Published in final edited form as:

Mol Carcinog. 2009 May ; 48(5): 465–478. doi:10.1002/mc.20493.

The 23rd Aspen Cancer Conference: Mechanisms of Toxicity, Carcinogenesis, Cancer Prevention and Cancer Therapy 2008

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INTRODUCTION

The 23rd Aspen Cancer Conference on Mechanisms of Toxicity, Carcinogenesis, Cancer Prevention and Cancer Therapy was held July 20–22, 2008 in Aspen, Colorado. The goals of the Aspen Cancer Conference are to understand the mechanisms of toxicity and carcinogenesis, to understand and minimize cancer risk, to assess the public health impact of cancer risk, and to develop the scientific basis for improving the diagnosis, prevention and treatment of toxic and neoplastic disease. The Aspen Cancer Conference fosters intense interaction, discussion and collaboration among key stakeholders in cancer research. One of the strengths of the conference is the sustained participation in and support of the conference by leaders in cancer research from government, academic and industry sectors. Because of its unusual qualities, the Aspen Cancer Conference provides an environment that is uniquely conducive to generating novel insights related to current issues in cancer prevention, intervention and therapy.

The scientific program for the 23rd Aspen Cancer Conference included eight Conference Sessions, a Poster Session by Aspen Cancer Conference Fellows with presentation of the Theodore T. Puck Award, a Special Public Conference Session on Breast Cancer at the Aspen Institute, a Special Tribute to the late Benjamin F. Trump, presentation of the First Annual Benjamin F. Trump Lecture, and presentation of the First Benjamin F. Trump Fellowship Award. As a long standing tradition at the Aspen Cancer Conference, conference sessions allotted equal time for slide presentations and open discussion. Session topics in the 23rd Aspen Cancer Conference were: Metabolism and Obesity in Cancer, Personalized Oncology, Cancer Genomics, Hypoxia, Senescence and Cancer, Immunology, Companion Animals in Oncology and Centrosomes, Chromosomes and Nuclear Architecture. At the close of the Conference, the Scientific Advisory Committee, chaired by Curtis C. Harris, met to select topics for the 24th Aspen Cancer Conference. This meeting summary concisely describes the content of each of the Conference Sessions.

SESSION 1: METABOLISM AND OBESITY IN CANCER

Chairperson: Thea Tlsty (University of California, San Francisco)

The cancer phenotype is thought to involve metabolic reprogramming, altered bioenergetics and alternative pathways for procuring nutrients to support rapid cell growth. This idea was

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advanced early in the study of cancer biology; for example, as early as 1924, Otto Heinrich Warburg hypothesized that enhanced glycolysis, and suppressed or defective respiration (via mitochondrial oxidative phosphorylation), is a fundamental characteristic of cancer cells.

Molecular evidence that cancer cells consume abnormally high amounts of glucose has been obtained using fluorodeoxyglucose positron emission tomography (FdG-PET). Most actively growing tumors have much higher levels of glucose uptake measured by ^{18}F -FdG-PET than control cells. The etiology of metabolic reprogramming in cancer cells may involve inactivating mutations in genes that are essential for mitochondrial respiration; alternatively, mutations in oncogenes and tumor suppressor genes may promote metabolic dependence on glycolytic ATP generation. Cancer cells could gain a growth advantage through increased dependence on glycolysis, because such a switch would decrease dependence on oxygen, and because increased production of bicarbonic, pyruvic and lactic acids could promote tumor invasion, suppress immune surveillance, and support stromal cell metabolism. Autophagy, another type of metabolic reprogramming, may be an alternative mechanism by which cancer cells obtain nutrients and energy. Autophagy may also be an alternative pathway towards tumor cell death.

Metabolic reprogramming may play an important role in obesity in humans. Energy balance, the ratio of caloric intake to caloric output, is a key determinant of body weight and body mass index (BMI). Energy balance can be modulated by dietary, microbial and life-style factors, but it can also be modulated by metabolic factors. A substantial body of evidence links obesity in humans and animal models with increased cancer risk. However, the mechanism by which obesity and obese life-style factors alter cancer risk is poorly understood.

Two talks in this session, by Julian J. Lum (BC Cancer Agency) and Stephen Hursting (University of Texas Health Sciences Center), explored the link between cancer and autophagy and the link between cancer and obesity, respectively.

Autophagy

Julian J. Lum (BC Cancer Agency)

Metabolic plasticity is essential for cell growth and survival in variable environmental conditions. A lymphocyte cell culture model can be used to study metabolic plasticity induced by exposure of quiescent lymphocytes to essential growth factors and/or altered cell culture conditions. In particular, interleukin-3 (IL-3) promotes increases in cell size and induces cell proliferation in Bak-Bax-double knockout (DKO) lymphocytes in vitro. In the presence of IL-3, these cells activate the PI3K/AKT/mTor pathway, which stimulates mitochondrial respiration and glucose uptake. In the absence of IL-3, glucose uptake decreases over 2–5 wk in cell culture, and cell size and cell growth is maintained for up to 14 wk, in an autophagy-dependent manner. Autophagic vesicles accumulate in growth-factor-deprived cells, and the autophagy-specific marker LC3 is re-localized to these vesicles. The autophagic vesicles support catabolic cell survival in a time-limited manner. Autophagy-dependent growth can be inhibited in these cells, by knockdown of essential autophagy factors, such as ATG5.

The availability of oxygen also influences metabolic programming in cultured lymphocytes. In IL-3-stimulated lymphocytes grown under hypoxic conditions, hypoxia inducible factor 1 α (HIF-1 α) is stabilized, mitochondrial respiration is inhibited, and mTor activation is suppressed. HIF-1 α plays a direct role in activating autophagy, because knockdown of HIF-1 α causes rapid cell death under hypoxic conditions. HIF-1 α may directly upregulate genes involved in autophagy, because HIF-1 knockdown inhibits hypoxia-induced expression of ATG5 in IL-3 treated lymphocytes. Reporter assays suggest that the promoter of the gene encoding ATG5 may contain a HIF-1 α target binding site.

Other metabolic changes may be associated with induction of autophagy in growth-factor deprived lymphocytes. These include upregulation of fatty acid oxidation and increased mitochondrial processing of succinate and pyruvate.

Cultured lymphocytes grown in the absence of IL-3 exist in a re-activatable quiescent state. Upon re-exposure to IL-3, these cells reactivate normal cell growth pathways. This suggests that autophagy may allow cells to recover from growth suppression in other biological contexts. This idea was tested in a B-cell lymphoma mouse model, in which tamoxifen-sensitive *c-myc*-dependent B-cell lymphoma cells were injected into host mice, allowed to progress, and then treated with tamoxifen. Tumor regression and relapse were monitored before, during and after treatment with tamoxifen. In this system, the duration of tamoxifen-induced tumor regression was significantly prolonged by treatment with an inhibitor of autophagy. Other experiments suggest that memory T cells may induce autophagy during periods of quiescence, because memory T cells upregulate autophagy-specific proteins such as ATG5, ULK1 and ULK2 after exposure to lymphocytic choriomeningitis virus.

Obesity and Energy Balance in Carcinogenesis

Stephen D. Hursting (University of Texas, MD Anderson Cancer Center)

Obesity and obesity-related adverse health effects have reached epidemic proportions in both adults and adolescents in the U.S. population. Data reported by the Centers for Disease Control indicated in 2006 that 66.3% of the adult U.S. population is obese or overweight (BMI >25.0 kg/m²) and approximately half of those individuals are obese, with BMI >30.0 kg/m². Obesity is associated with an altered metabolic state characterized by high levels of insulin-like growth factor 1 (IGF-1), insulin resistance, dyslipidemia, elevated pro-inflammatory cytokines, and associated with increased risk of atherosclerosis, hypertension and type 2 diabetes. Obese individuals also have a significantly higher risk of developing many types of cancer, including breast, endometrial, cervical, uterine and ovarian cancer in women, prostate, stomach cancer in men, and colorectal, kidney, liver/gall bladder, pancreatic, esophageal and hematopoietic cancer in both men and women.

