Meeting Report: The International Conference on Tumor Progression and Therapeutic Resistance

Wafik S. El-Deiry

Department of Medicine (Hematology/Oncology), Genetics, and Pharmacology, The Abramson Cancer Center, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

Abstract

A multidisciplinary conference was held November 7 to 9, 2004 in Philadelphia, PA to focus on the problem of drug resistance in cancer. A great deal of knowledge is beginning to unravel the complex molecular and cellular changes associated with malignant tumor progression. With this comes many opportunities for therapeutic development. Featuring the latest tools, models, and research findings, this conference which included over 250 members of both academia and industry was a great opportunity to learn and develop new approaches and collaborations. The Keynote speaker was Dr. Robert Horvitz (Massachusetts Institute of Technology), who won the 2002 Nobel Prize in Medicine for his pioneering work on the cell death pathway in *Caenorhabditis elegans*. Speakers covered various aspects of tumor progression and therapy from simple models to clinical trials. (Cancer Res 2005; 65(11): 4475-84)

Tumor Progression and Metastasis

The Opening Scientific Session on tumor progression and metastasis was chaired by Mary Hendrix (Northwestern University). Bruce Zetter (Children's Hospital of Boston) spoke about markers of tumor progression, first distinguishing between oncogenes and genes that promote metastatic progression, as well as tumor suppressors versus genes that suppress metastases. There was a discussion about the need to understand the signature of metastatic disease as well as the need for biomarkers for cancer outcome prediction based on the molecular signature of more aggressive cancer, cancer recurrence detection, markers of susceptibility to therapy, and markers of therapeutic resistance. The β-thymosin family seems to have predictive value in determining the probability of prostate-specific antigen failure (1). The SILAC procedure (2) using heavy/light carbon lysine ratios was mentioned as a way to use mass spectroscopy and proteomics to study metastases. The current challenge is no longer high throughput discovery but rather high throughput validation. It is also becoming clear that in terms of translation to the clinic, effective use of 5 to 10 markers may offer the same sensitivity as 1,000 markers.

Mien-Chie Hung (M.D. Anderson Cancer Center) described several novel observations that are unraveling mechanisms of cancer cell survival and invasion. One mechanism involves suppression of Foxo3a, which is associated with greatly reduced 5-year survival in breast cancer. He showed that IKKα phosphorylates Foxo3a at Ser^{644} and that the tumorigenicity of IKK requires its suppression of forkhead (3). A second mechanism discussed involved the G protein–coupled receptor CXCR4 which is overexpressed in breast cancer and the ligand SDF for CXCR4 which is enriched in organs where metastases occur (lung, liver, and bone). He showed that HER-2/*neu* enhances CXCR4 expression and that CXCR4 mediates invasiveness due to HER-2/*neu* overexpression using CXCR4 small interfering RNA (siRNA) to show decreased invasion both *in vitro* and *in vivo*.

Further evidence was presented to support a correlation in primary breast cancer between CXCR4 expression and HER-2/*neu* overexpression as well as a correlation between CXCR4 expression and poor survival (4). Dr. Hung mentioned that GSK-dependent phosphorylation of Snail leads to cytoplasmic localization and degradation of Snail, which leads to inhibition of the epithelial-to-mesenchymal transition (EMT). The β-Trcp E3 ligase in the cytoplasm is involved in degradation of Snail and then the cells express E-cadherin whose expression is normally repressed by Snail. Thus, Snail, which normally represses E-cadherin, promotes EMT. GSK3β, which targets Snail for degradation, inhibits the EMT and GSK3β is negatively regulated by Wnt, Akt, and mitogen-activated protein kinase (MAPK), which ultimately promote the EMT (5).

Ruth Muschel (Children's Hospital of Philadelphia) presented data challenging the mainstream view for *in vivo* hematogenous metastasis that this occurs by capillary trapping, extravasation of tumor cells, colony formation, and angiogenesis. Her alternative model involves vascular attachment, intravascular proliferation, intravascular colony formation, and ultimately loss of vascular integrity. Using video microscopy of green fluorescent protein (GFP)–tagged tumor cells, she showed that blocking antibodies to either α3 or β1 integrins inhibited attachment of HT1080 cells. She hypothesized that adherence of tumor cells was mediated by exposed basement membrane and used both electron microscopy as well as a laminin 5 antibody to inhibit pulmonary arrest of tumor cells (6). Interestingly, she found that after pulmonary arrest, clots form with platelet aggregation. The clots then dissolve and tumor cells flatten becoming integrated in the vessel wall. She mentioned that anticoagulants are known to inhibit metastasis and that coagulation facilitates tumor cell spreading (7). She finds that extravasated cells are rare and are cleared. However, she found that GFP-labeled tumor cells could grow into capillaries and that vascular leakage occurs with large colonies. Her models are amenable for drug testing and may be generalizable although the effects on α3β1 were shown in fibrosarcoma.

Host-Neoplasm Interactions and Angiogenesis

The second session on host-neoplasm interactions and angiogenesis was chaired by Robert Kerbel (University of Toronto). Mary Hendrix spoke about the plasticity of aggressive melanoma cells and showed how this undifferentiated phenotype is capable of transdifferentiating into an endothelial-like cell type with the ability to form vasculogenic-like networks *in vitro* (called

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Requests for reprints: Wafik S. El-Deiry, University of Pennsylvania, 415 Curie Boulevard, CRB 437A, Philadelphia, PA 19104. Phone: 215-898-9015; Fax: 215-573-9139; E-mail: wafik@mail.med.upenn.edu.

vasculogenic mimicry). Although aggressive melanoma cells and endothelial cells share common markers for a vascular phenotype, they differ in the expression of endostatin receptors, resulting in endothelial cells responding more effectively to angiogenesis inhibitors compared with melanoma cells. She also presented experimental studies showing that the microenvironment of metastatic melanoma cells can exert an epigenetic effect on poorly aggressive melanoma cells, resulting in their transdifferentiation into a more aggressive cell phenotype. Lastly, she showed the developmental plasticity of these tumor cells in an embryonic zebrafish model, which highlighted the importance of this microenvironment in reversing the tumorigenic/metastatic phenotype of aggressive melanoma.

