

Profile: Helmut Bartsch, PhD



Dr. Helmut Bartsch

I received my academic education and my Ph.D. at the University of Heidelberg in 1968 with a doctoral thesis on the elucidation of the structure of phorbol, the parent diterpene of the tumor promoter, 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Shortly after starting my work as a doctoral student in the newly founded German Cancer Research Center in Heidelberg (Institute of Biochemistry, Dir. Prof. E. Hecker) I could quickly prove in 1964 (it was Mardi gras) by circular dichroism that a structural formula for phorbol proposed earlier was incorrect. My successful synthesis of crystals of a phorbol heavy atom derivative allowed in 1967 the absolute structure to be determined by X-ray diffraction analysis (1). Attempts to isolate and structurally elucidate the tumor promoting phorbol

esters in croton oil had been pursued nearly for one century. In 2004 the PubMed data base listed over 5800 articles published on the pleiotropic effects of TPA, now also considered for treatment of myelocytic leukemia. My handling of large (gram!) quantities of TPA at that time caused red ears and skin irritation. I later assumed, however, that I had inverted the steps of the initiation-promotion model of skin carcinogenesis by Berenblum. Thereafter I worked on new metabolic activation pathways of aromatic amine carcinogens by one electron oxidation and *N*-acetyltransfer (2). This happened as a postdoc at the McArdle Laboratory for Cancer Research in Madison / WI under the unforgettable guidance and inspiration of the late Elizabeth C. and James A. Miller.

Biochemical and molecular epidemiology: difficult birth of a new discipline: In 1973 after returning from USA I began research at IARC in Lyon, France, under L. Tomatis, Unit Chief, with a spirit of inquiry and the hope that my efforts would contribute to the prevention and cure of human diseases. I soon realized the gap between bench work and

studies in humans themselves. In order to bridge the two groups I organized a series of multidisciplinary meetings, one of the first was a 'think tank' called Host Factors in Human Carcinogenesis (3). Judged retrospectively, the meeting held in 1981, its participants and the proceedings volume all substantially contributed to further development of a multidisciplinary approach, first introduced as 'geographical pathology' by J. Higginson (1972; the term was originally used by A. Hirsch in 1864), then 'biochemical epidemiology' (Wynder and Reddy, 1974) and 'molecular epidemiology' (Perera and Weinstein, 1982).

Below are summarized some achievements in cancer etiology and prevention research that included i) search for hitherto unknown carcinogens and mechanisms, relevant for human disease onset, ii) development and application of biomarkers for exposure and susceptibility and iii) characterization of new agents for cancer chemoprevention. Most of what I may have achieved depended heavily on the skills of my co-workers of whom it has been my good fortune to have had so

(Continued on page 4)

HIGHLIGHTS

Inside this issue:

Profile—Helmut Bartsch,	1
Profile—Michael Pereira,	4
Society Update: Structural and Name Changes for ISCaC	11

Editor:

Vernon E. Steele,
PhD, MPH

Managing Editor:

Shannon Daley

Profile — Michael A. Pereira, PhD



Dr. Michael Pereira

Dr. Michael A. Pereira is a widely recognized authority in environmental carcinogenesis and cancer chemoprevention. He is a Professor in the Department of Medicine, Division of Hematology and Oncology, Cancer Chemoprevention Program of The Ohio State University, College of Medicine and Public Health, Columbus, OH. Dr. Pereira returned to The Ohio State University in October, 2003 where he had received his Ph.D. in Pharmacology and Toxicology (1971). After a Damon Runyon Cancer Research Fellowship at NIH, he joined the New York University Medical Center, Department of Environmental Medicine where he started his research in environmental carcinogenesis. From there he went to the US Environmental Protection Agency where he focused his research on drinking water contaminants and disinfection by-products and on the mechanism of action of nongenotoxic carcinogens. This research focus has continued through his employment at Environmental Health Research and Testing, the Medical College of Ohio in Toledo, OH and currently at The Ohio State University, Columbus, OH.

In the early 1990s, Dr. Pereira added another dimension to his research that of cancer chemoprevention, especially in the colon, liver and lung. This addition was synergistic to his carcinogenesis research, since molecular pathways and techniques used in the evaluation of carcinogenic mechanisms of carcinogens were similar to those used for evaluation of chemoprevention. Chemopreventive agents are expected to have opposing mechanisms to chemical carcinogens and to modulate molecular pathways and biomarkers including surrogate end-point biomarkers in contrasting directions. Hence, a molecular pathway that is modulated by both carcinogens and chemopreventive agents, albeit in opposing directions, is likely to be an important pathway for chemoprevention. His NCI funding for chemoprevention studies has been increasing since 1990/1991 when he was awarded his first NCI grant and his first NCI contract in chemoprevention, respectively. Since then he has collaborated with NCI scientists in the Chemopreventive Agent Development Research Group on a wide variety of preclinical chemoprevention studies.

Laboratory

Dr. Pereira's laboratory has recently moved from the Medical College of Ohio in Toledo to The Ohio State University in Columbus, although he still maintains a laboratory in Toledo to perform chemoprevention studies in experimental animal models. His laboratory is divided into two sections. The Chemoprevention Bioassay Section performs animal bioassays to identify and determine the efficacy of proposed chemopreventive agents and to obtain tissue specimens for the

evaluation of surrogate end-point biomarkers. This section also performs the histology and immunohistochemistry required for the bioassays. The section is led by Paula Kramer at MCO and by Dr. Bruce Casto at OSU. The Molecular Mechanism and Biomarker Section is led by Dr. Lainhui Tao. This section performs biochemical and molecular analyses to understand the mechanism of chemopreventive agents and to develop and validate surrogate end-point biomarkers. Studies have included the evaluation in colon, liver and lung tumors of the ability of chemopreventive agents to modulate DNA hypomethylation, the hypermethylation of tumor suppressor genes, histone acetylation, and the mRNA expression and protein levels of genes related to molecular pathways associated with carcinogenesis and chemoprevention. Presently, Dr. Pereira's laboratory is supported by three post-doctoral fellows, two graduate students, and three technicians.