The phenotype of obesity develops when energy intake exceeds energy output. In contrast, when a calorie-restricted diet is imposed or elected, energy intake is lower than energy output. While obesity is associated with increased risk of multiple cancers in animal models and humans, 20% calorie restriction (CR) is associated with 2- to 44-fold lower risk of spontaneous cancers in rodent model systems, as well as significantly lower risk of chemically induced and transplanted cancer in multiple animal models.

The mechanism by which obesity and CR influence cancer risk is not well understood. However, it has been proposed that IGF-1, leptin as well as other cytokines and adipokines may modulate energy balance and energy metabolism in obese humans and animals, and that these factors may also influence cancer risk. For example, in a Wnt-1 mouse model for mammary tumorigenesis, there is a positive correlation between percent body fat and serum levels of IGF-1, leptin and insulin in lean (29% body fat), overweight (35% body fat) and obese (47% body fat) mice, respectively. In these animals, mammary gland tumor volume also correlates positively with percent body fat. Conversely, liver-specific IGF-1 knockout mice (LID) demonstrate low serum IGF-1 and much lower tumor susceptibility.

The role of adipocyte-generated factors in obesity and carcinogenesis was examined using the A-ZIP/F-1 fatless diabetic mouse. This genetically engineered mouse expresses a dominant negative protein that is a specific transcriptional repressor targeting adipose tissue-specific gene expression. Because adipose-specific gene expression is repressed in the A-ZIP/F-1 mouse, these animals have no white adipose tissue and reduced amounts of dysfunctional

brown adipose tissue. A-ZIP/F-1 mice also express abnormally low levels of leptin, resistin and adiponectin and abnormally high levels of insulin, growth hormone, IGF-1, IL-4, IL-6, IL-1b and other cytokines. Interestingly, A-ZIP/F-1 mice remain highly susceptible to chemically induced skin cancer and genetically initiated mammary tumors. Pathway analysis suggests that LID1 mice and CR-treated wild-type mice suppress signaling through the AKT/mTor pathway, while RTK-dependent signaling through the AKT/mTor pathways is upregulated in diet-induced or genetically induced obese animals. The latter effects are independent of autocrine or paracrine functions of adipose tissue (i.e., A-ZIP/F-1 mice). High levels of IGF-1, insulin and some cytokines appear to correlate with increased cancer risk in these mouse models, while adipokines are dispensable for the obesity-cancer link. Thus, the IGF-1 or AKT/mTor pathways may be suitable targets for future attempts to disrupt the obesity-cancer link in animal model systems.

SESSION 2: PERSONALIZED ONCOLOGY

Genetic Predispositions to Cancer

Peter Emanuel (University of Arkansas)

Juvenile myelomonocytic leukemia (JMML) is a rare mixed-type clonal childhood leukemia, characterized by poor survival and high relapse after transplantation. Myeloid progenitor cells from JMML patients characteristically have an abnormally high rate of spontaneous colony formation in vitro, resulting from hypersensitivity to granulocyte macrophage colony stimulating factor (GM-CSF). Because this hypersensitivity is not linked to upregulation or altered sensitivity of the GM-CSF receptor in JMML patients, it has been proposed that JMML patients carry defects downstream of the GM-CSF receptor that confer GM-CSF hypersensitivity.

Searches for the molecular bases of GM-CSF hypersensitivity in JMML cells led to the discovery that 10–14% of JMML patients have neurofibromatosis, and that these patients carry mutations in *NF-1*, the causative gene for neurofibromatosis. *NF-1* encodes a tumor suppressor that inhibits activation of Ras. Thus, mutation in *NF-1* is the functional equivalent of an activating Ras mutation. Similarly, neurofibromatosis patients are at 500-fold higher risk of having JMML than non-neurofibromatosis patients, and *NF-1*-deficient mouse cells are hypersensitive to GM-CSF. However, the cellular response to IL-3 is similar in isogenic wild-type and *NF-1* mouse cells. This parallels the cytokine response profile of human JMML cells. The molecular mechanism that confers selective responsiveness to GM-CSF in these cells is not understood.

JMML is also linked to genetic alterations in *PTPN11*, the gene encoding the cytosolic protein tyrosine phosphatase SHP-2. Mutations in *PTPN11* occur in approximately 35% of JMML patients and in approximately 50% of patients with Noonan syndrome, an autosomal dominant human disorder characterized by short stature, heart disease, dysmorphic facial features and mental retardation. SHP-2 has two src homology domains and a catalytic tyrosine phosphatase domain. Molecular studies show that the cellular phenotype associated with activating mutations in *PTPN11* mimic the cellular phenotype of JMML, including hypersensitivity to GM-CSF. This is consistent with the fact that SHP-2 modulates Ras activation.

Approximately 20% of JMML patients carry genetic alterations in *Ras*, 35% carry genetic alterations in *PTPN11*, and 20% carry genetic alterations in *NF-1*. All JMML cells, regardless of genotype, demonstrate activation of downstream effectors of Ras signaling, including proteins in the PI3K/AKT and MAPK (Erk) pathways. These results have led to the hypothesis that therapeutics targeting Ras, such as farnesyl transferase inhibitors (FTIs), might be useful for treating JMML and JMML-related disorders. While clinical studies with FTIs in patients with

AML, MDS, NSCLC and various solid tumors showed low or no overall response, JMML cells are hypersensitive to FTIs in vitro. Furthermore, up to an 81% overall response rate was observed in a clinical trial involving 46 Tipifarnib-treated JMML patients conducted using a Children's Oncology Group Protocol. Unfortunately, FTIs such as Tipifarnib do not significantly inhibit farnesylation of Ras in vivo, which raises doubts about the mechanism by which tumor regression is achieved in these JMML patients. Future studies are planned to target other proteins, including PTEN, which might regulate downstream signaling via the AKT/mTor pathway.

Regulatory Issues and Solutions

John Leighton (U.S. Food and Drug Administration)

Worldwide development, testing and implementation of therapeutic pharmaceuticals has been facilitated by guidance documents issued by the International Conference on Harmonization (ICH). In most therapeutic areas, non-clinical requirements are described by ICH M3. ICH S6 discusses the development of biologicals. Recently, the ICH process for anti-cancer pharmaceuticals was initiated, and a Business Plan and Concept Paper were developed. ICH S9: Preclinical Guideline on Oncology Therapeutic Development was endorsed by the ICH Steering Committee in May 2007 (www.ICH.org). Progress to harmonization is continuing through ongoing discussions, and Step 2 of the ICH process is expected to be completed by October 2008, with final release of ICH S9 targeted for March 2009.

ICH S9 provides guidance for development and implementation of oncology drugs and biologics. However, ICH S9 excludes botanicals, chemopreventive agents, radiopharmaceuticals, imaging agents, supportive care, gene therapy, somatic cell therapy and vaccines. General topics discussed in ICH S9 include the following: route of administration, dose, species, schedule, duration, non-clinical safety, GLP compliance, pharmacology, pharmacokinetics (PK), toxicokinetics, toxicology, reproductive toxicology, genetic toxicology and carcinogenicity, first dose in man, dose escalation in clinical trials, formulation, drug combinations, use in children, drug metabolites and drug impurities. Specific concerns for specialized drug subgroups are also discussed, as well as timing of non-clinical and clinical studies.

The intent of ICH S9 is to provide information but not to educate, to highlight differences between oncology and non-oncology pharmaceuticals and between drugs and biologics, and to provide cross-references to other ICH guidance documents. In agreement with FDA policy, emphasis was placed on the "three Rs": reduction, refinement and replacement of experimental animals in non-clinical research settings. Thus, development and use of alternative non-animal based assays and tests are encouraged in ICH S9 and other ICH guidance documents.