Meenhard Herlyn (Wistar Institute) discussed communication between melanoma cells and keratinocytes specifically focusing on genes and signaling pathways involved in cell communication and their role in melanoma. HGF and platelet-derived growth factor (PDGF) from fibroblasts suppress E-cadherin and Desmoglein expression. Melanoma cells produce various growth factors for autocrine growth, stimulation of fibroblasts, and endothelial attraction. He showed that overexpression of growth factors in fibroblasts plus UVB exposure induce melanocytic lesions in skin, enough to get invasion typical of human melanoma. Dr. Herlyn also discussed BRAF and a clinical trial combining BAY 43-9006 with cisplatin and paclitaxel. He mentioned that it is necessary to combine the BRAF inhibitor with chemotherapy and that there is a 50% response rate lasting >1 year. He also mentioned that the National Cancer Institute (NCI) is initiating a large phase III trial in 800 patients.

Dr. Herlyn commented that therapeutic strategies for melanoma need to target several pathways, including Raf, signal transducers and activators of transcription 3, nuclear factor-κB (NF-κB), phosphatidylinositol-3 kinase (PI3K), and Bcl2 among others. For example, fibroblasts produce growth factors essential for melanoma growth. Insulin-like growth factor-I induces cells to produce matrix proteins leading to cross-talk through N-cadherin and linking to the cytoskeleton. Such signaling supports survival, growth, invasion, and motility. He mentioned that whereas LY294002 at 10 μmol/L inhibits adherent melanoma cells, spheroids were resistant. He also mentioned that one of the open questions in the field is whether there are stem cells in melanoma. Dr. Herlyn concluded with mention of data showing that BAY 43-9006 seems to work well in renal cell cancer and is expected to be approved as a single agent through an antiangiogenic mechanism, although in melanoma it needs to be combined with more drugs such as cisplatin and paclitaxel.

Kornelia Polyak (Dana Farber Cancer Institute) discussed the role of the stroma in breast cancer. The stroma is not simply an innocent bystander but potentially an active contributor to tumor progression and as such, a potential target for cancer therapy and prevention. She described her laboratory's efforts to purify all cell types from normal and cancerous breast tissue using magnetic beads and cell type–specific cell surface markers resulting in over 90% pure cell populations. Analysis of SAGE libraries generated from these purified cell types resulted in the identification of 63 highly cell type–specific transcripts (8). Clustering the SAGE libraries using these genes showed that gene expression changes occur in each cell type during tumor progression. Interestingly, many of the genes differentially expressed between normal and cancerous tissue, particularly genes expressed in myoepithelial cells, encode secreted proteins. Among others, they found that CXCL12, the ligand for CXCR4, was overexpressed in tumor myofibroblasts and that both CXCL12 and CXCL14 were greatly increased in ductal carcinoma *in situ* (DCIS) myoepithelial cells.

Dr. Polyak mentioned that chemokine receptors are attractive therapeutic targets, because they are G protein–coupled receptors. The receptor for the CXCL14 chemokine is unknown. However, using a CXCL14-alkaline phosphatase fusion protein, they were able to show the presence of a high-affinity candidate CXCL14 receptor present on epithelial cells. Similar to other chemokines, CXCL14 promotes breast cancer cell migration and invasion and acts as a paracrine factor because it is produced by DCIS myoepithelial cells, but its receptor seems on the tumor epithelial cells. She discussed a "release" versus "escape" model of DCIS to invasive carcinoma transition and mentioned that myoepithelial cells may play a crucial role in this process.

Robert Kerbel mentioned that the Food and Drug Administration (FDA) approved Avastin in 2004 as anti–vascular endothelial growth factor (VEGF) therapy and discussed some of the issues in the use of antiangiogenic agents. Some of these include how to measure angiogenesis in humans and how to monitor antiangiogenic drugs. He made the point that the optimal biological dose is not necessarily the maximally tolerated dose and reiterated Dr. Zetter's comments about the need for biomarkers. Dr. Kerbel discussed low-dose metronomic chemotherapy as "endothelial cell centric" dose dense but minimally toxic therapy (9). He recounted the first description of the low-dose "antiangiogenic scheduling" from Judah Folkman's lab and the coining of the term "metronomic" by Hanahan.

Dr. Kerbel has been administering low-dose cyclophosphamide in the drinking water to breast cancer–bearing mice. He recounted the results of several clinical trials supporting the use of metronomic therapy including a trial in Milan by Colleoni et al. in 2002 where 64 patients with metastatic breast cancer received low doses of cytoxan plus methotrexate for up to 2 years and the overall response rate was 32%. He also mentioned a phase II trial at the Dana-Farber Cancer Institute (HJ Burstein, PI) using cytoxan, methotrexate, and Avastin and an NCI Cancer Therapy Evaluation Program trial (HG Augustin, PI) using cytoxan and Avastin. Dr. Kerbel discussed the dosing of low-dose metronomic therapy and issues about choosing or even defining the best dose. He mentioned as possible aids to this end functional imaging, molecular detection of circulating TSP-1, as well as circulating peripheral blood endothelial cells or circulating endothelial progenitor (CEP) cells. He mentioned that CEP cells may be a target as well as a marker and may help establish the optimal dose for metronomic therapy.