From Environmental Carcinogenesis to Chemoprevention

An interesting observation of his environmental carcinogenesis studies was that some nongenotoxic carcinogens under certain conditions can be demonstrated to prevent cancer and that the reverse was also true that is some chemopreventive agents can promote cancer. Chloroform, the major drinking water contaminant was demonstrated by NCI to be a liver carcinogen and, being nongenotoxic, was proposed to be a tumor promoter. Therefore, Dr. Pereira decided to demonstrate that it did in fact promote liver tumors. Surprisingly, chloroform instead of promoting

tumors was demonstrated to prevent them, probably by altering metabolism of other promoters and carcinogens in the liver [1]. He has also found that putative chemopreventive agents, including quercetin, rutin and some retinoids possess tumor promoting activity in the colon [2-4]. Since chemopreventive agents are to be administered to relatively healthy individuals, albeit individuals at risk of cancer, it is important to ensure that the agents do not significantly increase the individual's risk of cancer in another organ.

Dr. Pereira was the first to demonstrate that the chlorine disinfection by-products, dichloroacetic acid and trichloroacetic acid were liver carcinogens and acted as tumor promoters [5]. One of the proposed epigenetic mechanisms of these chemicals that he investigated was their ability to induce DNA hypomethylation. To determine whether DNA hypomethylation was critical for the carcinogenic activity of the dichloroacetic acid and trichloroacetic acid in contrast to their other biological and molecular activities, he determined the ability of methionine and chloroform to prevent liver tumors, inhibit DNA hypomethylation and modulate other biological and molecular activities induced by the two chloroacetic acids [6-9]. His studies demonstrated that prevention by methionine and chloroform of liver tumors correlated with their ability to prevent and reverse DNA hypomethylation induced by dichloroacetic acid and trichloroacetic acid, but other biological and molecular activities of the two chloroacetic acids was not altered. Thus, the results of Dr. Pereira's studies demonstrated that DNA hypomethylation was critical for carcinogenic activity of at least some nongenotoxic carcinogens and

that prevention of DNA hypomethylation is a viable mechanism for chemoprevention.

Chemopreventive Agents Slow the Progression to Cancer

Prevention of liver tumors by methionine appeared to result from the slowing of progression of altered hepatocyte foci to adenomas [9]. This was indicated by the multiplicity of both foci and tumors being decreased by the high dose level of methionine, while the low dose decreased only the multiplicity of tumors with the yield foci being increased, although not statistically significant. In another study, the effect of budesonide, a glucocorticoid drug that had been shown to prevent mouse lung tumors, was further evaluated when administered after the carcinogen for the prevention of vinyl carbamate-induced lung tumors in Strain A mice [10]. At the first sacrifice, when mainly adenomas were present, budesonide caused a large reduction in tumor multiplicity. However at later sacrifices, the percent reduction in tumor multiplicity was barely significant. Furthermore, in mice treated with budesonide the tumors were smaller and a greater percentage of the tumors were adenomas than in mice not treated with the drug, where more of the tumors were adenocarcinomas. Similar results have been obtained with other chemopreventive agents in that they have a greater effect on the rate of progression and on tumor size than tumor multiplicity.

The fact that the chemopreventive agents appear only to slow the progression to cancer suggests that, even in the continuous presence of the agents, cancer would eventually occur. It also suggests

that combinations of agents acting at different stages or by different mechanisms might slow the progression to a greater extent than individual agents acting alone. He therefore, evaluated the ability of combinations containing budesonide and R115777, a farnesyl transferase inhibitor, on vinyl carbamate-induced lung tumors [Unpublished Results]. At early sacrifices, both drugs decreased the multiplicity and size of lung tumors, while at the final sacrifice only budesonide decreased the multiplicity of tumors. However, combinations of the two drugs reduced the multiplicity and size of the tumors to a greater extent than either drug administered alone.

In summary, the results of Dr. Pereira's studies generally indicate that chemopreventive agents delay the occurrence of overt cancer by decreasing the rate of progression so that combinations of agents might be a more efficacious way to prevent cancer.

Surrogate End-Point Biomarkers for Chemoprevention

After demonstrating that DNA hypomethylation induced by nongenotoxic carcinogens was critical for carcinogenic activity and could be reversed by chemopreventive agents, it was concluded that studies be conducted to determine whether hypomethylation in tumors could also be reversed and whether it could serve as a surrogate endpoint biomarker. Another alteration in methylation found in tumors is the hypermethylation of tumor suppressor genes, that also has the potential for being a reversible alteration modulated by chemopreventive agents. An advantage shared by

(Continued on page 5)

Profile—Bartsch, continued

(Continued from page 1)

many over the past 35 years.

Successful application of screening assays in drug safety testing:

Preclinical genotoxicity testing of an antischistosomal drug Praziquantel (a product developed by Bayer AG and Merck, Germany) was required as other drugs in use gave rise to serious side-effects. The companies through the WHO requested Praziquantel to be tested in a battery of short-term tests, and only if found to be devoid of any genetic effects they would proceed with further safety evaluations. When tested in the best assays available at that time, the uniform absence of any genetic activity of Praziquantel (4) led to clinical trials and later to approval by drug control agencies. This very efficient antischistosomal drug preventing parasite-associated disease is now used world wide and has and will continue to save millions of lives.