ICH S9 provides specific guidance on selection of dose and schedule for non-clinical and clinical studies of oncology drugs. The time frame of toxicology studies for oncology drugs is also discussed. While current FDA guidelines require 6-mo toxicology testing, ICH S9 indicates that 3-mo toxicology testing of oncology drugs may become an acceptable alternative in the future. This approach is being considered for both oncology drugs and oncology biologics. Embryo-fetal and peri- and postnatal studies are required for oncology drugs and biologics, and should be performed prior to Phase III trials.

Key areas of consensus in ICH S9 are: (1) similar approaches are appropriate for oncology drugs and biologics; (2) schedules for toxicology studies should be similar to schedules for proposed clinical studies; (3) toxicology studies should not limit the clinical dose or schedule for patients receiving benefit; and (4) the impact of impurities, metabolites and conjugated

agents associated with oncology therapeutics should be evaluated. ICH S9 is still under development and details of the recommendations are currently being refined.

SESSION 3: THE BENJAMIN F. TRUMP LECTURE

Modifiers and Modulators of Hepatocellular Carcinogenesis

James E. Klaunig (Indiana University School of Medicine)

Hepatocellular carcinogenesis is classically viewed as a multi-step process with at least three stages: initiation, promotion and progression. A rodent model was used to identify and characterize modifiers of hepatocellular carcinogenesis induced by non-genotoxic (or epigenetic) tumor promoters such as phenobarbital, Wyeth 14,643 and diethylhexyl phthalate (DEHP). Hepatic focal lesions in the rat or mouse were initiated by single or multiple treatments with diethylnitrosamine, followed by a period of approximately 6 mo latency. Histological studies showed that the hepatic focal lesions were hyper-susceptible to the tumor-promoting effects of Wyeth 14,643 and DEHP, showing higher tumor promoter-induced DNA synthesis associated with lesionexpansive growth, compared to surrounding hepatic tissue. The effects of tumor promoters on hepatic foci were inhibited by caloric restriction, and both cellular effects and subsequent tumor development were completely blocked in peroxisome proliferator activated receptor (PPAR)- α knockout mice.

Previous studies suggested that Kupffer cells are activated by various types of liver pathology including acute and chronic liver toxicity, alcohol liver disease, carcinogenesis, ischemia/reperfusion injury and idiosyncratic liver toxicity. Kupffer cells are also activated by exposure to peroxisome proliferators, silica, low temperature, lipopolysaccharide (LPS) and other types of liver-targeted agents. Activated Kupffer cells are required for liver regeneration and factors secreted by Kupffer cells, including reactive oxygen species (ROS), tumor necrosis factor- α (TNF- α), appear to play a significant role in peroxisome proliferator-induced hepatocyte proliferation. These data suggest that activated Kupffer cells may modulate the microenvironment of pre-neoplastic hepatic lesions, and promote hepatocellular carcinogenesis.

To examine the role of Kupffer cells in hepatocellular carcinogenesis, rat or mouse Kupffer cells were isolated by centrifugal elutriation followed by fluorescence-activated cell sorting using Kupffer cell-specific cell surface markers. This Kupffer cell-enriched population could be activated by LPS, and secreted TNF- α in response to tumor promoters (i.e., Dieldrin, MEHP or Wyeth 14,643.) Kupffer cells were depleted in vivo, by injecting mice with clodronate-saturated liposomes three times per week, beginning 1 d prior to treatment with tumor promoters. In this mouse model, Kupffer cell-depleted animals showed significantly lower basal levels of hepatocellular DNA synthesis, as well as complete suppression of LPS-stimulated DNA synthesis and liver cell proliferation. Similarly, tumor promotion and/or progression was blocked in Kupffer cell-depleted mice subjected to a two-stage liver carcinogenesis protocol using phenobarbital or Wyeth 14,643. The mechanism and specificity of the anti-tumor effects of Kupffer cells are currently being investigated.

SESSION 4: CANCER GENOMICS

Chairperson: Webster Cavenue (University of California, San Diego)

Cancer genomics is a gene discovery exercise, whose goal is to identify changes in genomic structure, organization and/or function that correlate with or are predictive of cancer susceptibility. The toolbox of cancer genomics includes techniques that analyze DNA sequence variation (single nucleotide polymorphism (SNPs) and mutations), genomic organization, copy

number variation, chromosome aberrations, gene expression and patterns of histone modification (i.e., chromatin signatures). The techniques used in cancer genomics include array comparative genomic hybridization (array-CGH), chromatin immunoprecipitation (ChIP), ChIP on ChIP, fluorescence in situ hybridization, microarray gene expression analysis and genome wide association studies (GWAS).

Genome wide association studies offer great future potential in the area of cancer genomics. GWAS is a complex multi-step process. Proper replication is required to establish the significance of gene regions identified by GWAS, and ultimately, functional studies must be performed to provide biological validation of markers or genes identified by GWAS.

Two talks in this session discussed recently identified markers that are linked to susceptibility to melanoma (Lynda Chin, Dana Farber Cancer Institute) and prostate, breast and other human cancers (Stephen Chanook, National Cancer Institute).

Malignant Melanoma

Lynda Chin (Dana Farber Cancer Institute)

The human oncogenome is highly complex, consisting of both key drivers of the disease as well as irrelevant noises. Therefore, a “cancer gene” identified by genomic aberration must be validated at the level of biological function and clinical relevance of such gene will require understanding of the mechanism by which altered biological function causes specific disease pathology. With high throughput genomic analysis identifying many new putative cancer genes, it is increasingly necessary to develop methods that provide functional analysis on the “omic” level.

In managing patients with melanoma, one of the clinical challenges is that patients presenting with “thin” melanoma (Breslow thickness <1 mm) have a heterogeneous risk of metastatic disease: while 85–90% of these patients have excellent prognosis after surgical removal of the cancer lesion, 10–15% of these patients die within 10 yr of diagnosis and surgery, due to aggressive metastatic disease. Currently, there are no prognostic parameters that can identify this group of high-risk patients. One hypothesis proposes that genetic variability in metastatic determinants in primary melanomas account for the observed clinical heterogeneity in metastatic potential of these primary tumors. If this is true, then it may be possible to distinguish thin melanoma subtypes based on copy number variation or gene expression signatures.

Two mouse models for melanoma have been developed which have significant differences in metastatic potential. One model has a doxycycline-inducible melanocyte-specific activated HRAS trans-gene (iHRAS), and the other model has a doxycycline-inducible melanocyte-specific Met oncogene (iMet). iHRAS melanomas do not metastasize in the mouse, while iMet melanomas are highly metastatic, with secondary tumors appearing in lymph node, lung and adrenal glands. Gene expression profiling on primary tumors from iHRAS and iMet mice identified approximately 1600 genes with >2-fold difference in expression. These mouse genes were triangulated with an independently determined set of human genes, whose copy number or level of expression was altered in metastatic versus primary melanoma tumors. This cross-species approach identified 295 upregulated/amplified and 65 downregulated/deleted genes, as candidate genes involved in melanoma metastasis in human and mouse.

Pathway and gene ontology analysis suggested that the candidate melanoma metastasis gene set was enriched in genes involved in cell cycle control, DNA replication and recombination, cell assembly, movement and invasion and cancer. Because metastatic potential is intrinsically linked to the process of cellular invasion, primary and secondary functional screens were performed on these genes in TERT immortalized melanocytes to identify 20 genes that can

confer enhanced invasion. When these 20 genes were used to query the Oncomine database, the results showed significant association for all genes with progression in one more non-melanoma solid tumors. For example, 12 of 20 genes demonstrated increased expression during progression of breast adenocarcinomas, and expression levels of these 12 genes successfully classified a preexisting cohort of 295 breast tumors with respect to Kaplan-Meier survival. Additional characterization of these candidate metastasis genes is in progress.

