant investment for progress in cancer prevention to occur. There is no substitute for understanding the biology of the carcinogenic process to allow rational selection of targets for intervention and the appropriate design of clinical trials. There exists a tremendous need to develop clinical trials models that can efficiently identify promising agents for cancer prevention in different target organs. This requires identification of biomarkers that reflect clinical benefit and, eventually, validation of these markers if they are to be used as surrogates. Simultaneous attention to biology, risk assessment, and trial design lies at the core of further progress.

Concurrent Session 8: New Approaches for Old (And New) Drugs as Cancer Preventive Agents

CN08-04 Insulin resistance: Influence on cancer risk and cancer prognosis. M. N. Pollak. McGill University / Jewish General Hospital, Montreal, Quebec, Canada.

Hyperinsulinemia is usually associated with reduced insulin signalling in classic target tissues for insulin action, such as liver, muscle, and fat. Intake of energy in excess of requirements often leads to insulin resistance, obesity, and hyperinsulinemia, although this is influenced by genetic factors and varies between individuals in human populations and between mouse strains in laboratory models.

Hyperinsulinemia and obesity are becoming more common in affluent societies, largely due to decreasing physical activity coupled with ample availability of calorie-dense foods. It is important to recognize that hyperinsulinemia is not always associated with obesity: in affluent societies, many individuals meet criteria for characterization as "metabolically obese, normal weight" (MONW).

Recent studies (1) provide early evidence hyperinsulinemia is associated with poor prognosis for common cancers. These studies are consistent with earlier observations suggesting that obesity (2) and hyperglycemia (3) are associated with increased cancer mortality. While they are many metabolic abnormalities in subjects who are hyperglycemic, hyperinsulinemic, and obese that might be causally associated with the increased cancer mortality observed, one obvious candidate is insulin itself. This involves the hypothesis that in hyperinsulinemic, insulin resistant subjects, neoplastic tissue may not share the insulin resistance present in the normal host tissues, but rather remain insulin sensitive, in a hyperinsulinemic milieu. As an early step to explore this hypothesis, we have confirmed and extended recent reports from several groups in documenting the presence of insulin receptors on primary human cancers, including those of breast, colon, and prostate. We also recognize that drugs known to lower insulin levels, such as metformin, would be predicted to have antineoplastic activity if this hypothesis is valid. Early population studies (4-5) are consistent with this possibility. I will describe recent studies with laboratory models that are also consistent with the hypothesis. These models suggest that the *in vivo* antineoplastic activity of metformin is restricted to hosts rendered insulin resistant by overfeeding; little activity was seen under control dietary conditions. The *in vivo* activity of the drug is correlated with reduction of insulin receptor activation in neoplastic tissue, suggesting reduction of insulin levels is a contributing mechanism, although the growth inhibition via AMPK activation described *in vitro* (6) may also play a role.

Taken together, the ongoing work is consistent with the possibility that excess insulin is a risk factor for poor cancer outcome. As hyperinsulinemia is common and is modifiable by lifestyle and drug therapy, further research is justified. The early results suggest that benefits of interventions in this area may be restricted to metabolically defined subsets of patients, a point which should be taken into account in the design of future intervention trials.

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Concurrent Session 9: Application of Mass Spectrometry to Detection and Prevention

CN09-02 Analysis of DNA lesion bypass by mass spectrometry. C. J. Rizzo. Vanderbilt University, Nashville, TN.

Formamidopyrimidine lesions can arise from either oxidative or alkylative damage to purines. We have developed chemistry for the site-specific incorporation of N6-(2-deoxy-D-erythropentofuranosyl)-2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine (MeFAPy-dG) into oligonucleotides. This lesion is derived from the initial N7-methylation of dG followed by hydroxide induced imidazole ring opening. The *in vitro* by-pass of the MeFAPy-dG lesion was examined by prokaryotic and human Y-family DNA polymerases; the by-pass products were characterized by tandem mass spectrometry. The MeFAPy-dG lesion was found to be highly miscoding.

Concurrent Session 11: Helicobacter pylori and Gastric Cancer

CN11-02 H. pylori eradication in the prevention of gastric cancer. B. Wong. University of Hong Kong, Pokfulam, Hong Kong.

Gastric cancer remains one of the top cancer killers in the world. Chronic *Helicobacter pylori* infection increases the risk of gastric cancer, by stepwise progression from chronic active gastritis to gastric atrophy, intestinal metaplasia, dysplasia and finally cancer. These stepwise progressions may take many years, and at present there is no proven effective treatment for the presence of premalignant lesions including intestinal metaplasia or dysplasia. Hence the two prevailing questions in gastric cancer prevention are (a) whether treatment of *H. pylori*-related gastritis can reduce the risk of gastric cancer, and (b) whether treatment of *H. pylori*-related IM or dysplasia can both reverse the premalignant lesions and reduce the risk of gastric cancer.

Our randomized placebo controlled trial in China started in year 1994 included both patients with *H. pylori* related gastritis and patients with *H. pylori* related premalignant lesions (1). After 7.5 years of follow up, patients receiving *H. pylori* treatment showed a non-significant trend of having less gastric cancer than those patients that received placebo. The sub-group analysis showed that patients with *H. pylori* related gastritis benefited most from treatment, with no cancer developing in 7.5 years. However, in patients with *H. pylori* related premalignant lesions, there was no difference in the risk of gastric cancer in both treatment and placebo groups. Hence our study suggests that the benefit of treating *H. pylori* in cancer prevention may be restricted to patients with gastritis only.

Correa et al performed another randomized placebo controlled trial in Columbia which included mainly patients with H pylori- related premalignant lesions (2). Their 12-year follow up result suggested that subjects who were H pylori negative after treatment had 14.8% more regression and 13.7% less progression than patients who were positive at 12 years ($p = 0.001$). Hence he concludes that it is beneficial to treat H pylori in patients with premalignant lesions. However the magnitude of benefit may be in the range of 15% only.

Based on these and other studies, the recommendation is that treatment of H pylori is beneficial in prevention of gastric cancer. The benefit is greater in patients without premalignant lesions. Hence treatment earlier in life may give better results.

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Concurrent Session 12: Pathway Discovery and Molecular Signatures for Cancer Risk

CN12-02 Pathway approaches to ovarian cancer risk discovery.

T. A. Sellers,¹ Y. Huang,¹ C. Phelan,¹ J. Schildkraut,² E. Goode³. ¹H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, ²Duke University, Durham, NC, ³Mayo Clinic, Rochester, MN.

It is generally accepted that for every type of cancer, there are at least two forms, one which is "hereditary" and one which is not. With regard to epithelial ovarian cancer, there are several genes that when inherited in mutated form are known to significantly increase risk. These genes, including BRCA1 and BRCA2, plus genes involved with the Lynch Syndrome, are rare. The general conclusion is that there exist additional genetic susceptibility factors that account for non-Mendelian familial risks that remain to be discovered. The prevailing hypothesis is that these will turn out to be common polymorphisms, each associated with low levels of increased risk.

Rapid advances in genotyping technology have greatly increased throughput capabilities at ever decreasing costs. In addition, collaborative efforts like the HapMap project have lead to the availability of large numbers of single nucleotide polymorphisms, some of which can serve as "tags" for haplotype blocks. Collectively, the ability to cover the genome is now feasible, either through genome wide association studies (GWAS) or more focused studies on particular pathways or networks. Both approaches are expected to increase our knowledge of the underlying biology of common cancers. We have formed a collaboration between several groups to apply both strategies in the study of epithelial ovarian cancer. This talk will focus on efforts considering specific pathways and networks.

The foundation for this work is based upon two large, ongoing case-control studies in North America. Cases had incident epithelial ovarian cancer ascertained at the Mayo Clinic in Rochester Minnesota or a 48-county region in North Carolina (Duke University). Cancer-free controls were frequency matched to the cases on age, race, and residence. After an interview to obtain data on risk factors, a sample of blood was collected for DNA. Among Caucasians, a total of 837 incident cases (388 at Mayo and 449 at Duke) and 941 controls were included in these analyses. Genes and pathways were identified through a number of sources including peer-reviewed published literature and the Cancer Genome Anatomy Project (CGAP) Biokarta and Kegg pathway databases. For each gene, chromosome and protein attributes were selected and the data mined from the Ensembl database version 34 (Biomart) using the gene reference sequence identification number (RefSeq ID) and the approved gene symbol from HUGO or Entrez Gene. The chromosomal location on build 35 and strand (forward or reverse) were provided to Illumina (San Diego, CA). Illumina verified chromosomal coordinates. We requested all

SNPs within each gene as well as up to 10kb in the 5' and 3' flanking regions and all non-synonymous SNPs with a MAF > 0.05 and Illumina Design Score > 0.6. The Illumina ADT (assay design tool) database includes all SNP data contained in the public domain, filtering out SNPs that are not suitable for the Illumina platform such as insertions/deletions, tri- and tetra-allelic SNPs, and SNPs that are not uniquely localized.

We excluded subjects and individual SNPs with genotype call rates <95%. All SNP genotypes among controls were tested against Hardy-Weinberg equilibrium. Data were analyzed considering individual SNPs under dominant, log additive, and recessive genetic models. Haplotype analyses were also performed. Selected results will be presented on the association of genetic polymorphisms in the glycosylation process and apoptosis with risk for ovarian cancer.

CN12-03 Unraveling the genetic etiology of mammographic breast density. C. M. Vachon,¹ V. Shane Pankratz,¹ T. A. Sellers². ¹Mayo Clinic College of Medicine, Rochester, MN, ²H. Lee Moffitt Cancer Center, Tampa, FL.

One of the most important risk factors for breast cancer identified to date is mammographic breast density. Breast density reflects variation in fat, stromal and epithelial tissues on the mammogram. Women with high levels of breast density have a 3-6 fold increased risk of breast cancer compared to women with little to no breast density. Increased breast density is common in the population, quantifiable and potentially modifiable but the biology underlying this trait is largely unknown. Risk factors identified for breast density to date only account for 20-30% of variation in the trait. Genetics and the interaction between genes and environment are hypothesized to account for the remaining variation. Evidence for a genetic influence on breast density to date comes from familial aggregation, family-based segregation, twin, molecular epidemiology and genetic linkage studies. These data together imply that genes for breast density may predispose women to high density across the lifespan, translating to increased breast cancer risk. The use of intermediate phenotypes such as breast density to identify genes for breast cancer may allow for increased power since the number of genetic and environmental factors influencing each intermediate marker is likely smaller than that influencing the clinical endpoint, namely breast cancer. As such, the variation attributed to genetic loci influencing breast density will likely be greater than for genetic loci influencing breast cancer risk.

This presentation will review the evidence for a genetic influence on breast density, discuss future genetic investigations and the importance of studying the genetic epidemiology of this marker of breast cancer risk.

CN12-04 CpG island methylator phenotype (CIMP) and colorectal cancer. M. L. Slattery. University of Utah, Salt Lake City, UT.

The methylation of CpG islands near promoter regions of genes and subsequent transcriptional silencing of these genes, has lead to the definition of a new molecular pathway for colon carcinogenesis. Using data from a large population-based study of colon cancer, the association of CIMP with other tumors mutations such as BRAF, microsatellite instability (MSI), Ki-ras, and p53 are presented. Several diet and lifestyle factors have been proposed as being associated with CIMP including dietary factors such as folate that may influence DNA methylation. Cigarette smoking appears to be most consistently associated with CIMP. Associations with dietary factors such as dietary folate as less consistently associated with CIMP in colon cancer. Diet and lifestyle factors associated with CIMP are reviewed to assess possible contributors to the CIMP pathway. Additionally, associations with germline polymorphisms in genes that may be involved in the CIMP pathway are presented.

Concurrent Session 13: Biological versus Social Constructs of Health Disparities

CN13-01 Moving toward the elimination of cancer health disparities. Judith S. Kaur, Mayo Clinic, Rochester, MN.

This review will provide: 1) A historical perspective on cancer patterns identifying excess morbidity and mortality in underserved populations will be presented, starting with the initial SEER black vs white mortality data and ending with the recent Annual Report to the Nation with a special report on American Indian and Alaska Native cancer patterns; 2) NCI's approach to disparities research through the Community Networks Programs utilizing community based participatory research; 3) Annual Report to the Nation as a blueprint for research needs; and 4) Future needs and scientific questions to be answered

Conclusions: Only recently has disparities research become an area of scientific specialization. Resources for high quality research on basic science research defining the biologic basis of disease, community interventions and new screening techniques will be essential to meet national goals to reduce morbidity and mortality from cancer in all populations.

CN13-02 Genetics and prostate cancer risk. R. A. Kittles. University of Chicago, Chicago, IL.

Epidemiological data reveal that African-American men have the highest incidence and mortality rates for prostate cancer in the world. Prostate cancer, like many other common diseases likely has environmental components to their risk and thus results from currently unknown interactions between environmental factors and underlying genotypes. However, the observed population differences in prostate cancer incidence and mortality can not be explained completely by differences in access to and quality of health care, diet or other lifestyle characteristics. Whether differences in the distribution of known or undefined genetic risk factors among different populations explain the disparity is unknown. What is known is that many prostate cancer candidate genes exhibit large differences in allele frequencies across different populations. The significance of these patterns has yet to be fully understood. Many scientists are now exploring genetic ancestry in order to help distinguish between the genetic and environmental factors which contribute to population differences in disease risk.

CN13-04 Developing novel approaches to breast cancer: Are we there yet? O. I. Olopade. University of Chicago Medical Center, Chicago, IL.

Breast cancer is a genetically and clinically heterogeneous disease. Whether different target cells contribute to this heterogeneity in different populations, and which cell types are most susceptible to oncogenesis is still not well understood. In addition, recent DNA and tissue microarray analyses have identified basal-like breast tumor subtype and shown that these tumors portend a poor prognosis. Although geographically separated, there are strong similarities in the clinico-pathological features of early onset breast cancer in African American women as well as women in countries with low breast cancer incidence. Globally, early onset breast cancer is frequently associated with high nuclear atypia, high S-phase fractions, poor histological differentiation, and predominantly estrogen and progesterone receptor negative tumors. The clinical outcome of early onset breast cancer in women of African ancestry has been uniformly poor partly because they are non-screen detected and/or the inadequate treatment of basal-like or triple negative breast cancer. Nonetheless, the aggressive biologic behavior of breast cancer in young women may be due to previously unrecognized genetic risk factors. In ongoing work, we are examining the frequency and mutational spectrum of BRCA1 and BRCA2 germline mutations in a large cohort of women in the US as well as in Africa and also exploring BRCA1 pathways as a mechanism to examine disparities in breast cancer outcomes. Translating this research into clinical practice and prevention can be challenging as will be discussed during this session.

Concurrent Session 14: Colon Cancer Prevention: Current Clinical Applications

CN14-02 The methods of recognizing and assigning familial risk.

R. W. Burt. University of Utah, Huntsman Cancer Institute, Salt Lake City, UT. Familial risk is common in colorectal cancer (1). Almost one third of cases exhibit familial risk, probably from inheritance, as part of their pathogenesis (2). In a large portion of these genetic-environmental factors are likely important. About 10% of persons in the general population have a first-degree relative with colon cancer, while 20% of those with colorectal cancer have an affected first-degree relative.

Approximately 3% to 5% of colorectal cancer cases occur in the setting of one of the known inherited syndromes of colon cancer. The genes mutated in these conditions are known and genetic testing is available for clinical diagnosis. Both common and syndromic categories will be covered in this presentation.