Field and laboratory studies on risk factors for esophageal cancer in Iran and China:

Studies on risk factors in the Caspian littoral of Iran carried out by IARC researchers had implicated nutritional deficiencies, thermal injury by drinking hot tea and chewing opium pipe residues (opium dross, a black tarry material). In the 1970s we started to characterize DNA-damaging agents, shown to be present in opium dross. By activity-guided isolation and biological testing, we were able to characterize potent mutagens from these pyrolysates, all derived from the parent compound, morphine, by mild pyrolysis (5). The pyrolysate mixtures were also shown to produce tumors in rodents. In an attempt to link ingestion of these opium pipe residues to

esophageal cancer, we measured morphine metabolites in urine of over 1500 individuals as an indicator of opium use. High urinary concentrations of morphine metabolites were more prevalent in high than in low incidence areas for esophageal cancer, and in members of households with a case than in members of control households in the same village (6). These initial results provided some support that opium (dross) use is an etiological factor for esophageal cancer in this region,

orally, scavenges nitrosating agents to form NPRO which is excreted unchanged in the urine. We tried to determine whether endogenous NOC play a role in esophageal cancer in China and participated in a large survey on diet, lifestyle and mortality in 69 counties representing a 300-fold range of mortality rates from esophageal cancer (coordinated by J. Chen, R. Peto and T.C. Campbell). Biological specimens were collected from over 4000 subjects and markers for ex-

The policy 'Put Prevention First' was adopted in the rapidly successful control of much of the infectious disease that afflicted the newly established People's Republic of China, and it is just as relevant to the control of the old and the new causes of the non-communicable disease that are now responsible for most premature deaths. (Excerpt from Foreword to Monograph, Chen et al., 1990, ref. 8).

預防為主

but it became clear that this habit alone could not account for the incidence of esophageal cancer type II in the rest of the Asian 'cancer belt', such as China. Our attention was directed to carcinogenic *N*-nitroso compounds (NOC) endogenously produced from dietary precursors and/or as a result of chronic inflammatory processes. As they probably escaped detection new non-invasive detection methods were required to establish their etiological role.

To measure endogenous NOC formation in humans, the non-invasive *N*-nitrosoproline (NPRO) test was developed (7). Proline, when given

posure to carcinogens (NOC) were measured. The results of this ecological study (8) were printed as a monograph (in Chinese and English, see excerpt above). Some important results (9) included: (i) a significant positive correlation between mortality rates from esophageal cancer and exposure to endogenous NOC; (ii) less strong correlations between exposure to NOC and mortality rates from other cancers (nasopharynx and leukemia) and (iii) with respect to prevention, a marked reduction of endogenous NOC synthesis by ingestion of the nitrosation inhibitor vitamin C.

(Continued on page 6)

Profile—Pereira, continued

(Continued from page 3)

DNA hypomethylation and hypermethylation of genes are qualitative differences, i.e., there is or is not methylation at a CpG site. To determine whether chemopreventive agents modulate methylation in tumors, Dr. Pereira has been using chemical carcinogens to induce tumors in mouse colon, liver and lung and in rat colon. Once the tumors are present, chemopreventive agents are administered for a short duration, of one to a few weeks, to determine whether they reversed the DNA hypomethylation and decreased hypermethylation of tumor suppressor genes. This procedure has the advantage of mimicking clinical chemoprevention trials in which agents are administered to patients with lesions in order to demonstrate that the agent could exert activity in the lesion consistent with chemoprevention. He has investigated whether biomarkers are modulated in tumors using long-term treatment starting prior to the appearance of lesions and whether such treatment would modulate tumor biomarkers. However, these attempts were hindered by technical problems, e.g., in some studies the chemopreventive agents were too active yielding an insufficient number of lesions and tumors for use in evaluating biomarkers. Also, tumors present at the end of the chemopreventive studies possessed many of the molecular characteristics of tumors from untreated animals, hindering determination of statistically significant modulation of biomarkers.

The DNA in colon and lung tumors in mice or rats was hypomethylated relative to non-involved tissues. When putative chemopreventive agents were ad-

ministered for 5-14 days after the occurrence of the tumors, agents that had been shown to prevent tumors in the organ and species being studied reversed the hypomethylation. In contrast, agents that were ineffective in preventing tumors were also ineffective in reversing hypomethylation. Bexarotene, budesonide, R115777 and combinations of these agents prevented lung tumors in mice and, after 14 days of treatment, reversed DNA hypomethylation in mouse lung tumors, while rosiglitazone did not prevent lung tumors and did not reverse the hypomethylation [10-13, Unpublished Results]. In rat colon, DNA hypomethylation in tumors was reversed by seven days of treatment with chemopreventive agents effective in preventing colon cancer (calcium chloride, celecoxib, α -difluoromethylornithine [DFMO], piroxicam, sulindac and combinations containing celecoxib and DFMO), while agents that did not prevent colon cancer in rats (low dose aspirin, 2-carboxyphenyl [retinamide], quercetin, 9-cis retinoic acid, and rutin) did not reverse the hypomethylation [14, 15]. Hence, Dr. Pereira's results indicate that reversal of DNA hypomethylation can be used as a biomarker for chemoprevention and distinguish susceptibility for chemoprevention. However, only a limited number of agents known to be either efficacious or ineffective in preventing tumors in the lung or colon and in mice and rats have so far been evaluated for the ability to reverse DNA hypomethylation. Hence, Dr. Pereira is continuing these studies to further determine the accuracy and specificity of reversal of DNA hypomethylation as a surrogate end-point biomarker.

Reversal by chemopreven-

tive agents of the hypermethylation of tumor suppressor genes in colon and lung is another area of active research by Dr. Pereira. He has demonstrated that the estrogen receptor- α is hypermethylated in rat colon tumors [15]. After short-term treatment with two chemopreventive agents, celecoxib DFMO the hypermethylation of the gene was reversed. Reversal of hypermethylation was dose-dependent as well as time dependent with 14 days of treatment being more effective than shorter durations. Combinations containing the two chemopreventive agents were far more efficacious than either agent alone resulting in a greater reversal and in a shorter time. The reversal of hypermethylation of the estrogen receptor- α gene was associated with increased mRNA expression of the gene. This would suggest that combinations containing the two drugs would be more efficacious than either drug acting alone in preventing colon cancer. Although Dr. Pereira's studies have, as yet, not yielded a clear mechanism by which chemopreventive agents modulate the methylation of DNA and the estrogen receptor- α gene in colon tumors, they do identify two surrogate end-point biomarkers for chemoprevention in the colon and other organs, i.e. reversal of global DNA hypomethylation and the hypermethylation of tumor suppressor genes.

