221 **CHARACTERIZATION OF A LUNG EPITHELIAL CELL LINE DERIVED FROM MUTA™MOUSE TO LUNG TISSUE USING DIFFERENT MICROARRAY PLATFORMS.** Yauk CL[1], Berndt L[1], Gingerich J[1], White PA[1], Douglas GR[1]. [1]Mutagenesis Section, Safe Environments Program, Health Canada, Ottawa, K1A 0L2.

Despite the value of *in vitro* toxicology and its widespread use, questions remain regarding extrapolations from cell cultures to living organisms. The detailed characterization of a cell line, and its comparison to cells from the original tissue can be extremely time-consuming. By using microarray technology, we can evaluate gene expression for the majority of the mouse genome, and rapidly characterize the attributes of cell lines derived from any tissue. Here, we show the results of our efforts to employ microarray technology to characterize our in-house developed Muta™Mouse epithelial lung cell line compared to its parent tissue (whole mouse lung). Confluent cells (5-10 day incubation after 100% confluence is reached) or saline-perfused lungs from mature mice were homogenized and RNA was isolated using TriZol extraction and RNEasy columns (Qiagen). Reference RNA (Stratagene) and either lung or cell line RNA was amplified in the presence of Cy5-dCTP or Cy3-dCTP and purified using PCR clean-up columns, as recommended by the microarray supplier. The cDNA probes were hybridized overnight, washed and subsequently scanned on a Virtek ChipReader (Virtek Inc., Waterloo, Ontario). Gene expression in the MutaTMMouse cells were compared to whole lung preparations using several different commercially available microarray chips, including both cDNA platforms (e.g., Ontario Cancer Institute, 15K NIA mouse cDNA microarrays) as well as oligonucleotide arrays (e.g., Agilent Technologies). We present the differences in gene expression between the cells and parent tissue, as well as an evaluation of the various commercial chips.

222 **EFFECT OF SMOKING HABIT ON THE FREQUENCY OF MICRONUCLEI IN HUMAN LYMPHOCYTES: RESULTS FROM THE HUMAN MICRONUCLEUS PROJECT.** Zeiger E[1], Bonassi S[2], Chang WP[3], Holland N[4], Kirsch-Volders M[5], Fenech M[6]. [1]Chapel Hill, NC 27517 USA. [2]Department of Environmental Epidemiology, Istituto Nazionale per la Ricerca sul Cancro,L.go R.Benzi,10,16132 Genova, Italy. [3]Institute of Environmental Health Sciences, National Yang Ming University Medical School, 155 Section2 Lih-non Street, 11200 Taipei, Taiwan. [4]School of Public Health, University of California, 217 Warren, Berkeley, CA 94720-7360, USA. [5]Laboratory for Cell Genetics, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussel, Belgium. [6]CSIRO Health Sciences and Nutrition, PO Box 10041, Adelaide, SA 5000, Australia.

The effect of tobacco smoking on the frequency of micronuclei (MN) in human lymphocytes has been the object of many population studies. In most reports, the results were unexpectedly negative, and in many instances smokers had lower frequencies of MN than non-smokers. A pooled re-analysis of 24 databases from the HUMN international collaborative project has been performed with the aim of understanding the impact of smoking habits on MN frequency. The complete database included 5710 subjects, with 3501 nonsmokers, 1409 current smokers, and 800 former smokers. The overall result of the re-analysis confirmed the small decrease of MN frequencies in current smokers (FR = 0.97; 95% CI = 0.93-1.01) and in former smokers (FR = 0.96; 95% CI = 0.91-1.01), when compared to non-smokers. MN frequency was not influenced by the number of cigarettes smoked per day among subjects occupationally exposed to genotoxic agents, whereas a typical U-shaped curve is observed for non-exposed smokers, showing a significant increase of MN frequency in individuals smoking 30 cigarettes or more per day (FR = 1.59; 95% CI = 1.35-1.88). This analysis confirmed that smokers do not experience an overall increase in MN frequency, although when the interaction with occupational exposure is taken into account, heavy smokers were the only group showing a significant increase in genotoxic damage as measured by the MN assay in lymphocytes. Quantitative data about smoking habit should always be collected because the simple comparison of smokers vs. non-smokers could be misleading. The sub-group of heavy smokers should be specifically evaluated whenever it is large enough to satisfy statistical requirements. The presence of an interaction between smoking habit and occupational exposure to genotoxic agents should be always tested.