Common familial risk. Common familial risk is determined by assessing which relatives have colon cancer as well as the age of cancer occurrence and the degree of relationship of each relative (1, 3). The population risk for colon cancer in the U.S. is 6%. Having a first-degree relative with colon cancer >50 years increases that risk two- to three-fold. An affected second-degree relative or third-degree relative increases the risk about 50%. If a person has a first-degree relative with an adenomatous polyp, particularly a high risk polyp (≥ 1 cm, villous histology or advanced dysplasia), the risk of colon cancer approximately doubles. A person with a first-degree relative with colon cancer at an age ≤ 50 years, or two or more affected first-degree relatives has a three- to four-fold increased risk (1).

Screening for persons with an affected first-degree relative >50 years should be the same as for average risk persons, but should start at age 40 years or ten years earlier than the earliest diagnosis in the family and be repeated every 5 years. In the higher risk setting, as outlined above, colonoscopy should be used, and should begin at age 40 or ten years younger than the earliest case in the family. An extended family history should also be done to see if one of the inherited syndromes should be considered.

Inherited syndromes of colorectal cancer familial adenomatous polyposis (FAP). FAP is autosomal dominant, arises from mutations of the APC gene and occurs in about 1 in 10,000 individuals. The phenotype is that of hundreds to thousands of colorectal adenomatous polyps with an average age of appearance of 16 years, and a virtually 100% risk of colon cancer without colectomy. The average age of colon cancer diagnosis is 39 years (4). Polyps also occur in the stomach, duodenum and small bowel, with a 5% lifetime risk of peri-ampullary cancer. Subtypes of FAP also arise from mutations of the APC gene and include Gardner syndrome, in which osteomas and benign soft tissue tumors occur in addition to the GI phenotype, attenuated FAP that averages 30 colonic polyps which appear about 10 years later than in typical FAP (5), and Turcot syndrome, that includes CNS tumors.

FAP or attenuated FAP should be considered in any person who develops 10 to 20 or more adenomatous polyps or in all close relatives of an affected individual. Deleterious APC mutations are found in 70% to 90% of persons with the typical clinical phenotype (6). Once the disease causing mutation is found in the index case, relatives can be tested for the presence or absence of that mutation with virtually 100% accuracy. Screening includes colon examination beginning at age 10 to 12 years in those who are found to have the mutated gene or in at risk persons where genetic testing is not possible or is uninformative. An appropriately timed colectomy is planned once polyps occur, but can often wait until after high school. Screening can be delayed until the late teens in families with attenuated FAP and surgery may not always be necessary.

MYH associated polyposis (MAP). MAP is an autosomal recessive disease, arising from biallelic mutations of the MYH gene (7). It is characterized by multiple colonic adenomas, usually less than 100, and an increased risk of colon cancer. MAP has been found in 5% to 40% of persons with 15 to 100 adenomas, and in approximately 1% of colon

cancer cases. It should be suspected when multiple adenomas occur in a recessive pattern or an APC gene mutation could not be found in a polyposis patient. Genetic testing is done looking for biallelic MYH gene mutations. Colonoscopy screening should be done every one to three years, probably beginning in the mid 20's. Colectomy is sometimes necessary.

Hereditary nonpolyposis colorectal cancer (HNPCC), or lynch syndrome. HNPCC is caused by autosomal dominant inheritance of mutations of any one of the four mismatch repair genes, MLH1, MSH2, MSH6 and PMS2 (8). It may account for up to 5% of colon cancer cases. HNPCC is clinically defined by the Amsterdam I criteria (Am), which are: 1) three relatives with colorectal cancer, two of them being first-degree relative of the third; 2) at least two affected generations; and, 3) at least one case having a diagnosis at an age <50 years. The average age of colon cancer diagnosis is 45 years, but may be older, and frequent synchronous and metachronous cancers are found. Adenomatous polyps precede the cancer, but only one or several are usually found. Other cancers that occur as part of the syndrome include uterine (40%), and 5% to 10% ovarian, gastric, biliary, renal, CNS, ureter and renal pelvis, and duodenum and small bowel. Amsterdam II criteria are similar to those listed above, but include colon or any of these other cancers. Am II criteria are more sensitive but less specific than Am I.

Germline genetic testing for mismatch repair gene mutations should be done on an index case, preferably the youngest colon cancer case in the family if the Am I or II criteria are met (6). DNA is usually obtained from lymphocytes through peripheral blood sampling. As this approach misses about 50% of cases, Bethesda guidelines should be applied to families with a strong family history of colon cancer who do not meet Am I or II (6). If one of the guidelines are positive, colon cancer tissue should undergo microsatellite instability (MSI) or immunohistochemistry (IHC) testing, both of which indicate mutation of one of the mismatch repair genes (6). If MSI or IHC is positive, then germline genetic testing should be done. Disease causing mutations are found in 50% to 70% of people meeting Am I or II, and 50% of those meeting Bethesda guidelines whose tumor is found to be MSI or IHC positive.

Another approach to finding HNPCC patients and families is to perform IHC testing on all colon cancers and then genetic testing if IHC is positive and then genetic testing if positive. Whether this approach is sufficiently cost and disease efficient remains to be determined.

Patients with HNPCC, determined either clinically or genetically should undergo colonoscopy beginning at age 25 years, or ten years younger than the earliest case in the family, whichever comes first. This should be repeated every 2 years. Screening for certain other tumors is also indicated (8). High risk families where the tumor MSI testing is negative, do not have HNPCC, and should have colonoscopy as outlined above under common familial risk (9).

Hamartomatous polyposis syndromes. These include Peutz-Jeghers syndrome (PJS), juvenile polyposis (JP) and Cowden's syndrome (CS), arising respectively from the STK11 gene; the BMPRIA, SMAD4, or ENG genes; and, the PTEN gene (10). All are extremely rare but all exhibit varying risks for colon and other cancers. PJS should be suspected anytime histologically characteristic polyps are found in the GI tract, JP when three or more juvenile polyps are found, and CS when any of the characteristic findings of this disease are present. Germline genetic testing is available for each of these diseases. It is advised that any person with one of these conditions should have at least one consultation at a center that deals with inherited colon cancer syndromes.

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CN14-03 The role of colonoscopy and polypectomy in the prevention of colorectal cancer. S. J. Winawer. Memorial Sloan-Kettering Cancer Center, New York, NY.

Adenomas are the most common neoplastic outcome in all screening modalities; they are far more common than cancer. Removal of these adenomas has been shown to reduce the incidence and mortality of colorectal cancer. In the National Polyp Study, patients were enrolled after one or more adenomas were removed and randomized into a more aggressive and less aggressive followup surveillance. The cohort was pooled and the number of cancers detected were 5 as compared to more than 40 in 2 reference groups that were adenoma bearing and more than 20 in the SEER database, showing a reduction in incidence of between 76 and 90% in the 1418 patients followed for 8401 person years of followup. These results supported the long standing belief in the adenoma-carcinoma sequence and the practice of removing adenomas to prevent colorectal cancer. Subsequent studies confirmed this finding. An Italian study of post polypectomy patients showed an incidence reduction of 68% and a U.K sigmoidoscopy study also showed a reduction in incidence of rectal cancer following rectal polypectomy. A Norwegian randomized study demonstrated an 80% reduction in incidence in people having polyps detected on sigmoidoscopy and removed by colonoscopy. Guidelines have been reported that recommend appropriate followup intervals following polypectomy based on risk stratification at baseline. However, colonoscopy is not a perfect examination. Interval cancers have been reported that range from less than 1% to more than 5%. The reasons for these interval cancers include; technological limitations of the instrument, the manner in which the procedure is performed, and biological factors such as cancers with rapid appearance. In one study, the proportion of MSI positivity was 30% in interval cancers as compared to 10% in non-interval cancers. The "fast track" cancers seen in HNPCC are considered now to occur in sporadic cancers without this strong family history. Aspects of the technique that impact on the finding of interval cancers include not reaching the cecum, poor preparation and a fast withdrawal time. A recent paper demonstrated that endoscopists who have a mean withdrawal time of less than 6 minutes tend to find fewer adenomas. Technological aspects of the colonoscope are being improved to increase its accuracy including: greater magnification, NBI, wider angle of vision, third eye retro-scope, and other modifications. The demonstration of prevention of colon cancer by colonoscopic polypectomy has been based on the use of mathematical analyses and modeling. This has shown not only a reduction in incidence of cancer, but also a reduction of mortality over a period of 20 years. These reductions appear to be the effect of the baseline colonoscopy with little additional effect of the followup surveillance until after 10 years following polypectomy.

Unfortunately there have been no prospective randomized trials of screening colonoscopy. There are several screening tests that if positive

lead to colonoscopy. However most of the tests are much more sensitive for cancer than for polyps. Direct screening colonoscopy is the test most likely to find adenomas and thus have the greatest impact on reducing colorectal cancer incidence and thus prevent the cancer entirely. Virtual colonoscopy has been shown to be equivalent to optical colonoscopy for the detection of polyps 6mm or larger. The miss rate for smaller polyps is substantial but the significance of this miss rate needs to be determined. Colonoscopy also has a miss rate for adenomas as well as for cancers, the former estimated as being 88-98%. The major problem today is the low screening rates for colorectal cancer, about 40-50% as compared to the higher rates for mammography which is in the range of 70-80%. It is not clear whether direct screening of the population with colonoscopy is feasible in this country. A two stage approach using other tests first is another option. Each country and community must decide on the best option based on resources, patient population, and other priorities. However, it is clear that any test will reduce the risk of dying from colon cancer. Any test is better than none. The best test is the one that gets done.

Concurrent Session 15: Melanoma and Non-melanoma Skin Cancer

CN15-01 New approaches to the prevention of cutaneous melanoma by regulation of redox. F. L. Meyskens, Jr., S. Yang, P. Farmer, Departments of Medicine (Hematology/Oncology), Biological Chemistry and Chemistry and the Chao Family Comprehensive Cancer Center, University of California, Irvine, Orange, CA.

Our studies of the role of reactive oxygen species (ROS) and transcriptional responses to redox stress in the pathogenesis of cutaneous of melanoma has led us to reconsider the role of ultraviolet light (UVL), to explore the role of metals in the pathogenic process and the possibility of using chelators to inhibit this process, and the role of Ref-1 as a key protein redox regulator of many transcription factors. A systematic exploration of the role of melanin and melanosomes in melanocytes and melanoma cells using electroparamagnetic resonance and other physical techniques has suggested that melanin is converted from an anti-oxidant to a pro-oxidant early in the pathogenic process which leads to melanosomal disruption that itself serves as an ongoing nidus for ROS generation. The involvement of redox-active metals is critical to this process and in conjunction with UVL leads to phenotypic changes in normal human melanocytes that resemble dysplastic nevi melanocytes in culture. A re-examination of the epigenetic risk factors for melanoma has identified a large (and neglected) epidemiology that implicates certain heavy metals and includes: markedly increased risks for melanoma in printers/lithographers (4 large studies), electrical industry workers (2 large studies), and hip replacements patients (2 large cohort studies and a meta analyses involving over one million years of follow-up). Clinical observations (imaging studies) and studies that document the prognostic strength metallothionein expression in primary melanoma offers ancillary support to a probable contribution of heavy metals to the pathogenesis of melanoma. Based on these and other considerations and the role of copper in the melanin synthesis pathway and the binding characteristics of metals to melanin we propose that the most likely culprits that accounts for a significant contribution to the etiology and pathogenesis of melanoma are Fe, +2, Fe+3, Cu+2 and Cr+6 but this remains to be definitively established. We are developing a chelator-based strategy to obviate the effect of heavy metals in this process and will review current results as well as our overall strategy. We are also developing new inhibitors of Ref-1, a major transcriptional regulator of the redox response in melanoma that likely plays a critical role in transformation. Our recent results will be reviewed.

CN15-02 Regulation of cyclic AMP as a prevention strategy for melanoma. J. D'Orazio. University of Kentucky College of Medicine, Lexington, KY.

Melanoma occurs mainly in fair-skinned individuals who burn rather than tan after ultraviolet (UV) exposure. This phenotype correlates with dysfunction in the melanocortin-1 receptor (Mc1r), the receptor on melanocytes for melanocyte stimulating hormone. When the activity of this Gs-protein coupled receptor is sub-optimal, there is muted cytoplasmic cAMP signaling and the melanocyte produces pheomelanin, a sulfated lighter pigment with poor UV-blocking ability, rather than eumelanin, the brown-black melanin polymer highly protective against UV injury. Our goal is to develop novel UV-protective strategies by pharmacologically rescuing eumelanin production in fair-skinned individuals who, because of Mc1r loss-of-function polymorphisms, cannot melanize effectively. Using a transgenic murine model of "humanized skin" of variant pigment composition, we show that UV damage in the skin (including carcinogenesis) is much greater in pheomelanotic animals than in eumelanotic animals. Furthermore, we find that topical application of forskolin, a direct activator of adenylyl cyclase, by-passes defective Mc1r signaling in pheomelanotic mice and rescues robust eumelanin production in the skin. Importantly, this pigment accumulation is highly protective against both acute and chronic UV injury. Pharmacologic eumelanization is well-tolerated by the animals over several months, and does not induce damage response signals in the skin in contrast to natural tanning responses after UV exposure. Forskolin-induced eumelanin persists as long as the drug is applied daily to the surface of the skin (now tested for four months) and is reversible upon discontinuation of topical treatments. Melanin deposition after forskolin application mimics the natural nuclear capping pattern found in UV-exposed skin of darker pigment phenotype, and induces thickening of the epidermis much like natural UV-induced tanning responses. Manipulating natural melanin pigments by small molecules (sunless tanning) holds great potential for being an important novel UV-protective strategy with particular benefit to those populations most at risk of melanoma and other skin cancers.

CN15-03 Molecular mechanisms of UV-induced non-melanoma skin cancer and prevention approaches. Z. Dong, University of Minnesota, Austin, MN.

Skin cancer is the most frequently diagnosed cancers in the U.S. Skin carcinogenesis is a multistage process consisting of initiation, promotion, and progression stages and each stage may be a possible target for chemopreventive agents. A significant outcome of these investigations on the elucidation of molecular and cellular mechanisms is the explication of signal transduction pathways induced by tumor promoters in cancer development. The current belief is that cancer may be prevented or treated by targeting specific cancer genes, signaling proteins, and transcription factors. The molecular mechanisms explaining how normal cells undergo neoplastic transformation induced by tumor promoters are rapidly being clarified. Accumulating research evidence suggests that many dietary factors, including tea compounds, may be used alone or in combination with traditional chemotherapeutic agents to prevent or treat cancer. The potential advantage of many natural or dietary compounds seems to focus on their potent anticancer activity combined with low toxicity and very few adverse side effects. I will review some of our recent work regarding the effects of the various cancer preventing agents on signal transduction pathways involved in neoplastic cell transformation and carcinogenesis.

Concurrent Session 17: Progression and Prevention of Barrett's Esophagus

CN17-01 Clonal evolution in Barrett's esophagus. C. C. Maley. The Wistar Institute, Philadelphia, PA.

Barrett's esophagus (BE) is both a clinically important intraepithelial neoplasm and one of the best models for studying neoplastic progression in human solid tumors. Because of the morbidity and mortality associated with esophagectomies, and the fact that only approximately 0.5% of BE patients progress to esophageal adenocarcinoma (EA) per year, the current standard of care includes endoscopic biopsy surveillance for the early detection of cancer. In addition to these longitudinal biopsies, it is common practice to take multiple biopsies per time point. The result is a unique opportunity to study the genetics and epigenetics of neoplastic progression over both time and space as well as intervene in those processes. Thus, we have devoted an entire session to the discussion of recent results from leaders in the field of Barrett's esophagus.