As further evidence of the need for better markers and targets he mentioned a landmark study from D'Amato's laboratory demonstrating remarkable variability in angiogenesis in mice. He showed that basic fibroblast growth factor and VEGF levels correlate well with levels of CEP cells and discussed their use as surrogate markers of antiangiogenesis (10). He mentioned studies using ATN-161, an anti-integrin agent to inhibit metastatic disease and showed additional data that viable CEP cells correlate with tumor volume and biological response.

Oncogenes and Tumor Suppressor Genes

The third session of the meeting focused on oncogenes and tumor suppressor genes and was chaired by Ramon Parsons (Columbia University). Tak Mak (University of Toronto) mentioned that *PTEN* mutations are rare in breast cancer, whereas 30% to 50% have

deregulated the pathway with phospho-Akt activation. Because Ras activation occurs in the *PTEN*-deficient state through Grb2/Sos, $PTEN^{+/-}Grb2^{+/-}$ mice were generated such that the $Grb2^{+/-}$ state rescued the partial lethality of $PTEN^{+/-}$ in the C57Bl6 background due to lack of neural tube closure and structural defects in placental development and chorioallantoic fusion. There was a discussion about mediators downstream of Grb2 that ultimately affect Ras versus Akt activation (Grb2 → Sos → Ras → Erk or p110 → Akt; Grb2 → Gab1→ p85 → p110 →Akt) as well as Grb2 mutations that may affect specific signaling pathways. Dr. Mak discussed relationships between PI3K and p53-dependent cell death. PTEN up-regulated by p53 can down-regulate Akt activity. Foxo is phosphorylated by Akt. He described both negative and positive links between p53 and forkhead. T32 phosphorylation of FKHRL-1 occurs after UV exposure but this is not observed in $p53^{-/-}$ cells after UV exposure, etoposide, or tumor necrosis factor (TNF) treatment. However, there is no difference in Akt phosphorylation. He showed that SGK1 is induced by wild-type p53 after DNA damage by a post-transcriptional mechanism and that siRNA against SGK reduces phosphorylation of FKHR. Genotoxic stress activates extracellular signal-regulated kinase (ERK) to activate SGK and activate forkhead.

Finally, Dr. Mak showed the results of a genetic screen in *Drosophila* to rescue the eyeless phenotype of PTEN-overexpressed flies and identified a gene called *DJ-1* (PARK7) that negatively regulates PTEN functions. In clinical samples, the enhanced levels of this protein correlate with the deregulation of the akt pathway and the frequency of relapse in lung cancers.

Charles Sherr (St. Jude Children's Research Hospital) spoke about the creation of a knock-in mouse with two Arf-GFP alleles. These mice develop green fluorescent tumors and all die of cancer by age 15 months. Irradiation or carcinogens accelerate tumorigenesis in these mice. He discussed p53-independent effects of Arf through effects on rRNA processing associated with Arf localization to nucleoli. Arf inhibits ribosomal biogenesis in a pathway that is negatively regulated by Bmi1, Twist, JunD, and TBX2. Dr. Sherr then discussed Arf turnover. Arf proteins contain >20% arginyl residues but only one lysine in the mouse and zero lysines in human Arf, and only one internal methionine. The δ2-14 mutant of Arf is degraded rapidly. Arf turnover does not depend on Mdm2 or p53. However, NPM/B23 binds and stabilizes Arf (11). Ubiquitinated seems important for Arf turnover. In human Arf only, the NH_2 terminus can be ubiquitinated and NH_2-terminal acetylation blocks ubiquitination. He discussed NH_2-terminal sequence rules predicting that Arf would be ubiquitinated and stated that Arf is the first example of a natural lysine-lacking protein that undergoes polyubiquitination (12). It is not yet clear what the E3 ligase for Arf is or whether Arf ubiquitination is regulated by oncogenic stress. Dr. Sherr speculated that Velcade may in part affect signaling through the Arf stabilization pathway.

Carlo Croce (Ohio State University) spoke about the identification of the Tcl1 protein through studies of loss of heterozygosity (LOH) at 13q12-3 in B-cell chronic lymphocytic leukemia (CLL). The Tcl-1 protein binds Akt and transports Akt to the nucleus. He generated mice with Tcl-1 expression in T cells and the mice developed a T-cell prolymphocytic leukemia not previously described in mice. Through efforts of the CLL consortium, Dr. Croce mapped translocation breakpoints at 13q14 and identified Mir16 and Mir15. These microRNAs encode small RNAs that might regulate the temporal expression of genes. The translocation disrupted the promoter of Mir16 in 60% of CLL. Dr. Croce described that his group had mapped >300 Mir genes and found that 53% mapped to regions involved in LOH in human tumors (13). He mentioned that the targets on chromosomes 7 and 5 in myelodysplastic syndrome might be *Mir* genes. Further work needs to examine the expression of the *Mir* genes in tissues and tumors and identify their targets. Dr. Croce mentioned that ZAP-70 is prognostic in B-cell CLL (14).

Ramon Parsons focused on a relationship between PTEN and Irs2. He found that partial *PTEN* disruption rescues *Irs2* knockout mice from diabetes and also found a decrease in prostatic intraepithelial neoplasia in $PTEN^{+/-}Irs2^{+/-}$ or $PTEN^{+/-}Irs2^{-/-}$ mice. He showed that Irs2 expression is up-regulated in the PIN lesions. Dr. Parsons also discussed *PIK3CA* mutations in 25% of breast cancers and their relationship to *PTEN* inactivation. He found that *PIK3CA* mutation correlated with wild-type *PTEN* status as well as a strong correlation between ER(+) tumors and *PIK3CA* mutation whereas *PTEN* loss is associated with ER(−) tumors (15). Dr. Parsons has also recently found that loss of *PTEN* and activation of Akt leads to impairment of CHK1 function through phosphorylation, increased cytoplasmic localization, and aneuploidy in primary breast carcinomas (16).