223 **MOLECULAR CYTOGENETIC CHARACTERIZATION OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA AND ITS RELATION TO *FHIT* GENE METHYLATION.** Zhang L[1], Zheng S[2], Ma X[1], Wiemels JL[2], Buffler PA[1], Smith MT[1], Wiencke JK[2]. [1]School of Public Health, University of California, Berkeley, CA 94720. [2]Laboratory for Molecular Epidemiology, University of California, San Francisco, CA 94143.

The chromosomal translocation, t(12;21)(p13;q22), and hyperdiploidy are the two most common (> 50%) cytogenetic abnormalities in childhood acute lymphoblastic leukemia (ALL). Classification is challenging because t(12;21) is cryptic to conventional cytogenetics, and in the case of hyperdiploidy, the leukemic cells are often difficult to grow *in vitro* for metaphase analysis. In a large population-based case-control study of childhood leukemia, we employed interphase fluorescence *in situ* hybridization (FISH) to simultaneously detect t(12;21) and hyperdiploidy in the diagnostic bone marrow smears of 143 childhood ALL patients (with normal or uninformative classic cytogenetic reports). To date, 45 cases have been revealed to be hyperdiploid that were unclassifiable or misclassified by conventional cytogenetics, whereas 55 cases have been identified as t(12;21) positive. In the t(12;21) positive subgroup, additional genetic changes such as a deletion of the second *TEL* allele (31/55), an extra copy of *AML1* (16/55), duplication of der(21)t(12;21) (13/55), and the loss or gain of chromosome X (9/55) were commonly observed. Having characterized the cases by FISH and conventional banding, we used methylation specific-PCR to examine whether aberrant methylation of specific genes occurred within specific cytogenetic subgroups. We found that hypermethylation of CpG sites within the promoter region of the fragile histidine triad (*FHIT*) gene was 20-fold more frequent in the hyperdiploid subgroup than in the t(12;21) positive group ($p < 0.0001$). Thus, FISH markedly improves the yield of definitive cytogenetic analyses. In addition, results suggest aberrant *FHIT* gene methylation is a common and perhaps early event in the etiology of specific cytogenetic subgroups of childhood ALL.

224 **APPLICATION OF A TWO-STAGE MORPHOLOGICAL TRANSFORMATION MODEL OF SYRIAN HAMSTER EMBRYO (SHE) CELLS TO DETECT CHEMOPREVENTIVE AGENTS.** Zhang H[1], Borman HD[1], Myhr BC[1]. [1]Genetic and Molecular Toxicology, Covance Laboratories Inc., Vienna, VA 22182.

Morphological transformation (MT) is the first identifiable stage of the multistage transformation of SHE cells *in vitro*. MT itself is known to be susceptible to a two-stage process akin to initiation and promotion. Our group has shown that treatment of SHE cells with a subtransforming dose of an initiator (BaP, 3.34 ng/mL) for 24 hrs followed by a subtransforming dose of a promoter (TPA, 1 ng/mL) for 6 days, resulted in a synergistic effect on MT. This interaction suggested the further possibility that chemopreventive agents might be identified by cotreatments during initiation or promotion and looking for a decrease in MT. Application of 100 μM α-tocopherol at the initiation stage (as a cotreatment with 3.34 ng/mL BaP for 24 hrs) significantly decreased MT induced by the sequential treatment with BaP and TPA. Similarly, application of α-tocopherol at the promotion stage (as a cotreatment with 1 ng/mL TPA for 6 days) also decreased MT significantly. When compared to the synergistic control (3.34 ng/mL BaP, 24 hrs + 1 ng/mL TPA, 6 days), cotreatment of α-tocopherol with BaP decreased the MT frequency by 57.5%, while cotreatment of α-tocopherol with TPA decreased the MT frequency by 63.0%. Thus, α-tocopherol has the ability to inhibit cell transformation at both initiation and promotion stages, with a slight preferential effect on the promotion stage. This result is consistent with the effects of α-tocopherol from *in vivo* studies. The two-stage MT model is being further explored for the detection of other chemopreventive agents in SHE cell cultures.