There are a variety of open problems in Barrett's esophagus. The origin of BE has been a long standing problem that Rebecca Fitzgerald has started to solve. Chronic gastroesophageal reflux disease (GERD) is known to predispose to the development of BE, and 10% of patients with GERD have BE when first endoscoped. However, it has been practically difficult to study the transition from GERD to BE, for there are no GERD cohorts with longitudinal endoscopic biopsies. Dr. Fitzgerald and her team have been able to keep both esophageal squamous and Barrett's esophagus biopsies alive long enough *ex vivo*, to be able to study the effects of exposures on those biopsies and discovered an important role of retinoic acid in their transformation.

Brian Reid and his team have recently conducted the first genome wide combined epigenetic and genetic assays of Barrett's epithelium and varying stages of progression. Little is known about the epigenetic modifications in Barrett's neoplastic progression, though given the frequency of epigenetic lesions and the evidence that it is often involved in silencing p16 (CDKN2A/INK4a), epigenetic alterations are likely to be important events throughout Barrett's neoplastic progression. Because BE takes so long to evolve into EA, it not only offers a model for studying neoplastic progression, but also a long window in which we can intervene so as to delay progression. Kenneth Wang is a world leader in the treatment of BE and the prevention of EA. He will report on recent advances in the treatment of BE at the Mayo clinic.

Thus, the speakers for this session will cover Barrett's neoplastic progression from initiation through treatment. I will set the context to help communicate the importance of the advances of the speakers in this session. I will also present some of our own recent work on the first longitudinal analyses of clonal evolution in a solid tumor, including fixation events, in which a clone expands to fill the entire neoplasm, the results of competition between Barrett's clones, and changes in the genetic diversity of the neoplasms over time. I will also discuss relationships between the evolutionary dynamics of these clones and exposures in their microenvironment associated with obesity, smoking behavior, non-steroidal anti-inflammatory drug use, age and sex.

CN17-03 The origin and prevention of Barrett's esophagus. R. Fitzgerald. Hutchison/MRC Research Center, Cambridge, United Kingdom.

The development of Barrett's esophagus is poorly understood at a cellular and molecular level. With the advent of endoscopic ablation therapies it is becoming increasingly possible to reverse the columnar epithelium, but in order to achieve long-term epithelial stability a better understanding of the pathogenesis of Barrett's esophagus is required. Key factors requiring elucidation with regards to the development of Barrett's esophagus include the cell of origin, an understanding of the transcription factors governing the cell fate of these cells and the trigger(s) for their commitment to a columnar state.

Cell of origin. The most likely cells of origin for the columnar esophageal epithelium are either direct conversion of differentiated cells in the absence of cell proliferation, so-called "transdifferentiation" or the conversion of a "stem" or "pluripotential cell," meaning a cell with the capacity for unlimited or prolonged self-renewal.

Transdifferentiation occurs as part of normal esophageal development with a columnar to squamous cell transition at 18 weeks gestation. Tosh and colleagues have shown that in the developing mouse esophagus, cells in the squamous basal layer arise directly from columnar tissue independent of squamous cell proliferation or apoptosis of columnar cells. A reverse transdifferentiation process could occur in the adult in the context of chronic reflux exposure.

If Barrett's instead originates from "stem" or "pluripotential" cells then there are several possible tissue locations for these cells. Some evidence exists for asymmetrically dividing stem cells arising in the inter-basal layer of the epithelium between the papillae. Alternatively it is possible that they are located in the glandular neck region of esophageal submucosal gland ducts analogous to those stem cells which reside within the bulge region of the hair follicle. Hence, it is possible that after ulceration or damage, stem cells grow out to form a new gland within the lamina propria, finally giving rise to a duct by which the glandular cells are carried to the surface. Our understanding of tissue specific stem cells is increasing rapidly. In the mammalian squamous epidermis instead of the stem cells giving rise to transit amplifying cells prior to differentiation tissue maintenance it has recently been suggested by Jones et al. that tissue maintenance may instead depend on a single population of proliferating cells. These observations may be germane to our understanding of differentiation in the squamous esophagus.

Transcriptional control of cell fate. The control of cell fate is likely to be achieved by a combination of internal cell signals, such as nuclear factors controlling gene expression, and external signals such as secreted factors, cell-cell adhesion molecules and extracellular matrix proteins. Interestingly activation of a single transcription factor can be sufficient to alter cell fate as shown by experimental evidence for conversion of pancreas to liver cells. Relevant transcription factors in the esophagus include p63 and homeobox genes.

p63 is a homolog of the tumor suppressor p53 and is normally absent in simple, columnar epithelia but expressed in the basal layer of squamous epithelia. Recently it has been shown that p63 is essential during normal esophageal development. When mice were produced which had been engineered without the p63 gene they developed highly ordered, columnar ciliated esophageal epithelium.

The homeobox or HOX family of genes are developmental transcription factors. Various evidence suggests that Cdx2 is a "master switch" gene whose normal expression determines the proximal and distal specialization of the gut in embryogenesis. In adulthood Cdx1/2 protein is predominantly expressed in the small intestine and colon but not in the stomach or esophagus. Transgenic mice over-expressing Cdx2 develop intestinal metaplasia in the gastric epithelium, and conversely loss of Cdx2 leads to the development of polyp like lesions in the intestine containing areas of squamous epithelium flanked by heterotopic gastric epithelium. In Barrett's metaplasia Cdx2 expression is observed in areas of specialized intestinal metaplasia and in a recent rat model Pera et al. demonstrated that chronic reflux induced glandular metaplasia coincident with nuclear Cdx2 expression. Epigenetic regulation of Cdx2 may be important as shown by data from Liu et al in which CDX2 was silenced in the squamous esophageal cell line HET1A via promoter CpG island methylation. Cdx2 was re-expressed upon DAC (5-aza-2'deoxyctidine) treatment, which was also reported to induce a crypt-like growth pattern *in vitro* in the same cell line.

It is relevant that Cdx1/2 may be activated by components of refluxate and inflammatory cytokines via NFκB. Chronic acid exposure has been shown to induce Cdx2 expression in primary mouse squamous esophageal cells and secondary bile exposure has also been shown to up-regulate it's expression in cell lines. This coupled with the observation that Cdx2 mRNA is expressed in esophagitis prior to the induction of Cdx1 or other intestine-specific genes, offers a possible link between gastro-esophageal reflux exposure and the molecular basis for metaplasia.

Experimental model for development of Barrett's. In embryogenesis retinoic acid is a powerful inducer of differentiation and mediator of the antero-posterior patterning of the gut via a number of cell

signalling pathways including Cdx2. We recently used an in vitro system to show that all-trans retinoic acid could induce columnar differentiation independent of proliferation using morphological and molecular criteria. Interestingly, in our model metaplasia could be induced from the stromal compartment alone with co-localization of cytokeratin and mesenchymal markers at an early time point. This observation could be explained by an origin from submucosal gland ducts but also raises the intriguing possibility of mesenchymal to epithelial transition. Mesenchymal to epithelial transition is a well described mechanism for RA-induced glandular differentiation and is well described within normal embryonic development. Previous work on the cell signalling pathways involved in RA induced columnar differentiation suggest that the first step in the mesenchymal to epithelial transition involves cell migration, followed by differentiation and cell cycle arrest; a process called condensation. In our culture system we observed the appearance of glands apparently fusing with the squamous epithelial surface which can also be observed in native Barrett's glandular tissues. We therefore proposed a hypothetical model for the development of Barrett's metaplasia from the stromal compartment in which an environmental stimulus leads to part of the squamous epithelium being sloughed. Glands then migrate and fuse with the breached surface. The glands may arise from migration of submucosal glands or from new gland formation secondary to mesenchymal-epithelial transition. As these glands unfold a new epithelial surface would be formed.

Stromal environment. Whether or not this proposed model is correct, the stromal compartment is likely to be important in the determination of cell fate. Conversion of embryonic stem cells to columnar or squamous epithelia has been shown to depend on the components of the extracellular matrix and in order to achieve asymmetrical stem cell divisions of the esophageal epithelium, it is necessary to reconstitute the esophageal squamous keratinocytes on denuded connective tissue containing esophageal laminin-2. As well as the extracellular matrix the supporting esophageal stroma is also infiltrated with inflammatory cells leading to an environment rich in cytokines and growth factors. Barrett's esophagus has impaired signalling through the TGF β cascade due to a decrease in the expression of the signalling components T β RII, Smad2 and Smad4. Targeted loss of TGF β signalling components in mice has revealed that this pathway has an important role in the development of various organs and tissues such as heart, bone and vasculature. In addition, Thrombospondin-1 knockout mice express low levels of active TGF β and have altered cell differentiation in their distal esophagus, amounting to focal areas of columnar epithelium. A reduction in TGF β signal propagation could decrease the expression of factors necessary for maintaining squamous differentiation, resulting in the formation of focal areas of Barrett's esophagus.

Reversal of Barrett's esophagus. Although it is possible to reverse Barrett's esophagus endoscopically it has not yet been possible to achieve this through the manipulation of specific cell signalling pathways. To determine whether inhibition of retinoic acid activity could reverse the columnar phenotype we extended our culture model to a retinoic acid inhibitor citral. In this system Barrett's explants had evidence of a sloughed villiform surface with remnant glandular structures overlain by squamous epithelium after 48 hours. The immunohistochemical characteristics resembled native squamous esophagus with expression of CK13 and CK14, but not CK8/18. Interestingly CK 7 which is usually expressed in the glandular mucosa, was selectively expressed in the superficial layers of the neo-squamous epithelium and these changes occurred independent of proliferation. These data are proof of concept for reversal of columnar differentiation via pharmacological manipulation of cell signaling pathways. The elucidation of the key pathways involved in the lineage specific differentiation pathways will be important for the development of novel therapeutic strategies in Barrett's esophagus.

Concurrent Session 18: Basic and Translational Advances in Lung Carcinogenesis and Chemoprevention

CN18-01 Examining the role of stem cell biology in lung cancer. C. F. Kim. Children's Hospital, Boston, MA.

It has been demonstrated that some types of tumors (e.g. breast, brain, and lymphoid tumors) harbor a cancer stem cell (CSC) population that is required to maintain malignancy and which may harbor resistance to current therapeutic strategies. However, it is unknown whether lung cancers similarly harbor a cancer stem cell population. The identification and characterization of Bronchioalveolar Stem Cells (BASCs), putative resident stem cells of the distal lung, has suggested that these cells are important in normal lung homeostasis, lung tumor initiation and cancer progression. In mouse models of lung adenocarcinomas, BASCs undergo expansion after oncogene activation and/or tumor suppressor loss, and BASC-like cells persist within the developed tumors. We hypothesize that BASC-like cells within these lesions play a critical role in tumor maintenance. To address this question, we have developed an assay to test for lung CSCs. Orthotopic transplantations of primary murine lung adenocarcinoma cells via intratracheal injections into nude mice yield secondary tumors that recapitulate the features of the primary lung tumors. Transplantation of specific cell subsets from primary tumors into secondary hosts is currently being performed for comparison of their capacity for tumorigenesis. Our current data suggest that the BASC-like cells and the remaining tumor cells, once present within an established tumor, are equally sufficient to function as lung cancer stem cells. Further work will be needed to identify markers that will be useful for isolating a lung cancer stem cell population.

Concurrent Session 21: Issues in the Use of Intermediate Endpoints

CN21-03 Targeted, label-free proteomic analysis of urine in a rat bladder cancer model. R.R. Townsend¹, Y. Liu², Y. Yi², H. Rohrs³, C. J. Grubbs⁴, R. A. Lubet⁵, and M. You². Departments of ¹Medicine, ²Surgery, and ³Chemistry, Washington University School of Medicine, St. Louis, MO 63110, ⁴Departments of Surgery, Genetics and Medicine, University of Alabama at Birmingham, Birmingham, AL, and ⁵Division of Cancer Prevention, National Cancer Institute, Bethesda, MD.

We have used a label-free quantitative nano-LC mass spectrometric method to perform comparative proteomics analysis on the urine from control and rats bearing 4-hydroxybutyl(butyl)nitrosamine induced bladder tumors. In this study the urinary proteins from control animals (n = 10) and from those with palpable bladder tumors (n = 10) were digested sequentially with endoprotease Lys C and trypsin after reduction and alkylation. The peptides were analyzed using nano-reversed phase-LC-linear quadrupole Fourier transform ion cyclotron mass spectrometry (FT-MS) by acquiring parent mass scans over a 2 h linear gradient of acetonitrile for each of the 20 samples. The mass spectra from the control and tumor-bearing groups were aligned and the individual accurate mass signals were quantified using Rosetta Elucidator™ software. The isotope groups with statistically significant different areas (P < 0.01) between the two groups were used to produce the accurate m/z values for directed acquisition of tandem mass spectra to sequence the peptide and identify the protein differences between the two groups. Among the proteins that were found to be increased in the tumor bearing urine (after excluding the hepatic-derived and well-recognized blood proteins e.g. albumin, hemopexin, hemoglobin) were meprin, E-cadherin, hyaluronan receptor, glycam, retinoblastoma binding protein.

CN21-04 Effects of combination chemoprevention on markers of cell turnover and agent action in colorectal mucosal tissue from patients in a Phase III trial of difluoromethylornithine and sulindac for prevention of colon polyp recurrence. E. W. Gerner,¹

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Ornithine decarboxylase (ODC) is the first enzyme in polyamine synthesis and is a target of the MYC gene. MYC is regulated by the adenomatous polyposis coli (APC) tumor suppressor gene in intestinal and colonic epithelial cells in humans and rodents (1). Non-steroidal anti-inflammatory drugs (NSAIDs) influence arachidonic acid metabolism in part by inhibiting the activities of cyclooxygenase 1 (COX1) and/or cyclooxygenase 2 (COX2), but NSAIDs also activate polyamine catabolism and export via COX-independent mechanisms (2, 3).

Difluoromethylornithine (DFMO) is an irreversible inhibitor of ODC, which has been developed as a cancer chemopreventive agent (4). DFMO or any of several NSAIDs alone reduces colon carcinogenesis in rodent models, and the combination of DFMO and NSAIDs acts at least synergistically in both rodent and human cell models of colonic neoplasia (1). A polymorphism affecting ODC expression has been associated with risk of polyp recurrence in humans with prior polyps, especially in individuals taking aspirin (5, 6), which further supports the rationale for combination chemoprevention with agents acting to suppress polyamine synthesis and/or activate polyamine catabolism and export.

A Phase III trial of combination DFMO (500 mg/day) and the NSAID sulindac (150 mg/day) versus placebo for three years for prevention of colon polyps was conducted in patients with prior sporadic polyps. Three hundred and seventy-five patients were randomized and stratified for aspirin usage in the trial. The treatment dramatically reduced the recurrence of total and advanced polyps, independent of aspirin. Treatment was associated with a statistically significant decrease in polyamine contents in apparently normal colorectal mucosa. Aspirin use was associated with a decrease in levels of prostaglandin E2 (PGE2) in normal colorectal biopsies at baseline. However, treatment with DFMO and sulindac was not associated a statistically significant further decrease in colorectal mucosal PGE2 in this trial. Measures of apoptosis and proliferation in apparently normal colorectal mucosa were unaffected by the treatment.

These data indicate that colorectal mucosal polyamine contents are markers of treatment effects mediated by the combination of DFMO and sulindac. Colorectal mucosal PGE2 levels, however, were not associated with treatment in this study. Measures of cell turnover (proliferation and apoptosis) in apparently normal colonic mucosa were not associated with treatment and its effects on polyp recurrence. The measures of colorectal biopsy polyamine and PGE2 levels support the hypothesis that combination DFMO and sulindac are acting to reduce tissue levels of the polyamines in a manner associated with a dramatic reduction in recurrence of neoplastic colon polyps.