References

1. Pereira, M. A., Knutsen, G. L. and Herren-Freund, S. L. Effect of subsequent treatment of chloroform or phenobarbital on the incidence of liver and lung tumors initiated by ethylnitrosourea in 15 day old mice. *Carcinogenesis*, 1985; 6: 203-207.
2. Pereira, M. A., Li, H., Grubbs, C.,

(Continued on page 7)

Profile—Bartsch, continued



(Continued from page 4)

Results from further international studies suggested to us that the burden of NOC exposure in humans is determined not only by ingestion of NOC and their precursors, but also by synthesis in the body through inflammatory processes *via* esophagitis, gastritis and chronic infections leading to overproduction of NO. We hypothesized (10) that chronic inflammatory conditions lead, *via* iNOS and activated macrophages, to a cascade of adverse reactions, including production of reactive oxygen (ROS) and nitrogen species that attack DNA. ROS were implicated in oral carcinogenesis as they were shown to be generated in substantial amounts *in vitro* and in the oral cavity of betel quid chewers for the first time (Nair et al., 1987; Nair et al., 1995). Whether such DNA damage, when chronically overproduced in cancer-prone tissues drives genomic instability and carcinogenesis, was investigated more intensively at the Division of Toxicology and Cancer Risk Factors at the DKFZ. After 20 years of service at IARC/WHO I was offered in 1993 a position as Division Head and Professor at the University of Heidelberg. Currently pursued projects and accomplishments by my research group (see photograph) are briefly summarized below.

DNA adducts as biomarkers in cancer etiology and prevention

Persistent oxidative stress enhances lipid peroxidation (LPO) implicated in the promotion and progression stages of carcinogenesis, particularly under conditions of chronic inflammation and infections. The mechanisms involve tissue and DNA damage caused by reactive oxygen/nitrogen species and LPO-endproducts resulting from unbalanced pro-oxidant state of the cells.

Our group was among the first to demonstrate conclusively that exocyclic etheno-DNA adducts are formed upon LPO of ω -6 polyunsaturated fatty acids (ω -6 PUFA), such as arachidonic acid and that such adducts are present in liver and other tissues of unexposed rodents and humans (11, 12). These miscoding etheno-DNA adducts are formed by the reaction of DNA-bases with 4-hydroxynonenal upon epoxidation, and also from the metabolites of the human carcinogen vinyl chloride and multi-organ rodent carcinogen urethane (compiled in 13). With seminal contributions from my co-workers, J. Nair, principal investigator, (DKFZ) and A. Barbin (IARC) we have developed ultrasensitive methods to measure etheno-DNA adducts (ethenodeoxyadenosine (edA) and ethenodeoxycytidine (edC)) by an immunoaffinity-³²P-postlabelling method (12), edA in urine by immunoenriched-HPLC-fluorescence detection and edA in cells by immunohistochemistry. Using these tools we have demonstrated that etheno-DNA adducts are elevated in an age- and copper-dependant manner in the liver DNA of LEC-rats (a model for human Wilson's disease) (14), in the liver of cancer prone Wilson's disease and primary hemochromatosis patients (Nair et al., 1998) and in those with alcohol abuse-related liver diseases (Frank et al. 2004). Elevated etheno-DNA adducts were found

in WBC in female subjects who are on a high ω -6 PUFA-diet (15), but intake of Vit. E and the ratio of different fatty acids in the diet effected their steady state adduct levels. Etheno-DNA adduct levels were elevated in the DNA isolated from colonic polyps of FAP patients suggesting an association of these adduct levels with COX-2 expression (Schmid et al., 2000). In rodent models we have shown that etheno-DNA adduct levels were markedly high as a result of nitric oxide overproduction (Nair et al., 1998) and of increased lipoxygenase-8 and -12 activities during mouse skin carcinogenesis (Nair et al., 2000). Taken together LPO-derived etheno DNA adducts are promising tools for quantifying increasing loads of promutagenic DNA damage in early stages of the carcinogenesis process and for verifying the efficacy of chemopreventive measures in humans. In order to facilitate such studies, we have developed a non-invasive urine assay which was successfully applied in human trials. A wide range of other biomarkers has been previously explored in human studies (reviewed in 16); new analytical methods were developed for detection of *O*⁴-ethylthymidine in smokers' lung and of the malondialdehyde-deoxyguanosine-adduct (M₁dG) in human tissues and WBC (17, 18).

Genetic polymorphisms and DNA repair capacity as modifiers of cancer risk: The overall aim is to evaluate the relevance of enzyme polymorphisms in relation to a) the risk of developing environmental cancers, b) the chemotherapy and radiation sensitivity, c) the prognosis of lung cancer pa-

(Continued on page 8)

Profile—Pereira, continued

(Continued from page 5)