The Gold Rush of Cancer GWAS: Sorting the Nuggets (The Devil is in the Details)

Stephen Chanock (National Cancer Institute)

Genome wide association studies (GWAS) offer great promise for advancing cancer genomics. This promise lies largely in the ability of GWAS to identify previously unrecognized genomic regions that could harbor cancer predisposition genes and reveal unrecognized biological functions. For example, since 2007, over 150 novel genomic regions have been identified that are statistically associated with risk of specific human diseases or that modulate human traits. These include genetic risk factors for type 2 diabetes, inflammatory bowel disease, multiple cancers (breast, colorectal, lung and prostate) and heart disease, as well as genetic modifiers of human traits such as height and weight. Of 284 human SNPs identified to date by GWAS, a small fraction (19) are in gene coding or gene promoter regions, while approximately 40% are in the vicinity of genes, such as the introns or nearby regulatory regions. Approximately, one-half of the SNPs reaching conclusive significance reside within non-genic regions, and many of these are hundreds of kilobases from the closest known gene. Thus, extensive genetic and functional studies are often required, in order to interpret GWAS data and understand its biological significance and/or potential clinical usefulness.

A survey of established breast cancer loci reveals a dichotomy and an inverse correlation between allele frequency and relative risk factor (i.e., odds ratio). Highly penetrant alleles such as *BRCA1*, *BRCA2*, *PTEN* and *p53*, identified and validated primarily in familial studies, have odds ratios in the range of 8–10; these alleles are very rare, with allele frequencies from 0.0001 to 0.001. In contrast, weakly penetrant alleles such as *FGFR2*, with odds ratios in the range of 1.1–1.3, have recently been identified by GWAS; these alleles are much more common, with allele frequencies in the range 0.3–0.8. A large “genomic space” separates these dichotomous groups. Furthermore, a similar pattern has been observed in studies of low and high penetrance alleles for other human cancers and complex diseases.

In some cases, low penetrance alleles for human disease risk are modified by genetic interactions with other human SNPs. For example, one study suggests that the impact of *FGFR2* genetic variants on breast cancer risk may be influenced significantly by ER status. Because such genetic interactions are not known a priori, the relative risk of weakly penetrant alleles may be underestimated in initial studies.

Recent GWAS studies have identified candidate genomic regions linked to increased risk of colorectal, lung, melanoma, neuroblastoma, prostate and breast cancer. Interestingly, the 8q24 region, which is relatively “gene-poor” except for *MYC*, includes three distinct regions linked to increased risk of prostate cancer, one of which overlaps with a marker of colorectal cancer susceptibility. The 8q24 region also has a marker for breast cancer susceptibility.

The National Cancer Institute (NCI) Cancer Genetic Markers of Susceptibility (CGEMS) project recently conducted a very large scale GWAS for prostate cancer genes. Using an initial cohort of 1150 cases and controls, and two secondary cohorts of 4000 or 5500 cases and controls, respectively, an initial set of 540 000 Tag SNPs, novel regions have been identified. Novel markers of prostate cancer susceptibility in this group include 19q13.41, encoding beta microseminoprotein; 10q11, a biomarker currently under consideration for early detecting

aggressive prostate cancer; as well as markers associated with *TCF2*, *JAZF1* and another region of chromosome 11. Additional studies suggest that 19q13.41 SNPs modulate levels of PSA. Extensive re-sequencing has been carried out in regions 1 and 3 in 8q24 linked to prostate cancer risk. This analysis yielded a large number of novel SNPs (non-dbSNPs) as well as previously reported dbSNPs. Thus, as noted above, much additional genetic and functional characterization is required, before GWAS data on candidate prostate cancer (or other cancer) alleles can be used to develop diagnostic or rational therapeutic approaches to managing or treating cancer.

SESSION 5: HYPOXIA

Chairperson: Cheryl Walker (University of Texas)

Models for cancer progression suggest that cancer cells acquire increased ability to survive under hypoxic conditions, and that this ability facilitates progression towards more aggressive stages of cancer growth (i.e., carcinoma in situ, invasive cancer). Metabolic changes associated with survival in a hypoxic environment are regulated by hypoxia-inducible factor (HIF), a heterodimeric transcription factor that upregulates expression of genes involved in glucose metabolism and angiogenesis. HIF activity is in turn regulated by prolyl hydroxylation and the von Hippel Lindau (VHL) tumor suppressor protein, which targets hydroxylated HIF-1 α for ubiquitin-dependent degradation. Expression of HIF activates a metabolic switch, that inhibits mitochondrial respiration and favors anaerobic glycolysis (i.e., metabolism of glucose to lactate via pyruvate). Increased glucose uptake associated with this metabolic switch can be detected in tumors using functional PET imaging. Functional PET imaging can also be used to monitor the efficacy of anti-cancer therapeutic agents that target HIF, or its upstream activators and downstream effectors/mediators.

VHL and Cancer

Bill Kaelin (Dana Farber Cancer Institute)

The human EglN protein family includes three protein hydroxylases (EglN1, 2 and 3), that hydroxylate prolyl residues on target proteins in an oxygen-, iron- and 2-oxoglutarate-dependent manner. EglN1/PHD2, which mediates hydroxylation of two conserved proline residues in HIF-1 α , is essential for viability in mice. Although human EglN2 and EglN3 are less well characterized than EglN1, recent studies suggest that they play roles in cell proliferation and apoptosis, respectively. For example, siRNA-mediated knockdown of EglN2 lowered expression of cyclin D1 and cyclin D3 in cell culture experiments, but did not reduce expression of CDK4 or CDK6. In addition, mammary gland development and cyclin D1 expression in mammary gland tissue was strongly reduced in EglN2 knockout mice, and mammary epithelial cells from EglN2 knockout mice were resistant to Her2- and PI3K-induced transformation in vitro. Conditional knockdown of EglN2 in MCF-7 xenograft tumors in nude mice also inhibited tumor growth and reduced tumor volume. These data suggest that EglN2 promotes cell proliferation and that it may be pro-carcinogenic in mice and humans.

In humans, *VHL*, neurofibroma 1 (*NF1*) and *c-RET* are linked to familial paraganglioma and pheochromocytoma. These genes define a pathway that is activated by growth factor withdrawal in sympathetic neuroblasts during embryonic development. Recent studies suggest that EglN3 acts as a downstream mediator of apoptotic cell death in this pathway. An unbiased screen for clones that are resistant to EglN3-induced apoptosis identified KIF1B- β , whose expression is required for EglN3-dependent apoptosis in vitro. The human gene encoding KIF1B- β maps to 1p36, a chromosomal region implicated as a tumor suppressor in several cancer types including neuroblastoma. This hypothesis is consistent with the observation that susceptibility of two neuroblastoma cell lines to vincristine-induced apoptosis is increased by

re-activation of KIF1B- β . The mechanism by which KIF1B- β and EglN3 induce apoptotic cell death is currently under investigation.

SESSION 6: SENESCENCE AND CANCER

Chairperson: Scott Lowe (Cold Spring Harbor Laboratory)

Cellular senescence is a form of permanent cell cycle arrest, associated with replicative exhaustion of human cells in vitro. Cellular senescence, which requires p53 and/or p16^{INK4a}, can be induced in response to environmental or genetic factors, including oncogene activation and DNA damage. Markers of cellular senescence include senescence-associated β -galactosidase (SA- β -Gal), a repressed chromatin state and global changes in secretory profiles. While cellular senescence was initially described as an in vitro phenomenon, recent studies suggest that senescence may be a biologically relevant mechanism of tumor suppression and a possible alternative to apoptosis. Evidence for senescence as a barrier to tumorigenesis has been obtained in many cancer models, including premalignant lesions in Ras-transgenic mice, chemically induced skin papillomas in the mouse and E2F-induced pituitary hyperplasia. While cellular immortalization (unlimited replicative potential) is viewed as an antithesis to cellular senescence (exhausted replicative potential), the mechanisms and genetic pathways that balance immortalization and senescence may overlap with those that balance self-renewal and differentiation in stem cells. The exact mechanisms involved in activating and maintaining cellular senescence have not yet been determined. Additional unanswered questions about cellular senescence include: by what mechanisms does senescence suppress tumorigenesis? Are there multiple senescence subpathways? What markers identify senescent cells in vivo? Is inactivation of senescence an obligatory step in tumor progression? Can senescence be activated or re-activated in tumor cells? Does senescence contribute to aging or non-cancer pathologies?