H. Robert Horvitz (Massachusetts Institute of Technology) gave the Keynote Address describing the genetic control of programmed cell death. He gave an overview of the cell death field and showed his classic experiments using Nomarski optics to visualize cell death in living animals. He described the cell lineages of *Caenorhabditis elegans* as well as the discovery of the *ced* mutants. The well-known pathway involves EGL-1 inhibiting CED-9, which inhibits CED-4, which activates CED-3 leading to cell death. The mammalian homologues are BH3 only proteins for EGL-1, Bcl-2 like proteins for CED-9, APAF-1 for CED-4, and caspases for CED-3. *egl-1* loss-of-function mutants lead to no programmed cell death, whereas specific gain-of-function mutants have a defect in egg laying due to the deaths of the neurons that innervate muscles required for egg laying. The *tra-1* gene determines sex and is "on" in hermaphrodites and is "off" in males. TRA-1 is a member of the Gli family of proteins, which have been implicated in glioblastoma. The DNA binding site of TRA-1 is disrupted in the gain-of-function *egl-1* mutants. In wild-type males, *tra-1* is inactive, *egl-1* is expressed, and HSN neurons die. In wild-type hermaphrodites TRA-1 binds and represses *egl-1* expression, whereas in the gain-of-function mutants TRA-1 does not bind, *egl-1* is expressed, and the HSN neurons die.

Dr. Horvitz presented evidence that both CED-9 and CED-4 localize to mitochondria and interact physically. When CED-9 is off, CED-4 no longer localizes to mitochondria but rather localizes to the nuclear membrane. Evidence was presented supporting that CED-4 translocation is downstream of CED-9 and upstream of CED-3. Nonetheless, a CED-4-GFP rescued the CED-4 mutant phenotype and hence was active but its localization to the nuclear membrane did not cause programmed cell death. He speculated that CED-4 translocation might be necessary but not sufficient for programmed cell death.

Dr. Horvitz discussed cell corpse engulfment describing a number of proteins including CED-7, CED-1, CED-6, as well as CED-2, CED-5, CED-12, and CED-10, which signal engulfment. These are proteins involved in cell shape changes and cytoskeletal reorganization. He showed that engulfment not only removes dying cells but also is part of the cell death process. This was first shown using weak *ced-3* alleles in worms that were then also mutant for other *ced* genes such as *ced-1*, *ced-2*, *ced-5*, *ced-6*, or *ced-7*, and these had a death defect indicating a synergy between *ced-3* and the engulfment genes. Dr. Horvitz showed results

using a *lin-11::GFP* worm, which allowed a demonstration of engulfment defects and visualization of the recovered dying cells. He speculated that in humans the engulfment aspect of promoting cell death may be suitable for therapeutic intervention. He described a mutational screen using weak *ced-3* mutant worms that carried the *lin-11::GFP* allele and this led to several targets including *mcd-1* (modifier of cell death) which has one zinc finger and *dpl-1* Dp which antagonizes Ras in *C. elegans* vulva development. Dp proteins heterodimerize with E2F proteins and can act in a complex with Rb proteins. Dr. Horvitz showed that both the *lin-35* Rb gene and the *efl-1* E2F gene promote cell death.

Finally, Dr. Horvitz discussed some recent work focused on studies of the male-specific CEM chemosensory neurons and genes that specify their death in hermaphrodites. His laboratory generated worms with *pkd-2::GFP* that is expressed in the CEM neurons and obtained 192 mutants that rescue CEM neurons in hermaphrodites. One of these genes is *ceh-30* which encodes a *C. elegans* homeodomain protein. These mutants disrupt the binding site of TRA-1 in an intron of *ceh-30*. Loss-of-function mutants of *ceh-30* cause CEMs to die in males. Human and mouse homologues of CEH-30 have been described. He mentioned a homologue called Barh11 and that mice deficient in Barh11 have hearing loss due to degeneration of cochlear hair cells, possibly, by analogy with what is known about CEH-30, as a consequence of programmed cell death.

Apoptosis and Growth Targets

The fourth session on apoptosis and growth targets was chaired by Craig Thompson (University of Pennsylvania). John Reed (Burnham Institute) described his recent efforts to develop XIAP-blocking molecules to inhibit binding of XIAP to active caspases. Results of the initial efforts were presented in detail and were published recently (17). These efforts led to the identification of phenylurea XIAP antagonists and these showed synergy with chemotherapy and TNF-related apoptosis-inducing ligand (TRAIL). Dr. Reed showed evidence that XIAP antagonists promote anoikis of human prostate cancer cells. The XIAP antagonists do not seem toxic in rodents. More recent studies showed that XIAP antagonists bind the Bir2 domain and the compounds can bypass Bcl-2 inhibition of apoptosis in cancer and leukemia cells (18, 19). Unlike staurosporine or serum withdrawal, the XIAP antagonists kill *bax/bak*-null cells. The death is not associated with changes in mitochondrial membrane potential at a time when Annexin positivity is evident but there is evidence of caspase processing in tumor cells.