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Controversy Session 1: SERMS versus Aromatase Inhibitors

CS01-02 Prevention of breast cancer using aromatase inhibitors.

J. Cuzick. Cancer Research UK Centre for Epidemiology, Mathematics and Statistics, Wolfson Institute of Preventive Medicine, London, United Kingdom.

Eight adjuvant trials have reported on the use of aromatase inhibitors for early breast cancer. In addition to benefits in reducing recurrence rates, all of them show a marked reduction in contralateral tumors compared to tamoxifen of about 50%. This, combined with the approximately 50% reduction in new tumors seen with tamoxifen, both in prevention trials, and for contralateral tumors in adjuvant studies, suggest aromatase inhibitors may be able to reduce the rate of ER positive breast cancer by as much as 75%. These drugs are also better tolerated and have fewer side effects than tamoxifen, suggesting they are very promising agents for breast cancer prevention. In particular they do not lead to the gynecologic side effects (including endometrial cancer) or thromboembolic complications associated with tamoxifen. However they are associated with increased rates if arthralgia and other joint symptoms, and decreased bone mineral density leading to increased fracture rates if not properly managed. These data will be reviewed the implications for ongoing chemoprevention trials will be discussed.

In particular these very promising results in the adjuvant setting have provided a strong rationale for using these agents in trying to prevent breast cancer. The IBIS II chemoprevention trial has been set up to compare the aromatase inhibitor anastrozole against a placebo in 6,000 high risk post-menopausal women, and against tamoxifen in 4000 post-menopausal women with completely locally excised oestrogen receptor positive DCIS. Both high efficacy and a low side effect profile are essential for a chemopreventive agent, and this trial is designed to address both of these issues. In particular, a detailed bone substudy in 1000 women is built into the trial, and will evaluate the use of the bisphosphonate risedronate on a randomized basis in osteopenic women. The rationale for, and design of, the trial will be described, and current recruitment status will be reported.

Educational Session 1: Carcinogens Cause Cancer: Recent Advances

ED01-02 Chemical carcinogens in the -omics era. J. D. Groopman. Johns Hopkins University, Baltimore, MD.

The exploration of the role of chemical carcinogens in the etiology and progression of human cancers has progressed enormously with the rapid discovery of the underlying biological mechanisms of cancer. Since it is becoming more apparent that each individual's cancer is unique, the application of our mechanistic understanding of the disease is opening a new venue for individual based biomarker development and application to prevention and therapy. A biomarker may be defined as a chemical, physical or biological agent in accessible body matrices, an *in vivo* response to an exposure or set of exposures, or a genotype or phenotype indicative of susceptibility to disease, all measurable in body fluids, cells or tissues. The promises of biomarkers are many fold, though, as yet, largely unrealized. Biomarkers have the potential to improve assessments of ambient environmental exposures; improve methods in risk estimation and classification of at-risk individuals, communities, and populations; define the mechanisms of exposure-disease linkages and underlying susceptibility

factors; define the interactions of multiple agents and exposures on disease outcomes; and, ultimately, improve and expedite methods for assessing the effect on disease outcomes of exposure remediation and preventive interventions (1-5). Of great interest is the translation of the myriad of -omics based platforms to the field of biomarker based strategies as they impact cancer prevention and control.

It is readily evident that population studies into etiology that use disease as the end point are of necessity large, lengthy and costly. While such investigations will always remain the ultimate standard for establishing linkage, they are unwieldy and inefficient for the timely translation of our accelerating understanding of the molecular basis of cancer towards preventive strategies. Thus, inclusion of biomarkers, despite some intrinsic limitations, into the paradigm of prevention and therapy are of central importance for the advancement of the field. To accomplish this end, validation strategies must be developed, refined and implemented.

The mere existence of a biomarker does not mean that it will be useful to the field. At present the opportunity for the biased use of biomarkers likely outweighs prospects for informed use. This concern arises from the simple fact that few of the biomarkers currently applied in either population, preclinical or clinical settings have undergone anything approaching rigorous validation. Indeed, paradigms for the validation of biomarkers, themselves, are still evolving (3, 6-9). Recognizing that considerable effort will be required for the validation of current and future biomarkers of potential use in environmental studies, this discussion seeks to highlight the types of approaches that are being used in the development and application of such biomarkers that, in turn, reflect different components of the multistage, multifactorial process of carcinogenesis. Of particular importance is the recognition of the concept that the utility of biomarkers in population and prevention studies are not dichotomous (i.e., good, bad), but rather continuous, with some markers being more informative than others depending upon how they are used. One of the cornerstones for development and validation of biomarkers can be built from a major set of tenets set out in epidemiology for defining causality. In 1965, Sir Austin Bradford Hill (10) set forth in his presidential address to the section of occupational medicine a systematic view to facilitate an analysis of the role of an environmental exposure to human disease. This view was outlined in the form of nine categories that have since become known as the Bradford Hill Criteria. These criteria now should be re-visited for the validation of biomarkers in the cancer etiology and disease linkage paradigm.

In the original Bradford Hill address there were several comments that looked to the future role for advances in understanding the mechanisms of disease and how they would impact upon the issues of causality. Reflecting upon the classic observation studies of John Snow, Bradford Hill commented that the effect of the water source was so strong upon the outbreak of cholera that the knowledge of the specific etiologic factor in the disease was not needed. Indeed it would be another 30 years before Koch characterized the *Vibrio cholera* as the specific organism leading to the disease. However, Bradford Hill recognized that the integration of experimental and mechanistic findings into this model will pose future challenges and opportunities.

The Bradford Hill Criteria were developed at a time when causality of disease was frequently measured in terms of exposure directly leading to disease. Thus, the questions being asked did not often incorporate mechanism based strategies. This is the extension to the validation paradigm that biomarkers bring to the field.

Strength of association was the first criteria listed and the discussion involved the examination of both the absolute difference in the disease outcome and the fold change of an incident disease linked to an exposure. Bradford Hill also raised the issue that a slight association may in fact have a profound impact on disease risk. The strength of the association is generally measured in terms of either relative risk or odds ratio which are both group or population based statistical analyses. Consistency is the next criteria and addresses whether the study had been repeated in different people/populations, places, circumstances and times. A strong consistency argument would be enhanced if different study designs, e.g. prospective

and retrospective analyses, were done with the same conclusions. Specificity is next major criteria. Often there will not be a one-to-one correspondence between an exposure and disease and such an occurrence is likely to be a fairly rare event. Nonetheless there should be specificity in the magnitude of the association. It would appear that biomarkers have a major role to play in sorting through the complexities associated with specificity. The issue of temporality involves the recognition that a disease outcome must be preceded by an exposure event. This type of analysis works well with a cross-sectional study design. However in longitudinal studies using biomarkers one can envision a more complex pattern of events when the outcome is driven by a multistage process. For example, an exposure that leads to an enzyme induction which is only relevant if there is a follow-up exposure could violate strict temporality rules. Biological gradient is addressed under the framework that a dose response relationship should exist for the exposure/biomarker/disease investigation. Plausibility is the next criteria. Unfortunately, what is biologically plausible depends upon the biological knowledge base of the day of the investigation. For single agents this is easier since animal or experimental models can address plausibility. This problem is much more difficult in gene-gene, gene-environment, vector-environment situations, thus the plausibility should be examined by testable experimental hypotheses. Coherence is invoked by the use experimental data to buttress human observations. Similarly, experiment involves the simple concept that the lower an exposure is the lower disease should occur. The final criteria was analogy and this implies that we can use data or biomarkers in a generic sense. Therefore, a commonly accepted phenomenon in one area can be extrapolated to another.

In conclusion, not all biomarkers are suitable for all purposes. Some will be helpful in understanding etiology, or selecting study cohorts, others will find use in assessing participant compliance, and others key to determining agent efficacy in prevention and therapeutic trials.

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Educational Session 3: Tumor Microenvironment

ED03-01 The role of the tumor microenvironment in tumor progression. I. P. Witz. Tel Aviv University, Tel Aviv, Israel.

The tumor tissue is composed of two compartments intimately associated with each other. The first compartment constitutes the malignant cells. The second is the tumor microenvironment. This compartment is composed of resident cells such as fibroblasts, endothelial

cells and other non-malignant cells; of infiltrating cells such as lymphocytes or macrophages and of numerous molecules such as those of the extracellular matrix, growth factors, cytokines, chemokines, antibodies, proteases, other types of enzymes and various metabolites. Cellular products released from necrotic cells (mainly tumor cells) are also present. All these molecules may be released from the tumor cells and/or from the non malignant cells. Some artificially administered molecules, for example drugs, may also be present in the microenvironment. The microenvironment of many solid tumors may be characterized by hypoxia; low extracellular pH and by low glucose concentration.

Although the term tumor microenvironment is used most often with respect to solid tumors, other types of malignancies have also their specific microenvironments. The bone marrow serving as a microenvironment for certain leukemias and for multiple myeloma is a case in point. The contemporary concept of tumor microenvironment postulates that it functions as an active "educational/inductive/selection" venue in which the tumor is directed into one of several molecular evolution pathways by microenvironmental factors. The interaction between microenvironmental components and tumor cells is a two way street. In addition to the regulation of genes in tumor cells by microenvironmental factors, tumor cells and their products are capable of regulating gene expression in non-tumor cells residing in or infiltrating into the microenvironment thereby altering their phenotype.

There are scores of tumor-microenvironment interactions that play anti or pro malignancy roles. These include interactions that lead to or drive cell proliferation or death, angiogenesis, motility, chemotaxis, invasion, protective immunity, inflammation and metastasis to name a few. Many such interactions await their discovery and the significance of other interactions has still to be elucidated.

It is now widely accepted that interactions of cancer cells with components of their microenvironment are crucial and pivotal determinants in the decision making process that determines if cancer cells will progress towards metastasis or whether they will stay dormant or disappear altogether.

The metastatic cascade is initiated when cells from the primary tumor detach and penetrate the extracellular matrix (ECM). Much of this invasive behavior depends upon the secretion of a variety of degrading enzymes, on alterations in the expression of adhesion molecules, on responses to cytokines and chemokines and on gene products regulating motility. A fraction of the invading cells penetrates into the vascular system (intravasation). The tumor cells in the blood form emboli with platelets and leukocytes, adhere to the endothelium of certain organs, extravasate and migrate into secondary sites to form site specific metastasis. Each of these steps involves an interaction between tumor cells and microenvironmental factors.

Can metastasis be prevented in a tumor bearer? In view of the fact that metastasis is, in many cases, present already when a primary tumor is diagnosed, the straight forward answer to this question is no. However, metastasis is a relatively inefficient process that proceeds throughout the tumor-bearer period. It is therefore reasonable to hypothesize that the metastatic process can be halted or at least hindered.

The realization that metastasis is controlled by interactions of tumor cells with microenvironmental components has allowed for the establishment of a new paradigm, namely intervention in the metastatic process by targeting interactions between the tumor cell and its microenvironment. Numerous preclinical and clinical trials attempt, on the one hand, to block tumor-microenvironment interactions that boost tumor progression and metastasis and on the other hand enhance interactions that counteract malignancy.

Aiming to develop rational and effective therapy modalities that would interfere with the metastatic cascade, we need to elucidate the molecular and cellular interactions that drive metastasis; identify the anti metastasis interactions operating in the tumor microenvironment and determine the net balance between them. We must also consider the unique properties of the tumor microenvironment. These include an overwhelming complexity of intertwined signaling pathways, an abnormality of its "normal", non-malignant compartment; opposing effects of some of its components on

metastasis and many circular chains of tumor-progression-enhancing events that may be described as vicious cycles.

In order to develop effective anti metastasis therapies and in view of the complexity of tumor-microenvironment interactions, combinatorial approaches used in the analysis of hyper complex systems will have to be employed.

ED03-02 TGF-betas as key mediators of tumor-stromal interactions: opportunities for prevention. J. Nam, Y. Yang, L. M. Wakefield. National Cancer Institute, Bethesda, MD.

TGF- β s as candidate master regulators of stroma/parenchymal interactions and environmental sensing within tissues. It is increasingly well-appreciated that normal tissue homeostasis is maintained through the integrated activity of many different cellular components, all of which have the potential to impact on the carcinogenic process if the integrative control mechanisms go awry. TGF- β s are highly pleiotropic polypeptide growth factors that may occupy a nodal position in the molecular networks that mediate this functional integration. The TGF- β s arose relatively late in metazoan evolution as organismal complexity was increasing, and it is plausible that they evolved to coordinate and fine tune higher order cell-cell interactions and organization. They have a number of properties that would particularly fit them for this role. Expression of TGF- β ligands and receptors is essentially ubiquitous, and nearly every cell type shows some biological response to TGF- β stimulation. TGF- β s are secreted into the extracellular milieu as biologically latent molecules where they must be activated in order to elicit a biological response. Typically the latent TGF- β complexes are bound to extracellular matrix components, where they are poised to act as local environmental sensors. Activation of TGF- β is an acute response to many forms of stress or injury that serves to restore normal homeostasis. However chronic activation of TGF- β is associated with the pathogenesis of many diseases, including fibroproliferative disorders and cancer.

Biological effects of TGF- β s on stromal cells. TGF- β s have effects on essentially all major cellular compartments of the stroma, including the vasculature, immune cells and fibroblasts. In the vascular compartment, TGF- β s promote blood vessel maturation through direct effects on endothelial cells, and by increasing pericyte and smooth muscle cell coverage. However, they can also impact indirectly on angiogenesis by regulating the local production of pro-angiogenic and anti-angiogenic factors. In the immune compartment, TGF- β s can initiate an acute inflammatory response by promoting leukocyte recruitment, but their overall effect on immune function is generally suppressive. Thus TGF- β s inhibit the generation or effector function of cytotoxic T-cells, NK cells, dendritic cells and macrophage/ monocytes, and they promote the generation and activity of immunosuppressive regulatory T-cells. Indeed, the TGF- β 1 null mouse dies of a multifocal inflammatory response, suggesting that tonic inhibition of the immune system by TGF- β 1 is critical to prevent self-reactivity and autoimmune disease. Finally, TGF- β modulates the phenotype of stromal fibroblasts, promoting differentiation to the myfibroblast phenotype, with particularly important effects on extracellular matrix and cytokine production. Clearly, these three activities, namely promotion of angiogenesis, immune suppression and modulation of the composition of the acellular microenvironment, if engaged chronically would tend to promote tumor progression.

Evidence that activity of the TGF- β pathway is critical for normal homeostatic interactions between stroma and epithelia. TGF- β s are potent inhibitors of epithelial cell proliferation, and initial studies on the role of TGF- β in tumorigenesis focused on loss of TGF- β response in the epithelial compartment. Unexpectedly however, recent work has suggested that stromal responses to TGF- β are critical for the maintenance of homeostasis in the overlying epithelium. Conditional knockout of the type II TGF- β receptor specifically in fibroblasts was shown to promote dysplasia or frank carcinogenesis in the epithelia of the prostate and stomach. Similarly, conditional knockout of the downstream TGF- β signaling component Smad4 in T-cells lead to development of colorectal carcinomas. In both cases, loss of TGF- β response in the stromal compartment resulted locally in the inappropriate secretion of growth factors or cytokines that were tumor promoting for the particular

epithelium. The data suggest that in normal homeostasis, tonic low level TGF- β activity is critical for maintaining an anti-carcinogenic phenotype in the stroma. Indeed, mice and humans with germline mutations or polymorphisms that reduce TGF- β pathway function tend to be tumor-prone, but since these lesions have a direct impact on the epithelial cells as well, it is difficult to dissect out the relative contribution of the impaired stroma to this phenotype.