- Barnes, L.H., Olson, G.R., Eto, I., Whitaker, L., Kelloff, G.J., Steele, V.E., and Lubet, R.A. Effects of the phytochemicals, curcumin and quercetin upon azoxymethane-induced colon cancer and DMBA-Induced Mammary Cancer in rats. *Carcinogenesis*, 1996; 17: 1305-1311.
3. Zheng, Y., Kramer, P.M., Olson, G., Lubet, R.A., Steele, V. E., Kelloff, G.J. and Pereira, M. A. Prevention by retinoids on azoxymethane-induced colon cancer and aberrant crypt foci and their modulation of cell proliferation in rats. *Carcinogenesis*, 1997; 18: 2119-2125.
 4. Tao, L., Kramer, P.M., Wang, W., Yang, S., Lubet, R.A., Steele, V.E., and Pereira, M.A. Altered Expression of c-Myc, P16 and P27 in Rat Colon Tumors and Its Reversal by Short-term Treatment with Chemopreventive Agents. *Carcinogenesis*, 2002; 23: 1447-1454.
 5. Herren-Freund, S.L., Khoury, M.D., Olson, G., and Pereira, M. A. The Carcinogenicity of trichloroethylene and its metabolites, trichloroacetic acid and dichloroacetic acid in mouse liver. *Toxicol. Appl. Pharmacol.*, 1987; 90: 183-189.
 6. Tao, L., Kramer, P.M., Ge, R., and Pereira, M.A. Effect of Dichloroacetic Acid and Trichloroacetic Acid on DNA Methylation in Liver and Tumors of Female B6C3F1 Mice. *Toxicological Sciences*, 1998; 43: 139-144.
 7. Tao, L., Ge, R., Xie, M., Kramer, P.M. and Pereira, M. A. Effect of Trichloroethylene and Its Metabolites, Dichloroacetic Acid and Trichloroacetic Acid on the Methylation and Expression of c-*Jun* and c-*Myc* Protooncogenes in Female B6C3F1 Mouse Liver: Prevention by Methionine. *Toxicological Sciences*, 2000; 54: 399-407.
 8. Pereira, M.A., Kramer, P. M., Conran, P.B. and Tao, L. Effect of Chloroform on Dichloroacetic Acid and Trichloroacetic Acid-Induced Hypomethylation and Expression of the c-*Myc* Gene and on Their Promotion of Liver and Kidney Tumors in Mice. *Carcinogenesis*, 2001; 22:1511-1519.
 9. Pereira, M.A., Wang, W., Kramer, P.M., and Tao, L. Prevention by Methionine of Dichloroacetic Acid-induced Liver Cancer and DNA Hypomethylation in Mice. *Toxicological Sciences*, 2004; 77: 243-248.
 10. Pereira, M.A., Li, Y., Gunning, W.T., Kramer, P.M., Al-Yaqoub, F.S., Lubet, R.A., Steele, V.E., Szabo, E., and Tao, L. Prevention of Mouse Lung Tumors by Budesonide and Its Modulation of Biomarkers. *Carcinogenesis*, 2002; 23: 1185-1192.
 11. Tao, L., Li, Y., Wang, W., Kramer, P.M., Gunning, W.T., Lubet, R.A., Steele, V.E., and Pereira, M.A. Effect of Budesonide on the Methylation and mRNA Expression of the IGF-II and c-Myc Genes in Mouse Lung Tumors. *Molecular Carcinogenesis*, 2002; 35: 93-102.
 12. Pereira, M.A., Tao, L.H., Wang, W., Gunning, W.T.Y. and Lubet, R.A. Chemoprevention: Mouse Colon and Lung Tumor Bioassay and Modulation of DNA Methylation as a Biomarker. *Experimental Lung Research*, 2004; In press.
 13. Gunning, W.T., Kramer, P.M., Ronald A. Lubet, R.A., Steele, V.E., End, D.W., Wouters, W., and Pereira, M.A. Chemoprevention of Benzo(a)pyrene-Induced Lung Tumors in Mice by the Farnesyl Transferase Inhibitor R115777. *Clinical Cancer Research*, 2003; 9: 1927-1930.
 14. Tao, L., Wang, W., Kramer, P. M., Lubet, R.A., Steele, V.E. and Pereira, M.A. Modulation of DNA hypomethylation as a surrogate endpoint biomarker for chemoprevention of colon cancer. *Molecular Carcinogenesis*, 2004; 39: 79-84.
 15. Pereira, M.A., Tao, L., Wang, W., Li, Y., Umar, A., Steele, V. E., and Lubet, R.A. Modulation by Celecoxib and Difluoromethylornithine of the Methylation of DNA and the Estrogen Receptor- α Gene in Rat Colon Tumors. *Carcinogenesis*, 2004. (In Press.)
-

Profile—Bartsch, continued

(Continued from page 6)

tients and d) the risk for attracting second primary lung tumors (within an EU-collaborative study). Investigations on tumors of the lung, larynx, breast, oral cavity with known or suspected environmental etiology are being conducted (A. Risch, P. Schmezer principal investigators, together with H. Dally, B. Spiegelhalter). In a large hospital based case-control study on lung cancer, differences in the frequency of genetic polymorphisms in xenobiotic metabolizing enzymes and in DNA-repair capacity are being investigated. Occupational and smoking history of lung cancer patients and hospital controls is recorded over 1800 blood (tissue) samples have been collected and genotyped for known and newly identified genetic polymorphisms including *NAT1*, *NAT2*, *GSTM1*, *GSTT1*, *GSTM3*, *GSTP1*, *CYP1B1*, *CYP1A1*, *CYP3A4*, *MPO*, *hOGG1*. Based on results one must distinguish between the different histological types of lung tumors, when evaluating the risk associated with different genotypes (19). The *MPO* genotype as well as the *CYP3A4* but not the *CYP3A5* genotype were identified as modifiers of risk of small cell lung cancer (20). *GSTM1/T1* null genotypes were identified as strong risk factors for oral leukoplakia in ethnic Indian betel quid / tobacco chewers (Nair et al., 1999). The impact of combinations of cancer-predisposing genes was explored in smokers and PAH-exposed workers by using PAH-DNA adducts as risk markers. The combined mutated *CYP1A1* and the *GSTM1* null genotypes were shown to lead to a stronger increase of benzo(a)pyrene diol-epoxide DNA adduct levels in lung and WBC than the wild-type combination (21). In Japanese this 'at risk' genotype combination was most susceptible to smoking-induced lung cancer, now also confirmed in Caucasians (Hung