Craig Thompson showed that nitrogen mustard and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) killed wild-type, *bax/bak*-null, or *p53*-null fibroblasts by inducing a necrotic form of cell death (20). Poly(ADP-ribose) polymerase (PARP) is essential for this form of cell death and seems involved in recognition of DNA strand breaks. Whereas PARP seems required for necrotic cell death as evidenced by the resistance of $PARP^{-/-}$ cells to the cell death inducing and proinflammatory effect of MNNG, the metabolic state of the treated cells was found important for the ultimate death of the cells. PARP consumes NAD in the cytoplasm and nucleus but not in the mitochondria. Depletion of PARP allows survival of nonproliferating cells. Wild-type cells treated with pyruvate survive the NAD block. HMG-B1 leaks out of the cells and induces a macrophage response not seen in $PARP^{-/-}$ cells.

Dr. Thompson further discussed the effect of cellular metabolism on PARP-mediated necrosis and its relevance to cancer-selective therapy. Using $bax^{-/-}bak^{-/-}$ cells that are dependent on interleukin 3 (IL-3) for proliferation but are otherwise resistant to apoptosis, his group found that in the presence of IL-3 the cells underwent necrotic cell death when exposed to MNNG, whereas the IL-3–deprived cells were resistant to PARP-mediated necrosis. Further experiments revealed that either glucose deprivation or the addition of methyl-pyruvate protected $bax^{-/-}bak^{-/-}$ cells from death due to exposure to MNNG in the presence of IL-3. Dr. Thompson discussed the implications of this work regarding cancer sensitivity to alkylating therapy. Tumor cells which maintain ATP levels through aerobic glycolysis and a dependence on glucose undergo necrosis upon exposure to alkylating agents which is tumor selective due to ATP depletion associated with PARP activation.

Mark Nelson (University of Arizona) discussed the anticancer effects of selenomethionine in the context of cancer chemoprevention trials against human colon adenoma recurrence using selenium. The original design of this trial included Celecoxib but the current trial uses high selenium containing yeast only following FDA action. Selenomethionine constitutes the major form of selenium in the yeast. Selenomethionine inhibited the growth of HCT-116 cells. He examined effects on the cell cycle in selenomethionine treated cells. Cyclin A levels were increased on days 6 and 8, whereas the inhibitory cdc2 Tyr^{15} phosphorylation was found increased. Phosphorylation of ERK at Tyr^{204} was increased by selenomethionine in a dose-dependent manner with physiologic concentration of selenomethionine range, whereas a MAP/ERK kinase (MEK) inhibitor blocked the effect of selenomethionine on ERK. ERK activation was associated with H3 phosphorylation by RSK not MSK (21).

Phil Hinds (Tufts University) discussed non–E2F-dependent effects of pRb on senescence and differentiation. He has generated several knock-in mice expressing mutant alleles of *cyclin D1* that allow a genetic assessment of different roles of cyclin D1 in development and tumorigenesis. Lysine 112 of cyclin D1 is within the cyclin box of cyclin D1 and the analogous residue in cyclin A directly contacts CDK2. K112E cyclin D1 binds but does not activate CDK4/6. K112E can inhibit senescence but not growth arrest mediated by pRb (22). Dr. Hinds has generated a K112E knock-in mouse that, like the cyclin D1 knockout animal generated by Piotr Sicinski et al., is small in size due to failure to thrive rather than small at birth. They also have a clasping response, and *KE/KE* $D2^{-/-}$ mice have defective cerebellar development. However, whereas the $D1^{-/-}$ mice have collapsed retinal layers and fail to undergo mammary epithelial cell expansion at pregnancy, the *KE/KE* retinas and mammary glands are essentially normal. Nevertheless, pRb phosphorylation is decreased in *KE/KE* retinas. Dr. Hinds discussed earlier results showing that cyclin D1 knockout cells are resistant to neu or ras-induced tumors. He found that *KE/KE* mammary glands are completely resistant to MMTV-neu. These results further support the conclusion that cyclin D/CDK4 is a good target for therapeutic development in breast cancer.

Animal Models

The fifth session on animal models was chaired by Terry Van Dyke (University of North Carolina). David Tuveson (University of Pennsylvania) spoke about the cellular and molecular events involved in progression towards pancreatic cancer. *K-Ras* and *p16* are involved early whereas *p53* and *SMAD4* mutations are late events. He discussed a murine model of early and advanced ductal

pancreatic cancer, based upon the endogenous expression of an oncogenic *K-RasG12D* allele in the developing pancreas. Preinvasive pancreatic cancer neoplasms, termed pancreatic intraepithelial neoplasia (PanIN) is observed in a totally penetrant fashion, with the progression to invasive and metastatic pancreatic ductal adenocarcinoma (PDA) in older mice. The PanINs express matrix metalloproteinase 7, Cox2, Shh1, and Hes1, suggesting potential therapeutic strategies. Epidermal growth factor receptor (EGFR) is increased and ErbB2 is increased in PanIN lesions but only in 50% of PDAs. He discussed a serum proteomics approach as a means to detect PanINs in mice. Finally, he showed an advanced pancreatic cancer model in the background of *p53* mutant mice (23) and mentioned the lack of increase in pancreatic cancer in FAP patients.

Sarki Abdulkadir (University of Alabama) spoke about mouse models of prostate cancer. He discussed NKX3.1, a homeodomain protein that is located at chromosome 8p21 where there is frequent LOH in human prostate cancer. *Nkx3.1* knockouts have branching morphogenesis defects and develop PIN lesions in the prostate; conditional knockouts get similar lesions. *Nkx3.1* haploinsufficiency leads to prostatic hyperplasia. Some work has been done which showed delayed cell cycle exit by differentiating prostate luminal epithelial cells in mutant mice in response to androgen. Microarrays were done in the *Nkx3.1*-deficient background. This unmasked androgen target genes and revealed dosage-sensitive target genes whose dysregulation may underlie haploinsufficiency. Additional work implicated Egr1 in the transition from PIN to invasive cancer.