The TGF- β -mediated parenchymal-stromal dialog becomes distorted in tumor progression. The mouse studies demonstrate that an intact TGF- β response in the stroma is important for normal epithelial homeostasis, but to date there is no clinical evidence that somatic inactivation of the TGF- β pathway occurs in the stromal compartment during carcinogenesis. However, the TGF- β -mediated dialog between tumor and stroma frequently gets distorted in a different way during cancer progression. Many advanced human tumors show elevated TGF- β expression, which correlates with metastasis and poor prognosis. Although TGF- β clearly can have direct pro-progression effects on the tumor parenchyma, preclinical studies suggest that the more important effect of elevated TGF- β is to generate a more permissive tumor stroma, primarily through suppression of effective anti-tumor immune surveillance and promotion of angiogenesis, as discussed in more detail below. Thus it appears that normal parenchymal/stromal interactions involving TGF- β are very delicately balanced, and that chronic resetting of the system away from its equilibrium position either by reduced or enhanced TGF- β pathway activation will result in the generation of a tumor-promoting stroma.

Targeting TGF- β to restore an anti-carcinogenic stroma: Prospects for tertiary prevention. If overexpression of TGF- β by advanced tumors does indeed generate a tumor-promoting stroma, then TGF- β antagonism should reverse this process and prevent further tumor progression. Clearly the delicate balance between the undesirable effects of too much and too little TGF- β , and the general complexity of TGF- β biology make the design of strategies for TGF- β pathway blockade *in vivo* somewhat tricky. However, a number of preclinical studies now suggest that this approach may be feasible. In one of the earliest studies of this type, our lab generated a transgenic mouse that chronically overexpressed an antibody-like TGF- β antagonist. We found that these mice were relatively protected from the development of metastatic disease, both in the context of spontaneously arising metastatic tumors driven by overexpression of the Her2/Neu oncogene, and when metastatic cells were injected intravenously. Unexpectedly, we saw no acceleration of primary tumorigenesis despite the fact that TGF- β functions as a tumor suppressor in the Neu-initiated mammary epithelium, and we saw little evidence for the autoimmune manifestations that would have been predicted from the phenotype of the TGF- β 1 knockout mouse. A number of other studies, using a variety of strategies to antagonize TGF- β have come to similar conclusions.

To address the mechanisms underlying this metastasis suppression, we have used the 4T1 transplantable model of metastatic mammary cancer in syngeneic Balb/c mice. By depleting select populations of immune cells, we can show that the efficacy of an anti-TGF-beta antibody is dependent on both the innate and the adaptive arms of the immune system, suggesting that TGF- β antagonism is acting to restore effective anti-tumor immune responses. We can further show that TGF- β antagonism has additional effects on the tumor cell itself that make the tumor more visible to the immune system and more susceptible to immune cell killing. Finally, we also found a decrease in microvessel density in tumors and metastases treated with anti-TGF- β , and at the RNA level we saw alterations in expression of extracellular matrix genes that have previously been implicated in metastasis susceptibility. Individually most of the effects of TGF- β antagonism were relatively small in magnitude and local to the tumor site. Thus, consistent with the notion that TGF- β occupies a nodal position in mediating mediating tumor-stroma interactions, TGF- β antagonism has effects on virtually every cell type in the tumor, and aggregately these effects combine to modify tumor/stromal interactions in such a way that further tumor progression is impeded.

Clinical prospects. Encouraged by these and similar preclinical results, a number of pharmaceutical and biotechnology companies are developing TGF- β antagonists for use in a cancer setting. A fully human monoclonal

antibody and a small molecule TGF- β receptor kinase antagonist are now in Phase I trials in cancer patients. It will be interesting to see whether this approach, which allows simultaneous targeting of so many cells in the cancer microenvironment, will offer advantages over more narrowly targeted approaches.

ED03-03 Angioprevention: Targeting angiogenesis and inflammation in prevention strategies. A. Albini. IRCCS Multimedica, Milano, Italy.

Angiogenesis is necessary for solid tumor growth and dissemination, a promising target not only in cancer therapy but also in prevention. The principle of cancer chemoprevention is based on the use of agents that, while devoid of collateral effects, are able to interfere with processes associated with malignant progression. In working with many chemoprevention agents, we recently observed that angiogenesis is a common and key target of most chemopreventive molecules. We termed "angioprevention" the concept that effective chemoprevention targets angiogenesis (1). The cancer microenvironment encompasses a series of complex interactions and communications between tumor cells and host cells, in particular endothelial and inflammatory cells, that promote progression to malignancy. We sought to identify molecules and pathways to prevent tumor development by targeting the microenvironment (2) and inflammatory angiogenesis (3).

We have shown that various molecules, including flavonoids, antioxidants and retinoids, act in the tumor micro-environment and inhibit the recruitment and/or activation of endothelial cells. We have shown that N-acetyl-cysteine (NAC), the green tea flavonoid epigallocatechin-3-gallate (EGCG) and the chalcone Xanthohumol (XN), and the Akt inhibitor deguelin all prevent angiogenesis in the Matrigel sponge angiogenic assay *in vivo* and inhibit the growth of the highly angiogenic Kaposi's sarcoma tumor cells (KS-Imm) in nude mice. The synthetic retinoid 4-hydroxyfenretinide (4HPR) also shows similar anti-angiogenic and anti-tumor effects.

To examine the molecular mechanisms involved in their activity, we have performed microarray expression profiling of endothelial cells in response to angiopreventive molecules using the Affymetrix GeneChipTM platform (4). NAC and EGCG showed similar effects no drastic changes in gene expression were observed, with consistent with the absence of toxicity. Many of the genes that respond to both drugs are involved in angiogenesis-related processes and give direct clues to the observed angiopreventive activity, in particular, we find repression of the NF- κ B pathway. Inhibition of the Akt and NF- κ B pathways are a consistent finding for most angioprevention molecules, including NAC, EGCG, XN and deguelin. The synthetic retinoid 4HPR followed a quite different pathway of regulation linked to the expression of anti-angiogenic molecules in the TGF-beta pathway, MIC1 and BMP2 (5).

We have now shown that the triterpenoid CDDO-Methylester is a remarkably potent inhibitor of angiogenesis and angiogenic tumor growth, effective at doses as low as 0.003 mg/kg body weight. The triterpenoids have also been shown to inhibit the NF- κ B pathway, consistent with the concept that endothelial NF- κ B is central to angiogenesis. The key and common mechanism of targeting NF- κ B by these agents suggest that the same group of molecules will show anti-inflammatory properties. We have shown that EGCG is a potent anti-inflammatory agent *in vivo* (6), and have now found similar properties for another angioprevention agent, Hyperforin (7).

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ED03-04 The role of inflammation and bone marrow progenitor cells in the pathogenesis of gastric cancer stem cells. T. C. Wang, S. Tu, S-W Wang, S. Takaiishi. Columbia University Medical Center, Department of Medicine and Irving Cancer Research Center, New York, NY.

The link between inflammation and cancer has long been recognized but poorly understood. In addition, cancers are heterogeneous with only a small subset of cells (cancer stem cells or CSCs) able to initiate and sustain disease. Epithelial cancers often arise in the setting of tissue loss and chronic inflammation, and are preceded by hyperproliferation and metaplasia. *Helicobacter pylori* infection induces chronic inflammation in the stomach and has been strongly linked to gastric cancer. Our group has developed the *Helicobacter* mouse model of gastric cancer to address the role of inflammation and the origins of epithelial cancer. *H. pylori* infection can activate NF- κ B signaling and cytokine production in macrophages, and a strong T helper 1 cytokine response appears to be necessary for progression to gastric cancer. Indeed, numerous studies of *Helicobacter*-infected mice suggest that a strong Th1 immune response is the common feature linking most *Helicobacter*-dependent gastric cancer mouse models. In addition, overexpression of pro-inflammatory cytokines in the stomach may be sufficient for the induction of gastric cancer in the absence of *Helicobacter* infection. Both *Helicobacter* infection and cytokine overexpression trigger changes in stromal (myofibroblasts) in the stomach, elevated circulating levels of IL-6 and TNF- α , and elevated levels of bone marrow-derived progenitor cells (BMDCs) in the circulation. Many of these changes can be inhibited by cytokine blockade. We examined the role of BMDCs in the pathogenesis of gastric cancer using lethally irradiated mice and used bone marrow reconstitution studies. Acute injury or acute inflammation did not result in significant engraftment, but mice with chronic (> 20 wks) *H. felis* infection did show BMDC engraftment. In these mice, BMDCs were shown to give rise to gastric metaplasia, dysplasia and cancer in a manner that suggested monoclonal conversion of gastric glands. Analysis of human gastric cancer cell lines suggests that the CD44+ fraction contains gastric cancer stem cells that are able to form spheroid colonies and tumors in immunodeficient mice. By immunohistochemistry, CD44 was strongly expressed in invasive gastric cancer lesions in *Helicobacter*-infected mouse models. Finally, CD44+ cells MSCs isolated from bone marrow appear to be able to reconstitute dysplasia in *Helicobacter*-infected mouse models. These findings from our laboratory suggest a link between bone marrow progenitors and cancer stem cells, and that epithelial cancers may originate from marrow-derived sources.

Educational Session 4: Preclinical Models for Prevention

ED04-02 Some recent findings of chemopreventive agents for the respiratory tract using the hamster and mouse models.

L. W. Wattenberg. University of Minnesota, Minneapolis, MN.

Two major strategies for advancing the field of chemoprevention will be discussed. The first focuses on identifying inhibitors that are effective when given at a late stage of precancer and could be administered over a short duration of time. The second deals with selection of agents for development that have been consumed or administered to human populations over a significant period of time with at most producing trivial side effects. Some recent findings pertaining to chemopreventive agents for the respiratory tract using animal models will be cited. For application of chemoprevention to human subjects there is a great advantage of utilizing agents that can be administered over a short period of time. Amongst the advantages is the increased likelihood that the subject will use the chemopreventive agent for a complete course. A second advantage is that a knowledge of the length of the course of administrations will facilitate evaluation and control of adverse effects. Currently most chemopreventive agents are used over a long period of time which is cumbersome and complicated. There are at least two classes of chemopreventive agents that fit the criterion of short duration of administration. One of these is termed interlockers. These are a hypothetical group of agents which act late in the precancer period and have the effect of producing the irreversibility of carcinogenesis which is characteristic of cancer (1). A second group of agents are those which affect tumor formation by aging as described by Campisi and others (2).

Of great interest are preventive agents for which a high degree of safety has been shown by intake in humans. A search for these has focused on three categories: a) Agents selected on a basis of mechanism. b) In a second group are agents for which there is epidemiological evidence for safety in humans as suggested by structural relationships and c) Compounds reported in literature which is difficult to access because of language or age. Examples of all three categories will be provided. The focus of research on agents likely to have applicability to the human increases the prospects of their ultimate utility for advancing the field of chemoprevention.

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ED04-03 Use of a carcinogen-induced rat mammary tumor model both to identify chemopreventive agents and to examine potential efficacy biomarkers.

R. A. Lubet. National Cancer Institute, Rockville, MD.

The chemically induced model of mammary cancer in rats was developed more than 40 years ago by Huggins and coworkers at the University of Chicago (1). The resulting tumors which appear within 6-20 weeks following treatment with carcinogen (methylnitrosourea, DMBA) are adenocarcinomas and are overwhelmingly ER+. The tumors appear histologically and by gene array analysis to be similar to highly differentiated ER+ tumors in humans. In contrast to humans roughly 50% of these tumors have mutations in the Ha Ras oncogene. The model was used by Huggins and coworkers to examine the role of hormonal manipulation in tumor development including role of estrogen, progesterone, pregnancy etc. The carcinogen induced model was subsequently employed to determine the efficacy of the SERMS (tamoxifen, raloxifene) (2). It has also been used to examine the efficacy of aromatase inhibitors (3). Not surprisingly, agents that have proven to be highly effective in prevention and therapy of ER+ tumors in humans have also proven to be highly effective in this specific model.

Because of the ease with which tumors can be generated and the substantial numbers of agents which have been tested in this model it has often been employed to screen for potential agents that might prove

relevant in the prevention of human breast cancer. As mentioned above it has proven susceptible to a wide variety of agents which alter the hormonal axis. Interestingly however it has proven susceptible to classes of agents which presumably do not function by altering the hormonal axis. Among the first class of non hormonal agents that was profoundly effective in this model were the RXR agonists (4). This initial observation has been confirmed by various investigators including Dr. Sporn. Thus a wide variety of RXR agonists, most of which increase triglyceride levels as their primary toxicity, have proven effective. We recently examined a number of synthetic RXR agonists, based on a 9cis retinoic acid backbone, and shown that these agents were effective in this model. The particular interest of this is that at least one of these effective agents (UAB 30) does not increase triglyceride levels (5).

In addition to results with the RXR agonists a number of other non hormonal agents appear to be effective in this model. Thus the farnesyltransferase inhibitor (tipifarnitib) was effective and preferentially inhibited development of tumors with an Ha Ras mutation (6). Also we have observed that a number of EGFR inhibitors (Iressa and Tarceva) are effective in this model.

In addition Dr. Sporn has observed that a number of triterpenoids have been highly effective in this model.

In addition to the large number of effective agents we have examined the widest variety of ineffective agents including various statins, Vitamin C, Vitamin E, 1,2 dithiol3-thione, phenyl butyrate etc. Finally we have used this model to determine various agents with moderate efficacy e.g. 5,6 benzoflavone, 9 cis RA, Celecoxib, various NSAIDS, etc.

The wide variety of agents which are both structurally and mechanistically varied has allowed us to use this model to examine for potential biomarkers in this model which we hope will prove applicable to humans. We initially observed that the aromatase inhibitor vorozole profoundly decreased tumor cell proliferation and increased tumor cell apoptosis in small palpable lesions treated short term (7). Human trials in a neoadjuvant setting have similarly shown that short term treatment with aromatase inhibitors in humans profoundly decreased tumor cell proliferation and was highly correlated with the overall efficacy of these agents (8-9). We have used these limited exposure studies to look at a wide variety of agents to determine whether short term treatment of palpable lesions followed by measurements of proliferation and apoptosis could predict long term chemopreventive efficacy. Initially we followed a variety of RXR agonists and showed that the short term markers could readily predict and differentiate highly active agents from agents that were less active (5). More recently we have expanded this to the widest range of agents in this model and again found that in this model that one could readily differentiate highly effective agents from less effective agents based on short term biomarker changes (10). We feel that this approach bears some similarities to what has been and can be employed in humans in the neoadjuvant setting.

Because of the wide and varied agents for which we have complete prevention data we have examined other potential biomarkers which might prove relevant to human trials e.g. gene array technologies and imaging techniques. Preliminary results with these alternative biomarkers will be discussed.

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Educational Session 5: Prevention Clinical Trials for the Non-clinician

ED05-02 Stacking the odds for success: Choosing biomarkers, endpoints, and designs for mid- to late-phase prevention trials.

J. J. Lee. UT M.D. Anderson Cancer Center, Houston, TX.

Upon the discovery of a handful of promising cancer prevention agents, a critical step is to conduct clinical trials to further test the agents and identify the most promising ones for large-scale prevention trials. The mid- to late- phase prevention trials are critical to the success of developing prevention agents. A few strategies will be introduced to maximize the odds for success.