Tumor suppression and cellular senescence

Scott Lowe (Cold Spring Harbor Laboratory)

A mouse model for hepatocellular carcinoma was developed, in which *p53*-deficient green fluorescent protein (GFP)-tagged fetal hepatoblasts were engineered to express pro- or anti-carcinogenic genes or targeted shRNAs, and transplanted into the spleens of recipient mice. The engineered hepatoblasts migrate spontaneously to the liver of the recipient mouse, and the chimeric mice develop GFP-labeled liver carcinomas at characteristic rates, depending on the genotype of the transplanted hepatoblasts. In this model, *p53*-deficient cells that constitutively express activated Ras, *c-myc* or AKT develop into GFP-tagged liver carcinomas that morphologically resemble human liver tumors. A conditional *p53* model was also generated, in which a doxycycline-regulated promoter drives expression of *p53*-targeted shRNA. In these engineered chimeric mice, *p53* expression can be turned on or off in the liver, and growth or regression of GFP-labeled tumors monitored over time. Four days after removal of doxycycline, p53 protein is no longer detected in extracts of liver cells by Western blot, and tumor volume increases dramatically. A pulse of p53 expression in these mice was associated with tumor shrinkage and expression of the senescence-associated marker SA- β -Gal, as well as infiltration of the tumor by natural killer (NK) cells and other immune surveillance cells. These data are consistent with the hypothesis that senescence is a *p53*-dependent mechanism of tumor suppression in vivo, and that senescence may trigger immune-mediated clearance of the senescent cells.

A mouse model was also used to examine whether senescence could play a role in fibrosis-associated liver cirrhosis in vivo. In this model, mice are chronically exposed to carbon tetrachloride for 6 wk, which causes liver fibrosis due to activation of stellate cells and

deposition of extracellular matrix (ECM). Liver fibrosis in carbon tetrachloride-treated mice was associated with expression of senescence-associated markers, including p21, p53, HMGa1 and SA- β -Gal, and these markers co-localized with des-min and smooth muscle actin, markers of activated stellate cells. When these experiments were repeated in *p53 p16^{INK4A}* DKO mice, the area of liver fibrosis increased by approximately 50%, the number of activated stellate cells increased, and activated stellate cells failed to senesce. Similar results were observed when *p16^{INK4A}* was selectively inactivated in stellate cells using Cre-Lox targeted gene inactivation. Liver fibrosis in these models appeared to be reversible, because areas of fibrosis decreased when exposure to carbon tetrachloride was terminated. These data suggest that stellate cells are involved in the early stages of liver fibrosis, and that senescence of stellate cells plays a role in limiting progression of liver fibrosis in the mouse.

Microarray gene expression analysis was used to identify genes whose expression are altered in senescent human stellate cells grown in culture. The results showed that genes involved cell proliferation and deposition of ECM were downregulated, while genes involved in degradation of ECM and immune surveillance were upregulated in senescent human stellate cells. In addition, when senescent stellate cells were incubated with NK cells in vitro, they were actively targeted for cell killing, while non-senescent cells were not. In vivo studies suggest that NK cells may also target senescent stellate cells during liver fibrosis in the mouse.

These studies suggest that senescence may play an important role in suppressing hepatocellular carcinogenesis and may modulate the process of liver fibrosis. Senescence of activated stellate cells may limit the extent of fibrosis in damaged liver, and immune-mediated clearance of senescent cells may also promote resolution of fibrosis. However, chronic production of senescent cells during wound healing may contribute to an inflammatory environment that is pro-carcinogenic.

Senescence as a Barrier to Cancer Prevention and a Biological Target for Therapy

Ron DePinho

Age is one of the strongest known risk factors for cancer in humans. Because age-associated cancers in humans are predominantly of epithelial origin, the laboratory mouse, which is resistant to naturally occurring epithelial cancer, has been viewed as a poor model system to study mechanisms of naturally occurring epithelial cancer. Recent studies show that *p53^{+/-} mTerc^{-/-}* mice have a humanized cancer spectrum, including up to 50% spontaneous epithelial cancers. This suggests that long telomeres, which are characteristic of mouse but not human epithelial cells, may provide a “telomere reserve,” which protects the mouse genome from instability that leads to age-associated epithelial cancer. In humans, it is postulated that telomere shortening in regenerating epithelial cells leads to an increased rate of DNA double-strand breaks (DSBs), which in turn cause chromosome rearrangements and oncogenic mutations during early steps of cellular transformation. Telomerase reactivation, which is associated with cancer progression in humans, may occur at a later step in cellular transformation.

Glioblastoma (GBM) is an aggressive brain malignancy in humans that is refractory to all known cancer therapeutics. GBM is subclassified into highly aggressive primary GBM and less aggressive secondary tumors, which progress more slowly. Molecular genetic studies predicted differential roles of *p53* and *PTEN* in primary and secondary GBM. This possibility was tested in conditional *p53 PTEN* DKO mice, in which *p53* and *PTEN* were selectively inactivated by Cre-lox-mediated deletion in neuroblasts. Surprisingly, the DKO knockout neuroblasts generated GBM-like tumors at a very high rate in the mouse, and the pathological presentation of these mouse tumors was very similar to primary human GBM. Subsequent examination of mutations reported in human GBM samples revealed that approximately 40%

have *p53* mutations, approximately 30% have *PTEN* mutations, and that primary GBMs are highly enriched for clones that have mutations in both loci. Additional characterization of *p53 PTEN* DKO cells suggest that these cells are incapable of activating pathways of neuroblast differentiation, which are coordinately suppressed by *Myc* and *Rb*. Thus, *p53 PTEN* DKO cells, and possibly premalignant GBM precursor cells, are locked into a highly proliferative undifferentiated state.

SESSION 7: IMMUNOLOGY

Chairperson: Richard Gaynor (Eli Lilly Company)

Cell intrinsic and cell extrinsic factors can influence tumor development and progression in humans and other mammals. When tumor cells express mutant or modified proteins, or aberrantly high levels of a normal protein, that protein can potentially be recognized and targeted for immune surveillance. However, because tumors are a chronic source of immunogen, the response to tumor antigens is often blunted by immune regulatory functions, whose biological role is to prevent deleterious autoimmune attack. In addition, tumors tend to secrete cytokines and other immunomodulatory factors that suppress dendritic cell function and generate a locally immunosuppressive micro-environment. These factors modulate the types of T cells and antigen-presenting cells (APCs) that are recruited to interact with tumor cells. Together these factors allow tumor cells to escape potent attack by the immune system, whose normal role is to target and destroy foreign, aberrant or pathological cells and/or molecules. Nevertheless, it remains possible to re-activate an immune response towards cancer cells in humans. Some recent approaches to enhance immunosurveillance of growing tumors include injection of cytotoxic T cells, vaccination with dendritic cells, injection of activated NK cells or injection of interleukin-2, interleukin-12 or interferon- α . Alternatively, it is possible to blunt immunoregulatory functions by blocking the activity of T regulatory (Treg) cells, blocking or removing immunosuppressive cytokines or generating a CTLA-4 blockade. Earlier emphasis on the former approach is now yielding to a more balanced two-prong approach, in which active immune functions are stimulated at the same time as suppressive immunoregulatory functions are blocked in cancer patients. Immunotherapeutic options may have promise in combination with other types of anti-cancer therapy; however, in some cases, cytotoxic agents may alter immune responses.

Two talks in this session by James Allison (Memorial Sloan Kettering Cancer Center) and Tyler Curiel (University of Texas Health Sciences Center) discussed immunotherapy for cancer based on CTLA-4 blockade and the role of T regulatory cells in immune surveillance of cancer, respectively.