Terry Van Dyke spoke about effects of targeting the *Rb* family in various cell types, including brain, mammary epithelia, and prostate epithelia. In experiments where the probasin promoter was used to drive the T121 T-antigen mutant (NH_2-terminal 121 amino acids; inactivates pRb, p107, and p130), 100% of mice went through the PIN stage to locally invasive prostate cancer. The T121 prostatic epithelium displayed increased proliferation and apoptosis. Because both *p53* and *PTEN* loss can lead to progressive events, experiments were conducted to determine effects of *p53* or *PTEN* inactivation on the phenotype of the probasin-T121 mice. *PTEN*, but not *p53*, seemed responsible for the observed apoptosis in the probasin-T121 prostatic epithelia, and this was associated with Akt activation. On a $PTEN^{+/-}$ background, the probasin-T121 prostates showed regions of loss of *PTEN* associated with a change from cytoplasmic to membrane-bound Akt and progression to increased invasiveness.

Dr. Van Dyke also discussed an astrocytoma model where the GFAP promoter was used to drive T121 expression. She found that the 100% incidence of astrocytoma was accelerated by *PTEN* loss that was associated with reduced apoptosis, increased invasiveness, and angiogenesis. PI3K inhibition restored apoptosis and inhibited cell invasion, whereas PKC inhibitors suppressed invasion but not apoptosis.

Margie Clapper (Fox Chase Cancer Center) discussed novel strategies for the chemoprevention of colorectal cancer (24). Her efforts have focused on sulindac sulfone, the irreversibly oxidized metabolite of sulindac that lacks cyclooxygenase 1/2 inhibitory activity, as a prototypic agent based on its ability to disrupt β-catenin/Tcf signaling and enhance the localization of β-catenin to the cell membrane. *In vitro* data have been validated *in vivo* using a unique strain of *Min* mice, which, unlike the conventional strain, develops colonic adenomas at a high multiplicity. Small animal magnetic resonance imaging and colonoscopy are currently being used to monitor β-catenin–mediated signaling during colon tumor formation and progression in the *Min* model (25).

Short Oral Presentations

A special session chaired by Mien-Chie Hung featured short oral presentations selected from the submitted abstracts. Frank Slack (Yale University) spoke about the role of let-7 and lin-4 microRNAs in repression of oncogenes such as Ras and their potential function as tumor suppressor genes. The *Ras* gene has multiple sites for potential inhibition by let-7 (26). Let-7 is located in fragile regions associated with lung cancer. Let-7 may target the 3′ untranslated regions of Ras or other oncogenes. His work is beginning to implicate *let-7* as a tumor suppressor gene in lung cancer.

Xiaolu Yang (University of Pennsylvania) spoke about mucosa associated lymphoid tissue lymphomas and the involvement of various translocations involving *Bcl10*, *cIAP2*, and *MALT1*. Bcl10 and MALT1 are involved signaling from the antigen receptor to NF-κB activation. TCR leads to formation of a complex between Bcl10 and MALT1, which signals to TRAF6 leading to IKK ubiqutination and NF-κB activation. He showed data on the role of cIAP2 in antigen receptor signaling and the dysregulation of this function of c-IAP2 in MALT lymphomas.

Eric Wickstrom (Thomas Jefferson University) has been developing noninvasive imaging methodologies to detect oncogene expression using Tc-99m-PNA-peptides for single-photon emission computed tomography imaging and Cu-64-PNA-peptides for positron emission tomography imaging. These efforts are providing proof-of-principle results to support the use of oncogene mRNA imaging in the clinic and are also paving the way for therapeutic targeting of oncogenes while using imaging for validation (27, 28).

Cheng Liu (The Scripps Research Institute) presented results regarding a novel legumain-activated, cell impermeable doxorubicin prodrug LEG-3 that was designed to be activated exclusively in the tumor microenvironment. The tumor microenvironment is notably enriched with a broad spectrum of proteases. Legumain, the only asparaginyl endopeptidase of the mammalian genome, is highly expressed by neoplastic, stromal, and endothelial cells in solid tumors. It is associated with matrix as well as cell surfaces and locally functional in the reduced pH of the tumor microenvironment. Upon administration of the prodrug, there is a profound increase of the end-product doxorubicin in nuclei of cells in tumors but little in other tissues. This prodrug completely arrested growth of a variety of neoplasms, including multidrug resistant tumors *in vivo*, and significantly extended survival without evidence of myelosuppression or cardiac toxicity.

DNA Damage, Repair, and Beyond

The sixth session on DNA damage, repair, and beyond was chaired by Thanos Halazonetis (Wistar Institute). Martin Brown (Stanford University) discussed a yeast screen using nearly 5,000 strains each carrying a unique 20-mer oligonucleotide cassette that can allow identification of each unique mutant strain and its differential sensitivity to nearly 60 different treatments through hybridization to an oligonucleotide array (29). He then performs hierarchical clustering to identify agents that work by similar mechanisms (e.g., drugs that affect homologous recombination versus nucleotide excision repair or topoisomerases). He showed that Tirapazamine, a hypoxia-activated topoisomerase II poison, clusters with Idarubicin and identified a new gene *Rad 61* that regulates sister chromatid cohesion. He also identified a new pathway in radiosensitivity involving Rad6 and Bre1 ubiquitination of Lys^{123} on histone H2B leading to methylation of Lys^{79} on histone H3 by Dot1.