Choosing the proper biomarkers. An effective prevention agent must be able to reach the target tissue and exert its effect. Standard PK-PD markers for measuring the drug delivery and metabolism can be used as a first step to measure the drug effect, for example PGE₂ level for COX inhibitors. Other molecular markers such as Ki-67 for proliferation, apoptotic index for apoptosis, viral load for anti-viral vaccine (e.g., HPV vaccine), and PSA for prostate cancer prevention have been used. The use of molecular imaging technique for target agents also offers a promising future.

Choosing the proper study endpoints. One of the major challenges in prevention trials is the low incidence of cancer during the trial. Using cancer as the study endpoint is not feasible in the mid-phase development and should be reserved for large-scale prevention trials. On the other hand, the intraepithelial neoplasia (IEN) can be an ideal alternative. IEN is often a precursor of cancer and can be modulated, for example, oral leukoplakia for oral cancer, polyp for colon cancer, and ductal carcinoma in situ for breast cancer.

Choosing the proper study designs. Key components for a successful trial design include: identify a high-risk population; enrich the study population for targeted agents; devise the best randomization scheme, such as the outcome-based adaptive randomization; and incorporate interim analysis for early stopping due to futility or efficacy. The use of neo-adjuvant design for prevention trials is also very appealing. Examples and practical considerations will be given to illustrate the above points with the goal of designing efficient and flexible trials to screen out losers and send off winners for further development.

Educational Session 7: Cancer Systems Biology

ED07-01 Role of systems biology in chemoprevention drug identification and implementation. G. B. Mills. UT M. D. Anderson Cancer Center, Houston, TX.

Systems biology is the study of the emergence of functional properties that are present in a biological system but that are not obvious from a study of its individual components. Systems biology represents a paradigm shift from an in-depth study of one molecule or a few molecules at a time to a study of a system of integrated, interacting molecules. Systems biology attempts to understand how a process, a cell, a group of cells, or an organism works at a global level and how different components of the process cooperate to attain the "correct" functional outcome. It is now recognized that component-by-component analysis is not sufficient for the study of signal transduction, gene regulatory and biochemical networks, oncogenic transformation, and other processes in which many genes and proteins interact. Understanding the dynamics of such systems, both qualitatively and quantitatively, and constructing mathematical models with robust predictive capabilities will result in powerful new tools for biomedical research. The systems approach will be directly applicable to patient care as we implement targeted therapeutics and molecular makers leading to personalized molecular medicine. At the lowest level, systems biology will develop models that can improve our ability to select patients likely to respond to particular therapies and also greatly improve our ability to develop rational drug combinations. In its maturity, systems biology will be able to predict unintuitive consequences of targeted therapeutics. An outstanding example of this is the recent termination of a clinical trial of an mTOR inhibitor due to apparent increased tumor growth. Our preliminary systems biology-based mathematical and experimental models of the phosphatidylinositol 3-kinase/mTOR signaling network accurately predict these consequences as well as the biochemical processes involved. Further, the models suggest combinations of targeted therapeutics likely to reverse the negative effects of the mTOR inhibitors converting the outcome from negative to positive in terms of tumor growth.

Many of the concepts of systems biology are not new. Biologists and biochemists have long known that the detailed (reductionist) study of individual proteins is just the first step toward an understanding of the overall (integrated) life process. Current advances in biology are a direct result of the success of the reductionist approach. The available experimental procedures necessarily forced a 'one protein at a time' analysis during the middle of the 20th century. Advances in experimental methodology (high-throughput screening and functional genomics technologies) have made the "global" view accessible for the first time, finally allowing scientific research at the overall level of the cell or the organism possible.

Recent developments in computational systems analysis are expanding the traditional hypothesis driven model in an intriguing new direction. By combining comprehensive representations of the data (with explicit ontologies) with machine learning tools, it becomes possible to add an automated capability for hypothesis generation. Critically, this integration between computational modeling and interventional laboratory bench studies provides an iterative approach to validate and constrain the computational models. New approaches are being developed by quantitative scientists, such as computational biologists, statisticians, mathematicians, computer scientists, engineers, and physicists, to improve our ability to make these high-throughput measurements and create, refine, and retest the models until the predicted behavior accurately reflects the phenotype observed. However, the successful implementation of this approach requires close collaboration between quantitative scientists and interventional biologists, who provide the original experimental data to develop the models and, more importantly, to test the hypotheses developed from the models. The importance of developing a cadre of scientists comfortable with both interventional bench studies and computational modeling is evidenced by a number of Roadmap, NCI and NIH training grants and RFAs related to this area.

It is now clear that systems biology is a data-driven process. Although

our analytical technologies have also improved over the last several years, most of them can generate only high-throughput data of low quality (i.e., microarrays), or low-throughput data of high quality (i.e., enzymatic assays). We need both high-throughput and high-quality data to create accurate cell response models. We also need technologies that simultaneously measure multiple parameters at the level of individual cells. Technological advances are beginning to provide the comprehensive databases at the DNA, RNA, and protein level necessary to integrate systems biology with cancer biology. Combining these patient and model-based databases with the ability to interrogate functional networks by a systematic analysis using siRNA libraries and chemical genomics provides an ability to link in silico modeling, computational biology, and interventional approaches to develop robust predictive models applicable to patient management.

Educational Session 8: Perception of Cancer Risk: Does It Matter?

ED08-01 Affect, cognition, superstition: Novel approaches to examining cancer risk perceptions in cancer prevention and control. J. L. Hay. Memorial Sloan-Kettering Cancer Center, New York, NY.

This talk draws from recent behavioral science and decision-making research to offer novel solutions to colorectal cancer screening non-adherence. I will first discuss research and theory asserting the importance of the perception of being at risk for colorectal cancer as an important prerequisite for screening adherence over and above the presence of epidemiological risk factors, such as family colorectal cancer history. Accordingly, many cancer prevention interventions aim to increase individuals' subjective appreciation of their own cancer risks in order to motivate cancer screening. I will next review recent research arguing that affective (emotional, feeling-based) and intuitive cognitive (automatic, non-rational) processing is important as individuals' think about their personal cancer risk. However, measurement strategies for cancer risk perceptions have not been revised to encompass this recent research. Assessment of affective and intuitive cognitive processing of risk may shed light on the manner in which cancer risk information is used - especially among those who are non-adherent to screening after physician recommendation. I will describe our qualitative and quantitative program of research aimed at validating a strategy to measure affective and intuitive cognitive processing of personal cancer risk (Cancer Risk Beliefs Scale) in diverse general population cohorts. I will present our findings regarding the reliability of the Cancer Risk Beliefs Scale, relationships with other key cognitive and behavioral variables, and preliminary findings relating the Cancer Risk Beliefs Scale to adherence with colorectal cancer screening. This program of research is aimed toward enhancing our understanding of diverse strategies for processing cancer risk and prevention messages, and providing the groundwork for new interventions to address screening non-adherence for this highly preventable and treatable cancer.

Educational Session 9: Signal Transduction Pathways as Targets for Chemoprevention

ED09-01 Sorting out roles for EGF receptor and its ligands in colonic neoplasia. R. J. Coffey. Vanderbilt University Medical Center, Nashville, TN.

We previously reported that there is iterative use of EGF receptor (EGFR) signaling in colonic neoplasia, both at a post-initiation, establishment phase and again later during tumor progression (1). Recent studies have examined trafficking of EGFR ligands in the context of polarizing epithelial cells, including a battery of human colorectal cell lines developed from well-differentiated cancers. Two EGFR ligands, TGF- α and amphiregulin, are delivered preferentially to the basolateral surface where they are cleaved by TACE/ADAM17 whereupon soluble ligand binds to

basolaterally restricted EGFRs. Thus, the EGFR axis (which we define as the proximal events in activation of the EGFR) is compartmentalized to the basolateral surface of polarized epithelial cells. Combined blockade of the EGFR axis (ligand cleavage, ligand uptake and EGFR tyrosine kinase activity) results in cooperative growth inhibition in colorectal cancer cell lines *in vitro*. We will present recent data demonstrating that Naked2, an inducible negative regulator of canonical WNT signaling, is a critical regulator of delivery of TGF- α to the basolateral surface of polarized epithelial cells and is downregulated as a late event in colorectal neoplasia.

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ED09-02 Stromal signaling pathways as targets for prevention.

H. L. Moses. Vanderbilt University, Nashville, TN.

Accumulating data indicate that the stroma can play a critical role in cancer initiation and progression. TGF- β signaling in both epithelial and stromal cells appears to be a key regulator of the stromal microenvironment. There is now compelling evidence from transgenic mouse studies and analyses of mutations in human carcinomas indicating that the TGF- β signal transduction pathway is tumor suppressive. Studies of human tumors have demonstrated inactivating mutations in human tumors of genes encoding proteins involved in TGF- β signal transduction, including DPC4/Smad4, Smad2, and the type I and type II TGF- β receptor (TBR1 and TBR2, respectively). However, there is some evidence that TGF- β signaling can promote tumor progression in the later stages. In order to examine the roles of TGF- β signaling in cancer more closely, we have generated mice with loxP sites flanking exon 2 of the type II receptor gene, *Tgfr2*, and crossed them with mice expressing Cre driven by different epithelial specific promoters. Loss of TGF- β signaling in six different epithelial cells gave a minimal phenotype. However, when challenged with oncogene expression or tumor suppressor gene impairment, there was rapid development of invasive and metastatic carcinomas supporting the hypothesis that epithelial cell autonomous TGF- β signaling is tumor suppressive in both early and late stages of carcinogenesis. One mechanism appears to be enhanced expression of chemokines by *Tgfr2* null carcinoma cells with resultant recruitment of bone marrow derived cells that express abundant TGF- β and MMPs in the tumor microenvironment. In contrast to the epithelial cell knockouts that gave a minimal phenotype, knockout of *Tgfr2* in stromal fibroblasts gave a striking epithelial phenotype in the mammary gland, prostate and forestomach, including epithelial pre-neoplasia and invasive carcinomas. One mechanism identified was paracrine stimulation of carcinoma cells by HGF, MSP and TGF- α . Another mechanism appeared to be over expression of chemokines by the knockout fibroblasts with recruitment of bone marrow derived cells. Thus, TGF- β signaling in fibroblasts modulates the growth and oncogenic potential of adjacent epithelia in selected tissues. The data indicate that targeting pathways that inhibit bone marrow cell differentiation or chemokine receptors may be useful in both therapy and prevention of cancer.

ED09-03 Targeting kinases in lung cancer prevention. J. M. Kurie. UT M. D. Anderson Cancer Center, Houston, TX.

The serine/threonine kinase AKT and its downstream mediator mammalian target of rapamycin (mTOR) are activated in lung adenocarcinoma, and clinical trials are underway to test whether inhibition of mTOR is useful in treating lung cancer. Here we report that mTOR inhibition blocked malignant progression in K-ras^{LA1} mice, which undergo somatic activation of the K-ras oncogene and display morphologic changes in alveolar epithelial cells that recapitulate those of precursors of human lung adenocarcinoma. Levels of phosphorylated S6^{Ser236/235} (p-S6), a downstream mediator of mTOR, increased with malignant progression (normal alveolar epithelial cells to adenocarcinoma) in K-ras^{LA1} mice and in patients with lung adenocarcinoma. Atypical alveolar hyperplasia (AAH),

an early neoplastic change, was prominently associated with macrophages and expressed high levels of p-S6. mTOR inhibition in K-ras^{LA1} mice by treatment with the rapamycin analog CCI-779 reduced the size and number of early epithelial neoplastic lesions (AAH and adenomas) and induced apoptosis of intra-epithelial macrophages. LKR-13, a lung adenocarcinoma cell line derived from K-ras^{LA1} mice, was resistant to treatment with CCI-779 *in vitro*. However, LKR-13 cells grown as syngeneic tumors recruited macrophages, and those tumors regressed in response to treatment with CCI-779. Lastly, conditioned medium from primary cultures of alveolar macrophages stimulated the proliferation of LKR-13 cells. These findings provide evidence that the expansion of lung adenocarcinoma precursors induced by oncogenic K-ras requires mTOR-dependent signaling and suggest that host factors derived from macrophages may contribute to adenocarcinoma progression.

To further test the role of inflammatory cells in lung tumor progression, we investigated the role of the chemokine receptor CXCR2 and its ligands KC and MIP-2. Recent studies have identified the CXC chemokine receptors and their ligands to be crucial factors in angiogenesis and the recruitment of inflammatory cells. The CXC chemokine family can be divided into two groups according to the presence (ELR+) or absence of (ELR-) of an ELR (Glu-Leu-Arg) motif located immediately before the first cysteine residue at the amino terminus. ELR+ CXC chemokines are known for their potent chemoattraction for neutrophils and macrophages as well as their ability to promote angiogenesis. CXC chemokines promote angiogenesis through the G protein-coupled receptor CXC chemokine receptor 2 (CXCR2) on endothelial cells. The CXCR2 ligand CXCL8 is present in freshly isolated specimens of human NSCLC and has been implicated as the dominant mediator of aberrant angiogenesis in a syngeneic murine Lewis lung cancer model and in human NSCLC/SCID mouse chimera. We investigated the role of CXCR2 and its ligands in the expansion of alveolar epithelial precursors in Kras^{LA1} mice. Malignant progression was associated with enhanced intra-lesional vascularity. CXCR2 and its ligands were highly expressed in macrophages and epithelial cells within alveolar lesions. Treatment of Kras^{LA1} mice with a neutralizing antibody against CXCR2 decreased the size and number of early lung lesions and, by histologic criteria, blocked the progression of early hyperplastic lesions to adenomas. Whereas the proliferation of an adenocarcinoma cell line derived from Kras^{LA1} mice (LKR-13) was resistant to treatment with the anti-CXCR2 antibody *in vitro*, LKR-13 cells established as syngeneic tumors were sensitive to treatment. Thus, CXCR2 activation was required for the expansion of early alveolar neoplastic lesions by oncogenic Kras, and regression of early lesions by CXCR2 inhibition required components of the tumor microenvironment.

Educational Session 10: Translational Regulation: A Promising Target for Cancer Prevention

ED10-02 Pcd4, a colon cancer prognostic that is regulated by a microRNA. H. Allgayer. Experimental Surgery, Medical Faculty Mannheim of University Heidelberg, Mannheim, Germany.

The novel tumor suppressor Pcd4 inhibits neoplastic transformation, tumor progression, and translation. Furthermore, we recently showed that Pcd4 suppresses invasion and intravasation, this at least in part by suppressing expression of the invasion-related urokinase receptor (u-PAR) gene via transcription factors Sp1/Sp3. However, relatively little is known about mechanisms regulating Pcd4 expression in cancer. Recently, microRNAs (miRNAs) have been discovered as naturally occurring non-coding RNAs, controlling gene expression via specific sites at the 3'-UTR of target mRNAs. We conducted a first study to investigate the regulation of Pcd4, and invasion/intravasation, by miRNAs.

A bioinformatic search revealed a conserved target site for miR-21 within the Pcd4 3'-UTR at 228-249 nucleotides. In 10 colorectal cell lines, an inverse correlation of miR-21 and Pcd4-protein was observed. Transfection of Colo206f-cells with miR-21 significantly suppressed a luciferase reporter containing the Pcd4 3'-UTR, whereas transfection of

RKO with anti-miR-21 increased activity of this construct. This was abolished when a construct mutated at the miR-21/nt228-249 target site was used instead. Anti-miR-21-transfected RKO-cells showed an increase of Pdc4-protein and reduced invasion. Moreover, these cells showed reduced intravasation and lung metastasis in a chicken-embryo-metastasis assay. In contrast, overexpression of miR-21 in Colo206f significantly reduced Pdc4-protein amounts and increased invasion, while Pdc4-mRNA was unaltered. Resected normal and tumor tissues of 22 colorectal cancer patients demonstrated an inverse correlation between miR-21 and Pdc4-protein. This is the first study to show that Pdc4 is negatively regulated by miR-21. Furthermore, it is the first report to demonstrate that miR-21 induces invasion/intravasation/metastasis.