et al., 2003). Other 'at risk' combinations (*MPO*, *GSTM1*, *GSTT1*, *NAT1*, *NAT2*) were investigated together with tobacco related DNA-adducts and more susceptible subgroups among smokers and coal-tar treated patients defined (Godschalk et al., 2001). After optimization of single cell microgel electrophoresis (comet) techniques for detecting genotoxic damage in small murine and human biopsies (Schmezer et al., 2000), this assay was used to monitor DNA repair capacity and mutagen sensitivity, induced by mutagens or ionizing radiation in peripheral blood lymphocytes (P. Schmezer and O. Popanda principal investigators together with G. Werle-Schneider, C. Mayer). In a case-control study increased bleomycin sensitivity and a reduced DNA repair capacity were found to be independent risk factors for non-small cell lung cancer (22, 23). Furthermore, as shown in a case-control study on laryngeal cancer, lymphocytes from cancer patients had a reduced capacity to synthesize poly (ADP)ribose (24). Comet assay, specific enzyme activities, and expression profiles generated by cDNA arrays (carrying 130 human repair related genes, Mayer et. al, 2002) together with quantitative real-time RT-PCR are now used to identify high risk individuals (e.g. radiotherapy patients, Popanda et al., 2003) characterised by impaired DNA repair capabilities. In several case-control studies different PCR techniques are applied to identify individuals with specific repair gene polymorphisms (*XPA*, *XPB*, *XRCC1/2/3*, *NBS1*, *OGG1*, *APE1*).

Cancer chemoprevention research and intervention trials: A research group 'Cancer Chemoprevention' was established in my Division in 1996, when C. Gerhäuser (principal investigator together with N. Frank) joined from John Pezzuto's lab at UIC in Chicago (25). Major aims are to identify

and evaluate new effective chemopreventive (lead) compounds, elucidation of their mechanism of action, demonstration of efficacy in animal models and of effects on biomarkers in human intervention studies. *In vitro* targets for the identification of novel agents include carcinogen-metabolism, reactive oxygen species, inflammation, hormones, proliferation, apoptosis (26) and angiogenesis. Constituents of *Brassica* vegetables, especially the isothiocyanate sulforaphane (SFN), and polyphenols from various sources (27) are compounds of major interest. Xanthohumol (XN), a prenylated chalcone from hop was identified as a novel potential chemopreventive agent acting by a broad spectrum of mechanisms involving all above mentioned targets. Also XN potentially inhibited DMBA-induced pre-neoplastic lesions in mouse mammary organ culture at 200-fold lower concentrations than resveratrol (28); investigations on *in vivo* efficacy are ongoing. We have described novel anti-inflammatory properties of SFN, including inhibition of iNOS, Cox-2 and TNF- α induction, by thiol-dependent inhibition of transcription factor NF- κ B binding to DNA (29). We also identified SFN, XN and a series of polyphenols, chalcones and Bibenzyl derivatives of lunularic acid as novel potent inhibitors of angiogenesis in a human *in vitro* test system (30). XN and SFN dose-dependently reduced newly formed capillary growth at physiological concentrations; they also potentially inhibited tube-formation and migration of microvascular endothelial cells *in vitro*. Both compounds strongly lowered mRNA expression of pro-angiogenic factors (VEGF, iNOS, Cox-2, HIF-1 α , c-

Myc and matrix-metalloproteases MMP-2 and -9). The anti-angiogenic and anti-tumor effect of XN against human MX-1 breast tumor xenografts was further demonstrated *in vivo* by intravital fluorescent microscopy in the dorsal skinfold chamber model in SCID mice (Bertl et al., 2004). The potent anti-angiogenic activities, detected for compounds of various structural classes might represent an important common characteristic of chemopreventive agents. Research is warranted to verify whether anti-angiogenic constituents of our diet can strengthen the host's defense mechanisms (*via* endostatin, angiostatin, etc.), so as to prevent the progression of pre-neoplasias or carcinomas *in situ* into life-threatening tumors.

Other milestones and highlights in the chemoprevention field (R. W. Owen and B. Spiegelhalter, principal investigators) were: i) the completion of a calcium/fiber intervention study (in collaboration with the European Agency for Cancer Prevention) in polypectomized adenoma patients: Calcium reduced adenoma recurrence while soluble fiber (Fybogel) significantly increased recurrence (31). ii) We established complete profiles (32, 33, Owen et al., 2003) of chemopreventive antioxidants in components of the Mediterranean diet (olives, olive oils, carob fiber) with full structure assignment by NMR, correcting mistakes published in the literature. iii) Also we initiated international collaborative studies on chemopreventive substances with scientific institutes in Thailand (tropical fruits and waste products), China (medicinal plants), Brazil (Amazonian medicinal plants), and Africa (Argan spinosa products and medicinal plants) and iv) we developed new liquid chromatography-electrospray mass spectrometry methods to assay chemopreventive antioxidants in serum and urine with minimal work-up procedures. The methodology was validated in a pilot intervention study with Linseeds in high-risk breast cancer sub-

jects.

Perspectives: Implementation of cancer chemoprevention requires more coordinated efforts world wide, to include: i) intense search for better chemopreventive agents (and combinations) with high efficacy and low or no long-term toxicity; ii) understanding of their mode of action at a molecular, cellular and *in vivo* level; iii) development and validation of cancer predictive biomarkers for evaluating the efficacy of new agents in shorter periods at lower cost; iv) a wider ethically approved clinical use of chemopreventive agents in smaller groups of high risk subjects (e.g. those with accessible pre-neoplastic lesions and v) propagation for use of safe agents in human populations at relatively low disease risk. Taken together, progress in genomic and post-genomic research is providing new agents and tools to be explored for early reversal of carcinogenesis offering a better quality of life than therapy of end-stage disease. So we can add life to years, not only years to life!