Bin-Bing Zhou (Incyte Corporation) spoke about targeting ligand cleavage in therapy of ErbB-dependent tumors. Because ErbB ligands are expressed as membrane-bound proforms and need to be proteolytically cleaved to be activated, his group has been targeting ADAM proteases whose blockade inhibits ErbB ligand activation. Both Heregulin and Her3 are overexpressed in non–small cell lung cancer (NSCLC). H1650 cells with EGFR mutation are sensitive to Iressa and this is associated with inhibition of phospho-Akt. A549 cells contain wild-type EGFR and are not sensitive to Iressa *in vitro*. Addition of Heregulin bypasses sensitivity to Iressa and inhibits apoptosis in H1650 cells. As a control, addition of EGF cannot bypass the Iressa sensitivity in H1650. A549 cells express Heregulin and Her3. Both Her3 and Akt are constitutively active in A549 cells. Blocking Heregulin binding to Her3 by an antibody inhibits Akt but not ERK phosphorylation in A549 cells. ADAM17 knockdown inhibits Heregulin signaling, suggesting ADAM17 as the major protease for heregulin. Dr. Zhou presented data using inhibitors of ADAM10 and ADAM17 and a dual inhibitor that blocks Her3 and Akt pathways in A549 cells. Addition of soluble recombinant heregulin can bypass signaling inhibition and apoptosis by ADAM 17 or dual inhibitors.

Vimla Band (Northwestern University) discussed the molecular basis of immortalization of different subtypes of mammary epithelial cells (MEC) using human papillomavirus (HPV) oncoproteins E6 and E7 as a model to examine the early steps in epithelial cancers. She showed that basal subtypes of MECs are immortalized by *E6* but not by *E7*, whereas luminal subtypes of MECs are immortalized by *E6* and *E7*, indicating differential susceptibility of MEC subtypes to different oncogenes. Her talk further focused on defining the biochemical pathways that are disrupted by the *E6* oncogene during immortalization. She discussed data that dominant-negative *p53* is not as effective as *E6* at immortalization, indicating that E6 immortalizes epithelial cells by targeting several other cellular proteins. She presented work from her laboratory on several novel E6 targets: a novel Rap1GAP designated as E6TP1; a novel transcriptional coactivator of p53, retinoid receptors, and estrogen receptors, hADA3 (30); a Rho-activated serine/threonine protein kinase, PKN; a coactivator for Notch-mediated transactivation, MamL1; and a novel regulator of p53, EIPR (E6-interacting p53 regulator). These proteins are associated with epithelial cancer-associated HPVs but not with benign lesion-associated HPVs. Moreover, mammary epithelial cell-immortalizing E6 mutants but not the nonimmortalizing E6 mutants bind to these proteins thus implicating these proteins as important targets in epithelial tumorigenesis.

Dr. Band particularly focused on EIPR, a novel p53 interacting protein that stabilizes p53 protein thus enhancing the p53-mediated transactivation of target genes. Overexpression of EIPR induces the p53-dependent cellular senescence and the knockdown of EIPR results in the down-regulation of p53 and p21. These studies suggest the role of EIPR as a novel p53 regulator. Dr. Band's laboratory is currently examining the role of the novel E6 targets in epithelial cell biology and oncogenesis.

Thanos Halazonetis spoke about DNA damage checkpoint pathways. He first showed that the DNA damage checkpoint pathway can be activated in human cancers. He showed that in lung and colon cancers histone H2AX and the checkpoint kinase Chk2 are phosphorylated, indicating that the DNA damage checkpoint pathway had been activated.

However, most of Dr. Halazonetis' presentation was focused on 53BP1, a DNA damage checkpoint protein that functions upstream of the checkpoint kinases ATM and Chk2. He proposed that 53BP1 is a sensor for DNA double-strand breaks (DSB). He showed the structure of the domain that is necessary and sufficient for 53BP1 to be recruited to DNA DSBs. This domain has a deep hydrophobic pocket that binds methylated Lys^{79} of histone H3. The interaction with histone H3 is necessary for recognition of DNA DSBs by 53BP1; furthermore, Lys^{79} is constitutively methylated in the genome. These observations led to the model that DNA DSBs affect chromatin structure resulting in exposure of methylated Lys^{79} of histone H3 and then to recruitment of 53BP1 (31). In support of this model, some of the genes identified by Martin Brown as affecting sensitivity to ionizing radiation encode enzymes that affect methylation of Lys^{79} of histone H3.

Drug Discovery and Development

The seventh session on drug discovery and development was chaired by Edward Sausville (University of Maryland). Dr. Sausville discussed current experimental approaches for treatment of bulky tumors, including combinations of antiproliferative agents, proapoptotic agents, and antivascular agents. For prevention, he mentioned approaches using antimutator, anti-telomerase, and anti-invasive drugs. As a recent good example, he discussed the Irinotecan, 5-fluorouracil/LV plus Bevacizumab combination, which increased median progression-free survival in metastatic colon cancer.

Dr. Sausville spoke about a recent NIH workshop "Road Map" to the clinic where he described steps in drug development. These include prioritization by *in vitro* effects and effects in synergy experiments, utilization of pharmacokinetics from early human studies to model clinical trials, and lack of necessity of *in vivo* tumor efficacy of combination therapies. He discussed a changing paradigm in drug development. The old paradigm involved combining drugs to reduce toxicity and maximize pharmacokinetics, whereas the new paradigm is targeting pathways. He mentioned surprising recent results with the Bayer Raf kinase inhibitor. As a single agent, response rates were 2% in melanoma, 35% in renal cell carcinoma, and 40% in thyroid cancer. However, in combination with carboplatin and taxol the Raf kinase inhibitor had a 40% response rate in melanoma.