Immunotherapy of Cancer

James Allison (Memorial Sloan Kettering Cancer Center)

The human immune system has complex regulatory circuits that both activate and suppress T cell proliferation. CTLA-4 is a T-cell membrane-associated protein that binds to B7-1 and B7-2 ligands on the surface of APCs, and specifically limits proliferation of APC-activated CD28-positive T cells. Recent studies show that antibody to CTLA-4 can block CTLA-4-mediated anti-proliferative effects on T cells. Thus, transplanted colorectal cancer cells fail to grow or regress, in mice injected with antibody to CTLA-4, and co-injection of anti-CTLA-4 and GM-CSF tumor cell vaccine (G-vax) synergistically eradicates established melanoma tumors in mice, while anti-CTLA-4 or G-vax alone does not block tumor progression in these animals. The mechanisms by which CTLA-4 blockade inhibits tumor growth include activation of tumor vasculature, stimulation of T cell infiltration into the tumor, increased ratio of interferon- γ to

IL-10, and a shift in the ratio of T effector (Teff) to T regulatory (Treg) cells, favoring more CD4-positive and CD8-positive T cells and fewer Foxp3-positive T cells.

Anti-CTLA-4 (MDX-010, Ipilimumab) is a human IgG1 monoclonal antibody to human CTLA-4 that is being co-developed by Medarex, Inc. and Bristol Meyers Squibb. Ipilimumab is currently being tested in Phase III clinical trials for metastatic melanoma and in Phase II clinical trials for melanoma, renal cell carcinoma, prostate and other cancers. Approximately 3700 patients have been treated with Ipilimumab (mostly melanoma patients), with an approximately 20% response rate, 1/4th of which have been complete responses. Adverse events have been limited primarily to colitis in patients given widely spaced treatments and to colitis, rash, hypophysitis and hepatitis in patients given more frequent treatment (i.e., monthly). Patients with ovarian cancer have also shown significant benefit from treatment with Ipilimumab. For specific clinical applications, dose and frequency of treatment with Ipilimumab with or without G-vax or other cancer therapeutics is still being optimized. The factors that predict responsiveness or non-responsiveness to Ipilimumab are also currently under investigation.

Recent studies in human bladder cancer patients treated with Ipilimumab suggest that drug efficacy correlates with increased expression of ICOS in CD4-positive T cells. In addition, the Teff/Treg ratio increased approximately 20-fold from an average starting value of 0.22 to >4.3 in three bladder cancer patients treated with Ipilimumab, and similar effects on ICOS expression and Teff/Treg ratio were observed in prostate cancer patients treated with Ipilimumab. These data suggest that the mechanism of action of Ipilimumab in humans may be similar to the mechanism of action of anti-CTLA-4 in mice.

Computational methods predict that the average human cancer cell expresses at least five and possibly as many as 50 neoepitopes that could formally be recognized and targeted for immune-mediated attack. Any agent that kills tumor cells will load APCs with these potential cancer antigens. However, cancer antigens cannot activate an effective immune response unless immune checkpoint mechanisms, such as CTLA-4, are disabled. Thus, treatment with Ipilimumab in combination with agents that selectively kill tumor cells could offer a promising therapeutic option for many human cancers in the future.

Regulatory T Cells in the Treatment of Cancer

Tyler Curiel (University of Texas Health Sciences Center)

The characteristics of cancer cells have been studied and described extensively. One therapeutically relevant characteristic of cancer cells is their ability to evade immune surveillance, despite acquisition of aberrant characteristics and markers that are not present in non-cancer cells. While the failure of immunosurveillance mechanisms to eradicate cancer has previously been attributed to defects in generating sufficient cancer-targeted T effector cells, it has recently been demonstrated in mouse models that T regulatory (Treg) cells blunt the efficacy of T effector cells in fighting and eradicating cancer. Thus, effective immunotherapy for cancer must inhibit Treg cells, as well as activate potent T effector cells, in order to slow or block continued cancer cell proliferation.

One component of the Treg cell population are CD4-positive CD25-positive Foxp3-positive Treg cells. In ovarian cancer patients, the number of CD4-positive CD25-positive T cells correlates inversely with survival outcome, and similar correlations have been observed in patients with other cancers. Tumors may enhance the protective effect of Treg cells by actively recruiting them towards the local tumor environment. This could be performed by generating a Treg specific attractant, or by generating a pro-inflammatory tumor microenvironment.

The simplest strategy to improve immunosurveillance of tumors is to non-specifically inactivate Treg cells (i.e., kill them all). Alternatively, it is possible to selectively target Tregs, inhibit Treg trafficking, inhibit Treg differentiation, convert Tregs to Teff cell subtypes, or increase the threshold required to activate Treg cells. Ontak is a covalent conjugate between diphtheria toxin and interleukin-2, which is FDA-approved for treating cutaneous T cell lymphoma, a rare form of non-Hodgkin's lymphoma (NHL). Because interleukin-2 binds to the CD25 cell surface receptor on Treg cells, it is possible that Ontak could deplete CD25-positive Treg cells and thereby stimulate immune clearance of cancer cells. This idea was tested in a phase 0/I trial with seven extensively pretreated advanced stage cancer patients. Patients were given a single intravenous infusion of Ontak, and blood levels of CD4-positive CD25-positive T cells, and interferon- γ -expressing CD3-positive T cells were determined before and three to 5 d after treatment. A functional assay for Treg cells was also performed. The results indicate that Ontak depleted CD25-positive Treg cells, and was associated with increase in the number of interferon- γ -expressing CD3-positive T cells in all seven patients. Clinical efficacy of Ontak in treating ovarian cancer has also been tested in a limited number of patients. In one patient with advanced refractory metastatic ovarian cancer, Ontak treatment was associated with a decrease in the ovarian cancer marker CA-125 1 mo after a single treatment, and sustained suppression of CA-125 levels after 6 weekly doses of Ontak. This patient also showed reduced burden of metastatic tumors 4 mo after treatment with Ontak. A more extensive phase II trial of Ontak therapy for ovarian cancer is in progress.

Although preliminary results suggest that Ontak depletes Treg cells and thereby improves clinical outcome in treated cancer patients, additional studies are needed to confirm whether and how Ontak modulates immune functions in treated cancer patients. Additional studies are also needed to determine optimal dosing and identify agents that might enhance Ontak-mediated Treg depletion. Preliminary studies suggest that interferon- α may augment the Treg depleting effects of Ontak in mice and humans. Future studies will explore other Treg cell subtypes as targets for depletion, alternative mechanisms for limiting Treg functions in cancer patients, and optimal combination therapies based on Treg depletion as an anti-cancer strategy.

SESSION 8: COMPANION ANIMALS IN ONCOLOGY

Chairperson: William Farland (Colorado State University)

In vivo models are essential tools for continued progress in oncology research, because they provide means for systematic hazard identification, quantitative risk assessment and mode of action analyses for large numbers of potentially oncogenic compounds; clearly, these are essential experiments that cannot be carried out in human subjects. In addition, in vivo animal models are used to develop and evaluate therapeutic and preventive approaches to specific human cancers. Rodent models have been used in toxicology research since the 1800s and beagles have been used for more than 70 yr. Recent advances in genetic engineering have led to increased use of transgenic and genetically engineered mouse strains that are designed to answer targeted questions about carcinogenic mode-of-action and human susceptibility to specific cancers. However, the relevance of rodent models for assessing human cancer risk has been questioned, because large carcinogen doses are often required to induce cancer in rodents, and because humans and rodents appear to have strong intrinsic differences in susceptibility to specific cancers.