Selected References (not all citations in text are listed)

- 1 Bartsch H, Hecker E. Circular dichroism und Röntgenstruktur analyse des Phorbols. In: Aktuelle Probleme aus dem Gebiet der Cancerologie II, Berlin, Springer-Verlag, 1968; pp. 162-169.
- 2 Bartsch H, Dworkin M, Miller JA, Miller EC. Electrophilic *N*-acetoxyaminoarenes derived from carcinogenic *N*-hydroxy-*N*-acetyl aminoarenes by enzymatic deacetylation in liver. *Biochim. Biophys. Acta*, 1972;286,272-298.
- 3 Bartsch H, Armstrong B. (eds.). Host Factors in Human Carcinogenesis IARC Scientific Publications No. 39, IARC Press, Lyon, 1982.
- 4 Bartsch H, Kuroki T, Malaveille C, Loprieno N, Barale R, Abbondandolo A, Bonatti S, Rainaldi G, Vogel E, Davis A. Absence of mutagenicity of praziquantel, a new, effective, anti-schistosomal drug, in bacteria, yeasts, insects and mammalian cells. *Mutation Research*, 1978;58:133-142.
- 5 Friesen M, O'Neill IK, Malaveille C, Garren L, Hautefeuille A, Bartsch H. Substituted hydroxyphenanthrenes in opium pyrolysates implicated in oesophageal cancer in Iran: structures and in-vitro metabolic activation of a novel class of mutagens. *Carcinogenesis*, 1987;8:1423-1432.
- 6 Ghadirian P, Stein GF, Gorodentzky C, Roberfroid MB, Mahon GAT, Bartsch H, Day NE. Oesophageal cancer studies in the Caspian littoral of Iran: some residual results, including opium use as a risk factor. *International Journal of Cancer*, 1985;35:593-597.
- 7 Ohshima H, Bartsch H. Quantitative estimation of endogenous nitrosation in humans by monitoring *N*-nitrosoproline excreted in the urine. *Cancer Research*, 1981;41:3658-3662.
- 8 Chen J, Campbell TC, Liu J, Peto R. Diet, Life-style and Mortality in China, Oxford University Press, 1990.
- 9 Wu Y, Chen J, Ohshima H, Pignatelli B, Boreham J, Li J, Campbell TC, Peto R, Bartsch H. Geographic association between urinary excretion of *N*-nitroso compounds and oesophageal cancer mortality in China. *Int. J. Cancer*, 1993;54:713-719.

(Continued on page 10)

Profile—Bartsch, continued

(Continued from page 9)

- 10 Ohshima H, Bartsch H. Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. *Mutation Research*, 1994;305:253-264.
- 11 Bartsch H, Barbin A, Marion M-J, Nair J, Guichard Y. Formation, detection and role in carcinogenesis of ethenobases in DNA. *Drug Metabolism Review*, 1994;26:349-371.
- 12 Nair J, Barbin A, Guichard Y, Bartsch H. 1, N⁶-Ethenodeoxyadenosine and 3, N⁴-ethenodeoxycytidine in liver DNA from humans and untreated rodents detected by immunoaffinity/³²P-postlabeling. *Carcinogenesis*, 1995;16:613-617.
- 13 Singer B, Bartsch H. (eds) IARC Scientific Publications No. 150 Exocyclic DNA Adducts in Mutagenesis and Carcinogenesis. IARC Press, Lyon, 1999; pp. 1-357.
- 14 Nair J, Sone H, Nagao M, Barbin A, Bartsch H. Copper-dependent formation of miscoding etheno-DNA adducts in the liver of Long Evans cinnamon (LEC) rats developing hereditary hepatitis and hepatocellular carcinoma. *Cancer Research*, 1996;56:1267-1271.
- 15 Nair J, Vaca CE, Velic I, Mutanen M, Valsta LM, Bartsch H. High dietary omega-6 polyunsaturated fatty acids drastically increase the formation of etheno-DNA base adducts in white blood cells of female subjects. *Cancer Epidemiol Biomarkers Prev.*, 1997;6:597-601.
- 16 Bartsch H. Studies on biomarkers in cancer etiology and prevention: a summary and challenge of interdisciplinary research. *Mutation Research*, 2000;462:255-279.
- 17 Godschalk R, Nair J, van Schooten FJ, Risch A, Drings P, Kayser K, Dienemann H, Bartsch H. Comparison of multiple DNA adduct types in tumor adjacent human lung tissue: effect of cigarette smoking. *Carcinogenesis*, 2002; 23:2081-2086.
- 18 Sun X, Nair J, Bartsch H. A modified immuno-enriched ³²P-postlabeling method for analyzing the malondialdehyde-deoxyguanosine adduct, 3-(2-deoxy-beta-D-erythro-pentofuranosyl)-pyrimido[1,2-alpha]purin-10(3H)one in human tissue samples. *Chem Res Toxicol.*,2004;17:268-272.
- 19 Risch A, Wikman H, Thiel S, Schmezer P, Edler L, Drings P, Dienemann H, Kayser K, Schulz V, Spiegelhalter B, Bartsch H. Glutathione-S-transferase M1, M3, T1 and P1 polymorphisms and susceptibility to non-small-cell lung cancer subtypes and hamartomas. *Pharmacogenetics*, 2001;11:757-764.
- 20 Dally H, Edler L, Jäger B, Schmezer P, Spiegelhalter B, Drings P, Dienemann H, Schulz V, Kayser K, Bartsch H, Risch A. The *CYP3A4* 1B allele increases risk for small cell lung cancer: effect of gender and smoking dose. *Pharmacogenetics*, 2003;13,607-618.
- 21 Rojas M, Alexandrov K, Cascorbi I, Brockmöller J, Likhachev A, Bouvier G, Auburtin G, Mayer L, Roots I, Bartsch H. High benzo[a]pyrene diol-epoxide DNA adduct levels in lung and blood cells from subjects with combined CYP1A1 MspI/MspI-GSTM1 0/0 genotypes. *Pharmacogenetics*, 1998;8:109-118.
- 22 Schmezer P, Rajae-Behbahani N, Risch A, Thiel S, Rittgen W, Drings P, Dienemann H, Kayser KW, Schulz V, Bartsch H. Rapid screening assay for mutagen sensitivity and DNA repair capacity in human peripheral blood lymphocytes. *Mutagenesis*, 2001;16:25-30.
- 23 Rajae-Behbahani N, Schmezer P, Risch A, Rittgen W, Kayser KW, Dienemann H, Schulz V, Drings P, Thiel S, Bartsch H. Altered DNA repair capacity and bleomycin sensitivity as risk markers for non-small cell lung cancer. *International Journal of Cancer*, 2001;95:86-91.
- 24 Rajae-Behbahani N, Schmezer P, Ramroth H, Bürkle A, Bartsch H, Dietz A, Becher H. Reduced poly(ADP-ribosylation) in lymphocytes of laryngeal cancer patients: results of a case-control study. *International Journal of Cancer*, 2002;98:780-784.
- 25 Gerhäuser C, You Y, Liu J, Moriarty RM, Hawthorne M, Mehta RG, Moon RC, Pezzuto JM. Cancer chemopreventive potential of sulforamate, a novel analog of sulforaphane that induces Phase 2 drug-metabolizing enzymes. *Cancer Research*, 1997;57:272-278.
- 26 Gerhäuser C, Klimo K, Heiss E, Neumann I, Gamal Eldeen A, Knauff J, Liu G, Sitthimonchai S, Frank N. Mechanism-based *in vitro* screening of potential cancer chemopreventive agents. *Mutation Research*, 2003;523-524:163-172.
- 27 Gerhäuser C, Alt AP, Klimo K, Knauff J, Frank N, Becker H. Isolation and potential cancer chemopreventive activities of phenolic compounds of beer. *Phytochemistry Reviews*, 2002;1:369-377.
- 28 Gerhäuser C, Alt A, Heiss E, Gamal-Eldeen A, Klimo K, Knauff J, Neumann I, Scherf HR, Frank N, Bartsch H, Becker H. Cancer chemopreventive ac-