This study adds to the increasing knowledge about diverse important roles played by Pdc4 in suppressing different phenoma associated with cancer. However, only few translational or clinical studies so far have investigated or supported the potential use of Pdc4 as a prognostic factor. Also, the role of Pdc4 in the progression from benign to malignant tissue has rarely been supported by *in vivo* studies at resected patient tissues. Therefore, we conducted a second study to 1. determine Pdc4 as a diagnostic marker in the adenoma- carcinoma sequence of patients with colorectal cancer, 2. obtain the first prognostic evidence of Pdc4 in colorectal cancer. Since recent studies also suggested that Pdc4 is regulated by Akt, one of the most important antiapoptotic regulators within the PI3-kinase pathway, and that Akt is able to regulate the nuclear localization of Pdc4, this study was also conducted, to 3. support phosphorylated Akt (pAkt) - mediated Pdc4 regulation *in vivo*.

Tumor samples and normal tissues from 71 patients with colorectal cancer who were followed prospectively (median follow-up, 36 months) and 42 adenomas were analyzed for Pdc4, Akt, and pAkt in immunohistochemical and Western blot analyses.

A significant reduction in Pdc4 was observed between normal mucosa and adenomas and between adenomas and tumor samples ($P < .01$ and $P < .01$, respectively). Normal mucosa demonstrated strong nuclear Pdc4, which was reduced significantly in adenomas ($P < .01$) and almost was lost in tumors ($P < .01$). pAkt was correlated inversely with Pdc4 and with the transition of Pdc4 from nucleus to cytoplasm ($P < .01$). Kaplan-Meier analysis (using the Mantel-Cox log-rank test) indicated a significant correlation between the loss of total and nuclear Pdc4 in tumors and overall survival ($P < .05$ and $P < .02$, respectively), and with disease-specific survival ($P < .01$ and $P < .01$, respectively). In multivariate analysis, loss of total or nuclear Pdc4 was an independent predictor of disease-specific or overall survival.

This is the first study to demonstrate an independent prognostic impact of Pdc4 and its expression pattern in colorectal cancer. Data from this study support the regulation of Pdc4 localization by pAkt *in vivo*. Pdc4 immunohistochemistry may be useful as a supportive diagnostic tool for the transition between normal, adenoma, and tumor tissues. Taken together, tumor suppressor Pdc4 marks adenoma/carcinoma transition, is an independent prognostic factor in resected colorectal cancer, and is negatively regulated by miR-21 in colorectal cancer, this being associated with miR-21 inducing invasion and intravasation.

ED10-03 MicroRNA reprogramming by oncogenes and tumor suppressors. J. Mendell, Johns Hopkins University School of Medicine, Baltimore, MD.

Dysregulated microRNA (miRNA) expression is a ubiquitous feature of cancer cells and select miRNAs have been demonstrated to possess oncogenic and tumor suppressing activity. Yet the mechanisms that lead to altered miRNA expression in tumors are poorly understood. One possibility is that miRNAs are under direct control of oncogenes and tumor suppressors whose gain- or loss-of-function directly contributes to abnormal miRNA expression patterns. In order to investigate this question, we have focused on the transcription factor c-Myc, one of the most commonly activated human oncogenes. Expression profiling studies in human and mouse models of B cell lymphoma have revealed that a major consequence of c-Myc activation is extensive reprogramming of the miRNA

transcriptome, including the direct upregulation of oncogenic miRNAs and the direct downregulation of tumor suppressing miRNAs. We have also identified miRNAs regulated by p53 that appear to participate in the anti-proliferative and pro-apoptotic functions of this tumor suppressor. Together, these studies demonstrate that abnormal miRNA expression in cancer cells cannot be explained solely as an indirect consequence of the loss of cellular identity that accompanies malignant transformation. Rather, oncogenic events directly reprogram the miRNA transcriptome to favor tumorigenesis. Moreover, miRNAs appear to function as critical downstream effectors of multiple oncogenic and tumor suppressor pathways.

Opening Keynote Session

KN01 Inflammation and cancer prevention: Is there a link?

R. N. DuBois. UT M. D. Anderson Cancer Center, Houston, TX.

Long-term use of NSAIDs leads to a 40-50% reduction in risk for colorectal cancer. How do these "anti-inflammatory" drugs act to reduce cancer risk? NSAIDs effectively target inhibition of prostaglandin synthesis by the cyclooxygenase enzymes (COX-1 and COX-2). Prostaglandins, such as PGE₂, regulate the expression of several downstream effector genes, some of which regulate pro-inflammatory pathways. These bioactive lipids signal via G protein-coupled receptors (GPCRs), which in turn can transactivate growth factor receptors and regulate cell proliferation, migration, and cell survival. Prostaglandin E₂ (PGE₂) has been shown to directly activate components of the canonical Wnt signaling system. Additionally, PGE₂ can transactivate the epidermal growth factor receptor (EGFR) in colorectal carcinoma cells via a c-Src dependent mechanism that regulates cell proliferation and migration. We found that the pathway which regulates degradation of prostaglandins, namely 15-PGDH is also regulated by EGFR signaling. We investigated the mechanisms by which PGDH is down-regulated in cancer and showed that epidermal growth factor (EGF) represses PGDH expression in colorectal cancer cells. EGFR signaling induces Snail, which represses PGDH transcription. Induction of PGE₂ catabolism through inhibition of EGFR signaling blocks cancer growth *in vivo*. In human colon cancers, elevated Snail expression correlates well with down-regulation of PGDH. These data indicate that PGDH may serve as a tumor suppressor in colorectal cancer and provide a possible COX-2-independent way to target PGE₂ to inhibit cancer progression.

Plenary Session 1: Predicting Cancer Risk: Advances in Genetics, Biomarkers, and Behavioral Science

PL01-01 Genetic polymorphisms in breast cancer risk and prevention. J. Newell Ingle. Mayo Clinic, Rochester, MN.

Breast cancer is a common but complex disease that is of concern to all women. In the United States, a woman is diagnosed with breast cancer about every 2 minutes. Although numerous advances have been made many women, estimated in excess of 40,000, will die this year. Prevention represents the ultimate goal and clear progress has been made with the use of selected estrogen receptor modulators (SERMs) tamoxifen plus raloxifene in that they have shown the ability to reduce invasive breast cancer by about one-half when given to high risk women. However, in the three-quarters of women without any identifiable genetic risk, the currently used models are not robust. For example, in the large P-1 trial of tamoxifen versus placebo, about 56 women were treated with tamoxifen for 5 years to prevent invasive breast cancer in a single patient. Improved determination of risk is crucial in order to more effectively utilize currently available treatments and decrease the number needed to treat. A minority of cases have one of the known predisposition mutations such as BRCA 1 or BRCA 2. Identification of additional genetic abnormalities would not only identify patients at risk but also give direction to new strategies for prevention. This represents a formidable task given the large number of genes (~30,000+) and number of single nucleotide polymorphisms

(SNPs) (~7M).

Breast cancer is a complex disease but to date the emphasis for prevention and treatment strategies has been on interference with estrogens either by blocking their interaction with the estrogen receptor such as with tamoxifen or lowering estrogen concentrations with ovarian function suppression in premenopausal women or aromatase inhibitors in postmenopausal women. Aside from mutations in predisposition genes, the strongest indicators of risk have been hormonally mediated factors such as early menarche, late menopause and levels of estrogens in the blood. The prevailing opinion is that this relationship between estrogens and breast cancer risk has been estrogen receptor (ER) mediated in that estrogen stimulates cell growth leading to accumulation of mutations. Evidence is increasing for an alternative and possibly additional mechanism of estrogen-related breast carcinogenesis in which certain estrogen metabolites are genotoxic producing depurinating adducts, apurinic sites in DNA, error-prone base excision repair leading to mutations and subsequently breast cancer. The estrogen genotoxicity hypothesis provides another area for study of genetic differences between patients that could identify patients at increased risk and potential strategies for prevention. This review will focus on new information relating to polymorphisms in a gene related to tamoxifen biotransformation, arguably the most important drug to date for women (and men) with breast cancer. Emerging information relating to polymorphisms in the aromatase gene CYP19 will be considered as the aromatase inhibitors are a class of drugs that have become established as being a part of optimal management of women in the adjuvant setting following removal of an early stage breast cancer and are the main class being studied in the prevention setting for postmenopausal women. Given the great importance of estrogens in breast cancer risk, studies examining genes in the estrogen metabolic pathway will be considered.

In selection of therapy for women with breast cancer, the focus has been almost exclusively on the characteristics of the tumor, e.g., ER and HER-2. Until recently, essentially no attention has been paid to the host and her genetic makeup as it relates to the metabolism of different drugs. The first real clinical application of pharmacogenetics in breast cancer management relates to tamoxifen's biotransformation to active anti-cancer metabolites. New information has arisen on the metabolism of tamoxifen to the active metabolite, 4 hydroxy-N-desmethyl-tamoxifen (endoxifen). Endoxifen is a metabolite with antitumor activity and affinity for the ER that is similar to 4-hydroxy-tamoxifen, but one that is normally present in substantially higher concentrations. CYP2D6 plays a central role in the metabolism to endoxifen and one published study in the adjuvant setting in early breast cancer shows that genotypic differences in CYP2D6 and use of CYP2D6 inhibitors has an impact on outcomes of women treated with tamoxifen. These findings also suggest that polymorphisms in the other genes encoding enzymes involved with drug metabolism should be investigated.

There are clear implications of this information on tamoxifen and CYP2D6 for women considering tamoxifen in the prevention setting. This is particularly the case since completion of the STAR (Study of Tamoxifen And Raloxifene) in which the 2 agents were shown to be equivalent in terms of reducing the incidence of invasive breast cancers. Thus, if it was to be confirmed that CYP2D6 metabolism was important in the prevention setting then raloxifene, which is not metabolized by CYP2D6, would be preferable in the woman who was found to be a CYP2D6 poor metabolizer.

The aromatase inhibitors represent a major class of drugs in the armamentarium against breast cancer. The aromatase gene has been resequenced and functional genomics have been performed on the identified non-synonymous coding SNPs showing significant decreases in levels of activity. These findings are consistent with a hypothesis that genetic variation in the CYP19 gene might be important in the activity of aromatase inhibitors. Currently the emphasis is on examining multiple genes (thus pharmacogenomics) in pharmacodynamic and pharmacokinetic pathways in women receiving aromatase inhibitors for breast cancer. This information has the potential for great importance in patient management because of differences in efficacy and striking differences in tolerability with some patients developing severe side-

effects, especially musculoskeletal complaints. The aromatase inhibitors are currently a major focus of research in the prevention setting in postmenopausal women.

The estrogen genotoxicity hypothesis states that certain metabolites of estrogen, specifically catechol estrogen quinones, can react with DNA and lead to mutations. This estrogen genotoxic mechanism does not require the presence of the ER. The presence of pathways for deactivation of the catechol estrogen quinones would be expected to decrease their reactions with DNA. The Mayo group under the direction of Richard Weinsilboum has performed a study of catechol O-methyltransferase (COMT) utilizing a comprehensive approach of (1) COMT resequencing, (2) breast cancer association studies (involving 1482 patients), (3) functional genomic studies and (4) replication association studies. They found 2 SNPs in the COMT distal promoter region that were associated with breast cancer risk reduction. The 2 SNPs were found by functional studies to up-regulate transcription and alter DNA-protein binding and these findings are consistent with the hypothesis that deactivation of the catechol estrogen quinones would decrease breast cancer risk. These functional studies provide biologic plausibility for the decreased risk observed in the Mayo association study.

The Mayo group has also examined polymorphisms in the CYP19 (aromatase) gene and risk of breast cancer in an association study also involving 1483 patients. This study was based on resequencing of the CYP19 gene in which 88 polymorphisms were identified. Two haplotyping approaches were utilized (methods of Carlson et al. and Stram). No association with breast cancer risk was detected for individual variants, tagSNPs or haplotype-tag SNPs despite 80% power to detect odds ratios as low as 1.49 for minor allele frequency of 0.10. Thus, there was no indication that CYP19 variants were associated with risk of developing breast cancer.

The complexity of the vast number genetic interactions has led to a growing sophistication in the methodology for examining many hundreds of thousands of SNPs in association studies. Such genome wide association studies (GWAS) will allow the identification of moderate risk alleles. This approach does not require any knowledge regarding position or function of the SNP thereby giving leads as to what genes to study on a functional basis and opening up new avenues of research into the biology of breast cancer and identification of new strategies of risk assessment and prevention.

Plenary Session 2: Immune System as a Target for Prevention

PL02-01 Psychological stress, immune and endocrine function, and cancer risk. J. Kiecolt-Glaser. Ohio State University College of Medicine, Columbus, OH.

There is extensive evidence linking psychological stress and depression with immune and endocrine dysregulation. This presentation will primarily focus on stress-related changes in inflammation, a noteworthy component in tumor progression. Production of proinflammatory cytokines can be substantially enhanced by stress and depression. Furthermore, stress and depression also contribute to greater risk for infection, prolonged infectious episodes, and delayed wound healing, all processes that indirectly fuel sustained proinflammatory cytokine production. Compounding the risks, health behaviors including poor sleep are commonplace consequences of stress and depression; poor sleep enhances proinflammatory cytokine production. In addition to these pathways, evidence from animal and human studies suggest that stress and depression can permanently alter the responsiveness of the immune system; stressors can effectively prime the inflammatory response, promoting larger proinflammatory cytokine increases in response to subsequent stressors and/or minor infectious challenges. Furthermore, stress and depression have also been associated with two important processes for carcinogenesis, poorer repair of damaged DNA, and alterations in apoptosis. Stress hormones can modulate many different aspects of the tumor microenvironment, including local growth factors. These data have provided mechanistic explanations for some of the

epidemiological evidence linking stress and depression with cancer incidence and progression. These studies and others suggest that psychological or behavioral factors may influence the incidence and progression of cancer through psychosocial influences on immune and endocrine function and other physiological pathways.

PL02-02 Inflammation and cancer: Organ-specific regulation of cancer development. L. M. Coussens, Department of Pathology, Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA

The concept that leukocytes are components of malignant tumors is not new; however, their functional involvement as promoting forces for tumor progression has only recently been appreciated. We are interested in understanding the molecular mechanisms that regulate leukocyte recruitment into neoplastic tissue and subsequent regulation those leukocytes exert on evolving cancer cells. By studying transgenic mouse models of skin, lung and breast cancer development, we have recently appreciated that adaptive leukocytes differentially regulate myeloid cell recruitment, activation, and behavior, by organ-dependent mechanisms. Thus, whereas chronic inflammation of premalignant skin neoplasms is B cell-dependent, during mammary carcinogenesis, T cells appear to play more of a dominant role in regulating pro-tumor and pro-metastatic properties of myeloid cells. To be presented will be recent insights into organ and tissue-specific regulation of epithelial cancer development by adaptive and innate immune cells, and thoughts on how these properties can be harnessed for effective anticancer therapeutics.

PL02-04 Aspirin, other NSAIDs and cancer prevention: Where are we now? E. J. Jacobs. American Cancer Society, Atlanta, GA.

Nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin, inhibit a wide variety of cancers in animal models, an effect usually attributed to inhibition of COX-1 and COX-2 activity. A relatively recently developed class of NSAIDs, selective COX-2 inhibitors, have been proven in randomized trials to substantially reduce colorectal polyp recurrence in humans. However, these same randomized trials showed that selective COX-2 inhibitors can increase risk of cardiovascular events, resulting in withdrawal of some COX-2 inhibitors from the U.S. market, and greatly limiting their use as cancer prevention agents. Considerable evidence indicates that other non-aspirin NSAIDs may also increase risk of cardiovascular events. These results have refocused attention on aspirin as a potential cancer prevention agent. Aspirin, unlike other NSAIDs, has antithrombotic effects and is already widely used to prevent cardiovascular disease, although often at doses (e.g. 81 mg/day) that may be of limited value in cancer prevention.

Over the last nearly 20 years, a large number of observational epidemiologic studies have examined the association between regular use of aspirin, usually adult strength aspirin (e.g. 325 mg tablets), and risk of individual cancers. In these studies, aspirin use has consistently been associated with reduced risk of colorectal cancer. Randomized trials have also shown that aspirin reduces colorectal polyp recurrence. Observational studies of aspirin use and risk of other common cancers have not produced consistent results. However, a considerable number of studies have reported modest reductions in risk of breast and prostate cancer, the most common cancers among U.S. women and men.

More recent studies on aspirin and cancer have highlighted the importance of dose and duration. The Women's Health Study, a 10 year long randomized trial of low dose aspirin (100 mg every other day), observed no reduction in risk of any cancer. In contrast, a significant reduction in risk of colorectal cancer was observed in a recent analysis including long-term post-intervention follow-up from two early randomized trials of at least 300 mg/day of aspirin for cardiovascular disease prevention. No reduction in risk of other cancers was observed. In a large observational study (the Cancer Prevention Study II Nutrition Cohort), daily use of adult-strength aspirin (\geq 325 mg) for 5 or more years was associated with an approximately 15 percent reduction in risk of total cancer incidence in both men and women, driven mainly by reduced risk of

colorectal, prostate, and possibly breast cancer.

Current clinical recommendations for use of aspirin for disease prevention are appropriately based on balancing the known reduction in risk of cardiovascular events against the known increased risk of serious gastrointestinal bleeding, without regard to cancer risk. While there is relatively strong evidence that aspirin can reduce colorectal cancer risk, a reduction in risk limited to one cancer is unlikely to be sufficiently important to be considered when making clinical decisions about aspirin use. The session will include a discussion of what types of research are needed to best determine if and how cancer prevention should be taken into account when making decisions about who should use aspirin and at what dose.

Plenary Session 3: Obesity, Metabolism, and Cancer

PL03-01 Obesity, metabolism and cancer: Trends, targets and transgenics. S. D. Hursting. Division of Nutritional Sciences, University of Texas and Department of Carcinogenesis, UT M. D. Anderson Cancer Center, Austin, TX.

The prevalence of obesity, an established risk factor for several cancers, has risen steadily for the past several decades in the US, with nearly two-thirds of adult Americans now overweight and nearly one third obese. Obesity is associated with dysregulation of multiple metabolic parameters, including alterations in hormonal, growth factor and cytokine pathways regulating insulin sensitivity, glucose metabolism, and inflammation. Unfortunately, the mechanisms underlying the obesity and cancer connection are not well understood, and the identification of key targets and new strategies for offsetting the impact of obesity on cancer risk are urgently needed. Calorie restriction (CR), the best characterized energy balance modulation, has been shown to inhibit spontaneous, transplanted and chemically induced tumors in a variety of models, while diet-induced obesity enhances tumorigenesis in many of these same models. We have shown in a series of transgenic model systems, as well as genomic and proteomic studies, that the insulin/insulin-like growth factor (IGF)-1 pathway mediates many of the anti-cancer effects of CR, while elevated levels of these factors are associated with obesity-induced tumor growth. Furthermore, the Akt/mTOR signaling pathway downstream of insulin and IGF-1 receptors provides important dietary and pharmacologic targets for disrupting the obesity-cancer link. Physical activity (treadmill or voluntary wheel running) exerts anti-cancer effects in our transgenic models as well, but apparently through very different molecular mechanisms than CR. A better understanding of the mechanisms underlying the energy balance-cancer link will facilitate the development of novel prevention and treatment strategies for offsetting the effects of obesity on cancer.

Plenary Session 5: Understanding and Preventing Cancers Due to Microbes

PL05-01 Burden of cancer attributable to infection. S. Franceschi. IARC, Lyon, France.

The most recent estimates attributed 18% of cancer (1.9 million cases) worldwide in 2002 to infectious agents (1). This fraction varies substantially between developed (7.7%) and developing (26.3%) countries. Estimates of infection-associated cancers are, for a number of reasons, conservative ones: 1) the prevalence of infection in cancer patients and in the general population (on whom the computation of attributable risks is based) are not always accurate and generally tend to be underestimated; 2) carcinogenic and non (or less) carcinogenic types of infection are not well distinguished; 3) infections may be involved in the aetiology of other cancers (e.g., skin, gallbladder, colon, bladder, and certain types of lymphomas and leukemias).

The most important contributors to these fractions are human papillomavirus (HPV, 5% of the cancer burden, nearly 10% among women, worldwide), hepatitis B and C viruses (HBV and HCV, 5%) and *Helicobacter pylori* (Hp, 6%). These infections will be discussed in greater detail. HPV (2) and HBV (3) represent good examples of well understood infections

against which highly efficacious preventive vaccines have become available (4). HCV is responsible for a higher fraction of hepatocellular carcinoma than HBV in the vast majority of developed countries, but also in several developing countries, on account of increases in HCV transmission through unsafe injection practices (3). In respect to Hp, improvements in the possibility to distinguish cagA-positive from cagA-negative strains are leading to a steady rise in the apparent strength of the association (5) and might, ultimately, increase substantially the fraction of gastric cancer attributed to *H. pylori*.

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PL05-02 Viral and host factors and risk of hepatocellular carcinoma. M. Yu. Graduate Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan.

Viral hepatitis is a significant problem worldwide. There are about 350 million chronic carriers of hepatitis B virus (HBV) and 170 million chronic carriers of hepatitis C virus (HCV). The exact number of people infected with both HBV and HCV is unknown, although dual HBV and HCV infection is not uncommon in endemic areas of hepatitis B. Chronic HBV and/or HCV infection can progress to chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). Globally, approximately 78% of HCC cases are attributable to either HBV (53%) or HCV (25%). The relative importance of HBV and HCV in the etiology of HCC varies by region. Perhaps the most striking characteristic of HCC is its marked variation among geographical regions. About 80% of the HCC cases occur in developing countries, but the incidence of HCC is increasing in many developed countries. While the effect of mono-infection with HBV or HCV on the risk of HCC is well described, the relation of dual HBV and HCV infection to HCC is not fully characterized because of the paucity of adequate long-term cohort study. Chronic HBV or HCV infection increases the risk of the development of HCC 20 to 40-fold. However, only a small fraction of HBV or HCV carriers develop HCC in their lifetime. This suggests that chronic hepatitis virus infection is not a sufficient cause of HCC. It becomes evident that multiple factors, including host-related, virus-related, and external factors, have roles in the etiology of HCC among HBV/HCV carriers. This presentation will focus on current findings from prospective follow-up studies, including (1) investigation of the risks of incident HCC and liver-related death for mono-infection by HBV or HCV in comparison to dual infection of HBV and HCV in hospital- and population-based studies; (2) results of the analyses on the familial risk of HCC after accounting for the context of HBV exposure and adjusting for other potential confounders; as well as (3) recent investigations of serological viral markers derived from molecularly based assays, such as viral load, genotype, and intragenotypic variants, and the risk of HCC. These data highlight the importance of population-based study in evaluation of HCC natural history, and suggest that understanding the complex interplay between different viral factors and between host and viral factors is important for distinguishing individuals who are infected with hepatitis virus at the highest risk of progression to HCC.

PL05-04 A shot in the arm? Cervical cancer prevention in the HPV vaccine era. P. Castle. National Cancer Institute, Rockville, MD.

Worldwide, approximately 500,000 new cases of cervical cancer (~1 case per minute) are diagnosed and 275,000 related-deaths occur annually, making it the second or third most common female cancer and cancer-related cause of mortality. Cervical cancer has an especially profound societal impact because a substantial fraction of cervical cancer occurs in women in their 30's and 40's while they are still raising or supporting families. Cervical cancer is an even more vexing problem in developing countries and regions, where more than 80% of all cervical cancer occurs and is often the most common cancer in women.

Screening with cervical cytology (Pap tests) has led to drastic reductions in cervical cancer incidence in developed countries; in the U.S., only about 11,000 cases will be diagnosed and less than 4,000 will die from cervical cancer this year. However, cytology-based programs in low-resource settings have largely been unsuccessful. The unequal burden of cervical cancer in resource-limited populations and the success of cervical cytology in developed countries highlights its limitations, including poor sensitivity and reproducibility, difficulty in sustaining high-quality facilities and staffing, and the requirement for a repeated three-visit intervention cycle of cytology/colposcopy/treatment that is prone to losses-to-follow-up. These limitations contribute to a very impractical and costly program; the cost of the U.S. program was estimated to be \$6 billion/annually in 1992. Although triennial cytology screening and careful management of abnormalities by colposcopy, biopsy, and treatment of histologically-confirmed precancers throughout most of a woman's lifetime can reduce cervical cancer incidence by up to 90%, it seems unlikely that rounds of cervical cytology screening as a preventive measure against cervical cancer will reach most of those women in the greatest need, especially since there are competing needs for the few public health dollars available. New prevention tools will need to be employed in order to impact cervical cancer rates worldwide.

In the last 20 years, unprecedented, rapid progress has been made in understanding the etiology of cervical cancer. It is now widely, if not universally, accepted that cervical infections by approximately 15 cancer-associated ("carcinogenic") human papillomavirus (HPV) genotypes cause virtually all cervical cancer and its immediate precursor lesion, cervical intraepithelial neoplasia grade 3 (CIN3) or carcinoma in situ (CIS). HPV infection is the most common sexually transmitted infection although there can be significant regional variation due to differences in cultural and sexual norms. HPV infections, even those by carcinogenic HPV genotypes, are typically transient with virtually all clearing within 1-2 years. Carcinogenic HPV infections that do not clear (i.e., persist) pose a significant risk to a woman for developing precancer, which if undetected, can invade. Thus, cancer develops rarely (on a per event basis) from an almost universal exposure to HPV. A new paradigm of cervical carcinogenesis replaces the model of stepwise progression from low-grade to high-grade morphological changes and can now be summarized based on 4 reliably measured stages: 1) HPV acquisition, 2) HPV persistence (vs. clearance), 3) progression of a persisting infection to precancer, and 4) invasion. Based on the central role of persistent carcinogenic HPV infection in the development of cervical cancer, two promising technologies targeting HPV have been developed in the fight against cervical cancer: Vaccination for preventing HPV infection and carcinogenic HPV testing for cervical cancer screening. The development of prophylactic HPV vaccines is based on the self-assembly of recombinantly-expressed L1 protein into non-infectious capsids without genetic material. Two commercial HPV vaccines have been developed, both that target HPV16 and HPV18, which cause ~70% of all cervical cancer. One also targets HPV6 and HPV11, which cause approximately 90% of genital warts. Two vaccines have shown 90% or greater efficacy for preventing persistent HPV16 and HPV18 infections and related cervical precancer in women who are not infected with these genotypes. However, these vaccines do not treat preexisting carcinogenic HPV infections and therefore are very unlikely to treat precancer or cancer and therefore do not obviate the need for screening. The next generation of vaccines will target 6 carcinogenic HPV genotypes responsible for 90%

of cervical cancers.

Carcinogenic HPV testing offers several advantages over cytology including: (1) greater sensitivity for the detection of prevalent and incipient CIN3 or cancer (\geq CIN3); (2) as a consequence of greater sensitivity, has higher negative predictive value i.e., testing negative for carcinogenic HPV DNA implies an extremely low risk of prevalent or incipient cancer/CIN3 and permits an extension of screening intervals; and (3) has greater reproducible and more consistent clinical performance. Recent randomized clinical trials have emphatically confirmed these findings. Carcinogenic HPV testing has now been approved in the United States as an adjunct to cytology for triage of equivocal cytology at all ages and for general screening in women \geq 30 years old. Based on the strength of data, it is likely that carcinogenic HPV testing will be adopted as an option for the primary screening test in the near future.

Given the robustness of both new technologies, an integrated, age-targeted approach of HPV vaccination in young, naïve women and carcinogenic HPV-based screening in older women could significantly impact cervical cancer rates worldwide. In developed countries, these HPV technologies if employed wisely could make cervical cancer prevention programs more cost-efficient and reduce the need for treatment, which has a negative impact on reproductive outcomes. However, we will need to consider rational strategies to integrate vaccination and screening to ensure women's safety and avoid costly duplication of prevention efforts in the future. In developing countries, these technologies, if made affordable, could effectively replace a lifetime program of cytology with many fewer visits and at significant cost savings. Together, HPV vaccination and screening could reduce the population risk by reducing the endemicity of HPV infection, precancer, and cancer within a 10-20 year time period.

We stand at the threshold of a sea change in the worldwide approach to the prevention of cervical cancer but a number of challenges that must first be addressed to aid those who need it most. First, these technologies must be made affordable. The introduction of HPV vaccination will probably require the involvement of donors like WHO, the Pan American Health Organization, the GAVI Alliance, or the Bill & Melinda Gates Foundation to make vaccines available and affordable. Like HPV vaccines, existing HPV tests are unaffordable and need to be done in specialized laboratories. A new HPV DNA test has been developed for low-resource regions by the Program for Appropriate Technology in Health (PATH) through a grant from the Gates Foundation. This test will provide results within a few hours with sensitivity and specificity similar to current commercially available tests, but at a cost of under US\$5. Validation studies are on-going. Other tests are likely in development. However, in scarcest-resource settings, neither HPV technologies may be afforded. In such settings, only visual inspection with acetic acid for screening may be the only realistic screening method when the alternative is nothing. Second, strategies must be developed that reduce the number of clinical visits per interventional cycle are a must. As described for cytology-based programs, three-dose vaccination, as needed for the current HPV vaccines, is liable to result in lost follow-up and incomplete vaccination. Alternative vaccine schedules, such as two-dose vaccination (which also reduces the cost), must be proven effective. HPV testing used in one-day screen and treat programs could be highly effective. Third, innovative programs will be needed that provide population coverage. For example, mother-daughter programs in which mothers are screened and daughters are vaccinated might increase compliance. Bundled, low-cost interventions might further extend the health care provided for a fixed expenditure. Fourth, there must be a commitment to treatment of screen-positive women, and there must be the recognition and understanding that a screening intervention in an unscreened, high-risk population will uncover many cases of cancers and spike incident rates. Fifth, demonstration projects are needed to provide convincing data that these new technologies should replace the standard of care, cytology/colposcopy programs. Without these projects, adoption will be slow and resistance to change will be great. Finally, these programs will require government buy-in and long-term commitment in order to reap population benefits.

Finally, given the worldwide diversity in cultural and sexual norms and

the competing health care demands, it seems improbable that any one strategy for cervical cancer prevention will meet the needs of all populations i.e., "one size does not fit all". Any intervention must be designed to meet the demands of the population it is intended to serve rather than demanding the population to use any specific intervention. Thus, interdisciplinary teams of scientists, doctors, and public health advocates will need to work in concert to implement and sustain population-specific interventions to reduce the burden of cervical cancer. Only together can we win the war against cervical cancer.