In 2003, the NCI launched the Comparative Oncology Program (COP), whose goal is to facilitate assessment of novel cancer therapeutics in companion dogs and cats afflicted by spontaneous cancers. This program takes advantage of the fact that spontaneous cancer in dogs and cats shares many features with human cancer including histology, genetics, biology and responsiveness to conventional cancer therapeutics, and that the frequency of new cancer cases

in dogs and cats in the U.S. is high enough to provide sufficient numbers of cancer cases for cancer clinical trials. In addition, dogs are susceptible to many cancers that are common in humans, including osteosarcoma, prostate, breast, NHL, melanoma, soft tissue sarcoma, head and neck carcinoma, and virally induced lymphomas. Multimodal studies are possible and subject compliance is often better for companion animals than for human subjects enrolled in clinical trials. Another advantage of studying cancer in companion animals is that dogs and cats share environmental exposures with their human caretakers; this makes it possible to study environmental risk factors for cancer in companion animals. Release of the complete dog genome in 2005 was an important step in facilitating molecular genetic analysis of cancer susceptibility in canine companion animals. Lastly, state-of-the-art facilities for studying cancer in companion animals have been developed at Schools of Veterinary Medicine throughout the U.S., one of which is The Flint Animal Cancer Center at Colorado State University.

This conference session featured a presentation by Douglas Thamm (Colorado State University) on spontaneous canine cancer as a translational model.

Spontaneous Canine Cancer as a Translational Model: From the Laboratory to the Labrador

Douglas Thamm (Colorado State University)

Canine susceptibility to spontaneous cancer is potentially a very useful translational model for human oncotherapeutics. Some advantages of the canine model include the following. Dogs as a species are more outbred than rodent and other experimental models; however, specific dog breeds are more inbred than human populations, showing breed-specific increases in susceptibility to tissue-specific cancers. Dogs share environmental risk factors for cancer with their human caretakers. The most common cancers in dogs are mammary, skin, connective tissue, testes, melanoma, and NHL, with more than 25 annual cases of each of these cancers per 100 000 dogs and a U.S. canine population of approximately 75 million. Canine cancer has similar metastatic properties as human cancer, and response to conventional oncotherapeutics is similar in humans and dogs. Human telomere and hepatic biology resembles the biology in the dog more than in the mouse. The human genome has higher synteny to the dog genome than to the mouse genome. Dog body size is close enough to human body size, so that many identical techniques and pieces of diagnostic and therapeutic equipment can be used for dogs and humans. The cost of conducting veterinary-based clinical trials is significantly lower than the cost of comparable human trials, and outcomes are obtained more quickly in dog clinical trials than in comparable human clinical trials.

Infrastructure for studies of spontaneous cancer in dogs is rapidly expanding. Currently available resources include the NCI Comparative Oncology Program, the Comparative Oncology Trials Consortium and the Canine Comparative Oncology and Genomics Consortium. The latter group has begun an effort to archive biologic samples from canine cancer patients for ongoing and future studies.

Caveats and disadvantages of the canine model for spontaneous cancer include the following. Cancer drug trials in dogs require larger amounts of compound than comparable studies in rodents. The number of canine cancer subjects is limited (smaller N than for human Phase III studies). Biological differences exist between dog and human, some of which may influence cancer susceptibility or therapeutic outcome for oncotherapeutics. The U.S. pharmaceutical and biotechnology industries as well as U.S. regulatory agencies may be slow to accept the canine model for cancer studies.

The safety and efficacy profile of a guanine nucleotide analogue prodrug, GS-9219, was recently tested in dogs with spontaneous NHL, through a collaborative effort between Colorado

State University, University of Wisconsin-Madison School of Veterinary Medicine, and Gilead Sciences. GS-9219 is metabolized in vivo to 9-(2-phosphonylmethoxyethyl)guanine (PMEG), a potent anti-proliferative nucleoside analog with severe dose-limiting toxicity when given systemically. However, GS-9219 loads specifically into lymphoid cells, leading to low levels of PMEG in plasma and strongly reduced toxicity. Preclinical in vitro studies show that GS-9219 inhibits proliferation of myeloid and lymphoid cell lines, and preclinical in vivo studies in beagle dogs showed rapid uptake of GS-9219 into lymphoid cells/tissues with limited intestinal and no renal toxicity.

In the initial Phase I/II trial of GS-9219 in canine subjects with NHL, GS-9219 was given on several alternative dose regimens to 38 naïve or refractory dogs with NHL. Non-responsive animals were removed from the study after the initial efficacy evaluation, which was based on RECIST, PET/CT and biopsy/cytology data. Responsive animals were continued through five treatment cycles of GS-9219 with follow-up. The investigators reported an overall response rate of 79% (23/38 CRs and 7/38 PRs). In addition, 70% of CR dogs that relapsed after completing five treatment cycles were successfully re-induced with GS-9219. Adverse events were transient or reversible with reduced dose/frequency. These promising results were used to develop and implement Phase I human clinical trials of GS-9219. Additional preclinical studies are exploring the efficacy of GS-9219 for treating canine myeloma, as well as the efficacy of GS-9219 in combination with other oncotherapeutic agents.

SESSION 9: CENTROSOMES, CHROMOSOMES AND NUCLEAR ARCHITECTURE

Chairperson: William R. Brinkley (Baylor College of Medicine)

The centrosome, which consists of a pair of centrioles and pericentriolar material, is the cell's primary microtubule organizing center. The centrosome also plays a critical role in mitotic spindle assembly, cell division and regulation of cell proliferation. Normal cells replicate a single centrosome with each cell cycle, while cancer cells often have supernumerary centrosomes. Centrosomes do not replicate in normal non-dividing differentiated cells. However, in differentiated cells, especially those with cilia and flagella, the centriole component of the centrosome migrates to the cell surface, becomes associated with the plasma membrane, and generates basal bodies, cilia and flagella. In contrast to motile or sensory cilia, primary cilia are non-motile and are found on most differentiated cell types in vertebrates. Until recently, primary cilia were thought to be vestigial structures with no biological function. Recent studies contradict this view, suggesting that primary cilia are cellular "antennae" that play an important role in intracellular signaling and communication.

Intracellular Signaling Mediated by Cilia

Gregory Pazour (University of Massachusetts Medical School, Worcester, Massachusetts)

Primary cilia on vertebrate cells are non-motile surface structures, intimately connected with and surrounded by plasma membrane. Plasma membrane-associated receptors, such as opsin/rhodopsin, somatostatin receptor 3, serotonin receptor 5 HT, smoothened, patched, PDGF α , polycystin-1, polycystin-2 and others, are present on primary cilia-associated membranes, which is consistent with the hypothesis that primary cilia are sensory organelles in vertebrate organisms. This hypothesis was validated by the recent discovery that Tg737 mutant mice, which have structurally and functionally defective primary cilia, develop fatal kidney dysfunction resembling human polycystic kidney disease (PKD). Tg737 mice have a defect in the gene encoding the IFT88 subunit of a protein complex required for intraflagellar protein transport.

In humans, PKD is characterized by excessive proliferation and poor differentiation of kidney epithelial cells. To explain the similar phenotype of PKD and Tg737 mutant mice, it has been proposed that primary cilia regulate growth and differentiation of kidney epithelial cells. This idea was tested by generating a conditional mouse model for PKD, in which a floxed allele of IFT20 was conditionally deleted in kidney nephrons by HoxB7-promoter driven expression of Cre recombinase. These mice develop normally until postnatal day 5, but develop large cysts and excessive disordered proliferation in kidney collecting ducts between postnatal day 5 and postnatal day 21. Molecular studies correlated aberrant kidney cell proliferation in 22-day-old mutant mice with high levels of dephosphorylated β -catenin, as well as upregulation of downstream targets of β -catenin; control experiments showed that the level of dephosphorylated β -catenin remained low in 5-day-old mutant mice, when kidney cell proliferation was not yet dysregulated. This result suggests that canonical Wnt signaling regulates proliferation of normal kidney epithelial cells. Cytological studies also suggest that kidney dysfunction in these mice is associated with abnormal spindle alignment in mitotic cells in the kidney collecting duct. Although the exact mechanism for this effect is not known, it is possible that non-canonical Wnt signaling plays a role in this process in the normal kidney.

These studies provide strong evidence that cilia are sensory organs in the kidney and that defects in cilia biogenesis play a role in human PKD. Furthermore, aberrant Wnt signaling, via both canonical and non-canonical pathways, appears to play a role in the development of kidney dysfunction in mouse models of human PKD.