tivity of Xanthohumol, a natural product derived from hop. *Molecular Cancer Therapy*, 2002;1:959-969.

- 29 Heiss E, Herhaus C, Klimo K, Bartsch H, Gerhäuser C. Nuclear factor kB is a molecular target for sulforaphane-mediated anti-inflammatory mechanisms. *Journal of Biological Chemistry*, 2001;276:32008-32015.
- 30 Bertl E, Klimo K, Heiss E, Klenke F, Pescshke P, Becker H, Eicher T, Herhaus C, Kapadia G, Bartsch H, Gerhäuser C. Identification of novel inhibitors of angiogenesis using a human *in vitro* angiogenic assay. *Int. J. Cancer Prev.*, 2004, in press.
- 31 Bonithon-Kopp C, Kronborg O, Giacosa A, Räth U, Faivre J. [Experts:-Milan, C., Fenger, C., Piard, F., Belghiti C., Owen, R. W., and Pignatelli, M.]. Calcium and fibre supplementation in the prevention of colorectal adenoma recurrence: a placebo-controlled intervention trial from the European Cancer Prevention Organisation (ECP). *Lancet*, 2000;356:1300-1306.
- 32 Owen RW, Giacosa A, Hull WE, Haubner R, Würtele G, Spiegelhalter B, Bartsch H. Olive oil consumption and health: the possible role of antioxidants. *Lancet Oncology*, 2000;1:107-112.
- 33 Owen RW, Haubner R, Mier W, Giacosa A, Hull WE, Spiegelhalter B, Bartsch H. The isolation, structural elucidation and antioxidant potential of the major phenolic compounds in brined olive drupes. *Food Chem Toxicol.*, 2003;41:703-717.

Society Update: Structural and Name Change for ISCaP

Dr. Hans-Jörg Senn

The last ISCaC-Annual Symposium in St. Gallen, Switzerland—coupled with the 3rd International Conference on “Controversies in Tumor Prevention & Genetics” from February 12-14, 2004 was a real scientific and social success, although the attendance (around 165 in total from 32 countries) could have been better. The meeting was heavily competed by an ISPO Conference in Nice, France (not too far from St. Gallen) which was shifted to Southern France from Montreal, Canada—thus immediately preceding the St. Gallen meeting, which was an unfortunate and confusing action for many.

During the St. Gallen conference, there was also a meeting of the ISCaC board members present, as several changes had evolved since 2003, when the planned former ISCaC board meeting in Toronto (April 2003) was canceled together with AACR Congress due to SARS!

The ISCaC board discussed several ways to secure the future and status of our society, its potential affiliations with other similar societies and its lack of its own journal to “spread” its aim and scope. There were also discussions about changes in the presidency and the re-election/composition of the Board, which were, however, postponed until 2005. These deliberations went on very intensely after the St. Gallen TUP-2004 Conference, and resulted in several concrete decisions, taken recently by the Executive Committee of the Board:

1. ISCaC—in order to escape its too narrow perception—has changed its name to “ISCaP” (International Society of Cancer Prevention), since many other kind of interventions, such as lifestyle changes, endocrine, immunological and biological approaches, etc. are emerging even in primary cancer prevention, which

are not sufficiently represented by the term “chemo”, which is rather an “adverse” term in many parts of the world today.

2. We have adopted the new “International Journal of Cancer Prevention” (IJCP) to be the “official journal” of our re-named society. The editor-in-chief of this new journal is Dr. Alaa Badawi from Fox Chase Cancer Center, in Philadelphia, PA. The new journal’s publisher (Nova Science Publisher, Inc., Hauppauge, NY), which was contacted by us after the St. Gallen meeting in February 2004, was willing to also change its name from “International Journal of Cancer Chemoprevention” to “International Journal of Cancer Prevention”—thus enlarging its aims and scope likewise to ISCaP above. ISCaP was offered 5-6 editorial positions, which were meanwhile assigned by our president, Frank Meyskens. (This is in addition to several other ISCaP members, who were already selected for this editorial task by officials of the new journal.)

3. We are presently exploring potential affiliations/mergers with similar groups such as the International Society for Predictive Oncology (ISPO) and we are trying to link with ASCO, ASPO, ESMO and the NCI for future joint conference planning on both sides of the Atlantic and/or Pacific.

I hope you enjoy this message and we are looking forward to your reaction—as well as to the first issue of the new IJCP. If you have any helpful suggestions, please let us know.