Chromosome Aberrations and Genetic Damage Caused by Methylphenidate

William Slikker, Jr. (National Center for Toxicological Research, U.S. Food and Drug Administration)

Methylphenidate (Ritalin®) is a prescription drug used to treat attention deficit hyperactivity disorder (ADHD) in children. Methylphenidate has undergone extensive toxicology testing, and has been an FDA-approved drug for treating ADHD for more than 50 yr. However, the safety of methylphenidate has been questioned because of a number of reports suggesting that it has potential genotoxic effects in vitro and/or in vivo. In particular, it was reported that exposure to methylphenidate increased the frequency of chromosomal aberrations in Chinese hamster ovary cells in one laboratory (but not in a second laboratory) and that methylphenidate increased the incidence of hepatic adenomas in the high dose treatment group of a 2-yr mouse bioassay. Evidence of methylphenidate-induced DNA damage was also obtained by Comet assay of brain and peripheral blood leukocytes from methylphenidate-treated rats. Other data contradict the view that methylphenidate is genotoxic: for example, negative results were obtained with methylphenidate in the Ames assay, mouse lymphoma gene mutation assay, in vivo micronucleus assay and a rat carcinogenicity study.

Due to the widespread therapeutic use of MPH, these data raise concern about the safety profile of methylphenidate, and whether methylphenidate-treated children are at risk of genotoxic and/or carcinogenic effects from long-term exposure to the drug. To address this question, additional studies have been designed and initiated to assess genotoxic and behavioral effects of methylphenidate in juvenile monkeys. In these studies, monkeys were randomly divided into three groups (10 monkeys per group) and dosed orally twice a day with vehicle, low (0.15 mg/kg) or high dose (1.5 mg/kg) methylphenidate. Dose levels were adjusted to provide plasma levels near the human therapeutic value (low dose group) or five-to-ten times higher than the human therapeutic value (high dose group). On the current dose schedule, control animals receive 0.5 mL/kg of vehicle twice a day, low dose animals receive 2.5 mg/kg twice a day, and high dose animals receive 12.5 mg/kg twice a day. Lymphocytes are being collected and the following assays and tests performed: micronucleus assays, baseline and 1-mo intervals; chromosome painting assays, baseline and 3-mo intervals; HPRT mutation assay, baseline and

3-mo intervals; pharmacokinetic analysis. An operant test battery to assess the effects of methylphenidate on behavior (i.e., motivation, color and position discrimination, learning, short-term memory) is performed on individual subjects 5 days per week during the course of the experiment. The behavioral findings to date indicate that animals in the high dose treatment group exhibited diminished behavioral responses, perhaps due to suppressed motivation for food reward. It is anticipated that the complete results of these studies will be available in early 2009.

ASPEN CANCER CONFERENCE 2008 SPECIAL PUBLIC SESSION

The Aspen Cancer Conference held a special session entitled *Breast Cancer: Prevention Diagnosis and Therapy* on Monday July 21, 2008 at the Paepke Auditorium of The Aspen Institute. This session was open to the public and was intended as a community outreach event. The session featured three short lectures and a panel discussion, followed by a reception and silent auction. Raymond DuBois (University of Texas, MD Anderson Cancer Center) moderated the event. In the first lecture, Thea Tlsty (University of California, San Francisco) discussed breast cancer *Prevention and Diagnosis*, highlighting recent advances in molecular diagnostics for breast cancer patients. In the second lecture, Funda Meric-Bernstam, a self-described surgeon-scientist, discussed *Future Directions in Breast Cancer Therapy*, emphasizing breast-conserving and cosmetically acceptable approaches to breast surgery, as well as improved methods for diagnosing and treating lymph node involvement. In the third lecture, Carolyn Kaelin discussed issues in *Surviving Breast Cancer*, where she focused on identifying and coping with the adverse chemotherapy effect known as *chemobrain*. Tlsty, Meric-Bernstam and Kaelin were then joined by Allan Tralins, a local oncologist, and Georgia Hanson, a local breast cancer survivor, for a panel discussion. The panelists addressed questions from the audience on topics related to cancer susceptibility, diagnosis and treatment.

PRESENTATION OF THE 2008 THEODORE T. PUCK AWARD

The 2008 Theodore T. Puck Award was presented to Kenneth Scott (Dana-Farber Cancer Institute) for his excellent poster presentation entitled *Integrative Functional Genomics for Metastasis Determination*.

PRESENTATION OF THE FIRST BENJAMIN F. TRUMP FELLOWSHIP AWARD

The 2008 Benjamin F. Trump Fellowship Award was presented to Benjamin Cosgrove (Massachusetts Institute of Technology) for his outstanding poster presentation entitled *Multi-Cue Landscape Analysis of Cytokine-Induced Hepatocyte Proliferation, Apoptosis and Toxicity*.

A TIME TO REMEMBER BEN

Benjamin Franklin Trump, co-founder and spiritual leader of the Aspen Cancer Conference, died on February 25, 2008. This loss was felt heavily by the Aspen Cancer Conference Community; all who knew Ben through the Aspen Cancer Conference greatly appreciated his contribution to the Conference year after year, and the entire scientific community, particularly the field of pathology, lost one of its great leaders, experimentalists, clinicians and teachers when Ben passed away this year.

Ben's esteemed career as a pathologist included early training at the University of Missouri and the University of Kansas School of Medicine, followed by additional studies at University of Washington. After service in the U.S. Army Medical Corps, Ben held an Associate Professorship of Pathology at Duke University. Ben was then recruited in 1970 as Professor and Chair of the Department of Pathology at the University of Maryland School of Medicine.

Under his leadership, the University of Maryland Department of Pathology became one of the largest clinical research-based pathology departments in the U.S. Ben's wife, colleague and friend, Elizabeth Trump, was instrumental in establishing the RA Cowley Shock Trauma Center at the University of Maryland. This is only a partial list of Ben's many contributions to the field of pathology at the University of Maryland and beyond.

In Ben's honor, a fellowship award has been established, to be awarded annually to a poster presenter at the Aspen Cancer Conference. An annual lecture has also been established in Ben's honor, to be given during an Aspen Cancer Conference session by a preeminent scientist in cancer research. The 2008 Benjamin F. Trump Lecture was given by James Klaunig, Director of Pharmacology and Toxicology, Indiana University School of Medicine.

In a special evening session, friends and colleagues of Ben, including Curtis Harris, James Klaunig, Ray Jones, Gary Stoner, Carl Alden, Andrew Saladino, Tom Slaga and others, spoke warmly of Ben, sharing memories of Ben's remarkable scientific and personal achievements. In James Klaunig's tribute to Ben, he gave Ben "the highest tribute" of all...the wish that Ben could mentor his own son. Andrew Saladino lauded Ben's fulfillment of "Stephen Covey's criteria: to live, to love, to learn, to leave a legacy." Tom Slaga said of Ben's passion for and skill in fly fishing: "Ben was not a fisherman, Ben was a catcherman." All who shared their memories of Ben expressed deep gratitude towards him as a source of great leadership, mentorship and inspiration over the years.

Abbreviations

BMI	body mass index
DKO	double knockout
HIF-1α	hypoxia inducible factor 1 α
IGF-1	insulin-like growth factor 1
CR	calorie restriction
GM-CSF	granulocyte macrophage colony stimulating factor
FTI	farnesyl transferase inhibitor
ICH	International Conference on Harmonization
LPS	lipopolysaccharide
GWAS	genome wide association study
NCI	National Cancer Institute
VHL	von Hippel Lindau
PHD1	prolyl hydroxylase 1
SA-β-Gal	senescence-associated β -galactosidase
GFP	green fluorescent protein
NK	natural killer
ECM	extracellular matrix
GBM	glioblastoma

APC	antigen-presenting cell
Treg	T regulatory cell
Teff	T effector cell
PKD	polycystic kidney disease