Dr. Sausville provided an update on Flavopiridol and mentioned its effects to inhibit cyclin D1 and D3 mRNA expression. Cyclin D1 is rate limiting for foci induced by HER-2/*neu*. There is a study at MGH using Trastuzumab and Flavopiridol. He mentioned Perifosine that inhibits Akt recruitment to the cell membrane. UCN01 inhibits Akt phosphorylation by inhibiting PDK1. A combination of UCN01 and Perifosine targets both phosphorylation and membrane localization of Akt and this shows cytostatic effects in culture. Because it is difficult to predict pharmacokinetic interactions in humans based on mouse studies, the NCI workshop in 2003 failed to reach the conclusions that "enhanced" activity in an animal model on the part of a combination in comparison to single agents was a necessary prelude to clinical studies, but that it certainly would increase enthusiasm. Rather, a clear understanding of the pharmacology achievable in the human with reference to additive or synergistic effects of the single agents *in vitro* offered the best chance of not missing a valuable combination in the clinic.

Andrei Gudkov (The Cleveland Clinic) discussed strategies to protect normal tissues from radiation toxicity. He made a point that although p53-deficient mice are resistant to radiation at low doses of radiation that kill by inducing a hematopoietic syndrome, they

are actually more sensitive as compared with wild-type mice to higher doses (15 Gy) that induce a lethal gastrointestinal syndrome (32). Thus, at high doses of radiation, p53 plays the role of a survival factor for the GI tract, presumably protecting radiosensitive damaged cells from premature entrance into mitosis resulting in mitotic catastrophe, a frequent cause of death of p53-deficient cells.

Dr. Gudkov discussed the role of p53 as a survival factor in tumor endothelium exposed to radiation and chemotherapy and showed that pharmacologic inhibition of p53 can have antiangiogenic effect, improving the outcome of chemotherapy and radiotherapy in mouse tumor models. He described isolation of a new type of p53 modulating radioprotective small molecules that inhibit p53-mediated apoptosis by suppressing p53 translocation to the mitochondria, having no effect on p53-dependent transactivation. He also discussed a new approach to radioprotection acting via pharmacologic activation of NF-κB. This approach uses natural modulators of NF-κB produced by mammalian bacteria and involved in the interaction with host cells. He described strategies using derivatives of flagellin of *Salmonella*, the ligand of Toll-like receptor 5, to protect mice from both gastrointestinal and hematopoietic radiation syndrome. Importantly, the developed radioprotectant had no rescuing effect on irradiated tumors thus opening the possibility of future therapeutic applications of these types of radioprotective agents to reduce radiotherapy side effects.

Dihua Yu (M.D. Anderson Cancer Center) discussed *PTEN* deficiency and Herceptin resistance. Less than 35% of patients with ErbB2-positive breast tumors respond to Herceptin as a single agent and 2% to 5% of Herceptin-treated patients suffer from severe side effects including cardiac dysfunction. Thus, there is a need to identify patients who will not respond. Dr. Yu discussed mechanisms by which Herceptin inhibits Akt phosphorylation independent of ErbB2 down-regulation. There is evidence for increased PTEN phosphatase activity in Herceptin-treated SkBr3 breast cancer cells and increased membrane-bound PTEN. Herceptin induces src dissociation from ErbB2 that inactivates src tyrosine kinase leading to reduced tyrosine phosphorylation of PTEN and thus PTEN goes to the membrane where it is active. Activation of PTEN by Herceptin contributes to its antitumor activity and loss of PTEN in breast tumors confers Herceptin resistance in breast cancer patients (33).

E. Premkumar Reddy (Temple University) discussed polo-like kinase 1 and the possibility for its involvement in the mechanism of action of novel therapeutics his group has identified. Mutations in *Plk1* in *Drosophila* lead to abnormalities in the mitotic spindle, chromosomal segregation, and cytokinesis. Plk1 transforms 3T3 cells and is overexpressed in many tumor cells.

In esophageal, pancreatic, and liver cancer, Plk1 overexpression is associated with reduced patient survival. Down-regulation of Plk1 leads to mitotic arrest, spindle abnormalities, and apoptosis. Plk1 also promotes mitotic progression through positive regulation of cdc25 and negative regulation of Myt1. In turn, cdc25 positively regulates CDK1 and Myt1 negatively regulates CDK1. A screen for small molecules that induce apoptosis of tumor but not normal cells identified a number of cell cycle inhibitors of which ON01910 was found to be an inhibitor of Plk1 with an IC_{50} of 9 nmol/L.

Dr. Reddy presented data that ON01910 is effective against a wide variety of tumors including multidrug resistant tumors. ON01910 seems to act as an irreversible inhibitor of Plk1 such that tumor cells undergo mitotic arrest followed by apoptosis, whereas normal cells undergo G_1 arrest. A number of other kinases such as Abl, Flt1, PDGF receptor, and Met are also inhibited.

Dr. Reddy reported that xenograft studies using MCF7 and other tumor lines were done using combinations of ON01910 with doxorubicin which is a very effective combination. Studies were done using Bel-7402, which is an aggressive liver tumor model. ON01910 plus oxaliplatin was shown effective in this model. Using a pancreatic tumor model MiaPaca, ON01910 plus Gemzar gave dramatic results over Gemzar alone, which has activity in this model. An antiangiogenic effect was observed and attributed to the blockade of Flt3.

A phase 1 study has been ongoing at Johns Hopkins using 80 mg total dose and ON01910 seems to have good pharmacokinetics. The lack of toxicity to normal cells is related to their G_1 rather than mitotic arrest. Dr. Reddy reported that ON012380 is a non–ATP-competitive inhibitor of BCR-ABL that overrides imatinib resistance (34). ON012380 specifically inhibits BCR-ABL and induces apoptosis of Philadelphia chromosome positive chronic myelogenous leukemia cells at concentrations of <10 nmol/L.

Models and Emerging Technologies

The eighth session on models and emerging technologies was chaired by Vivek Rangnekar (University of Kentucky). Nicholas Nicolaides (Morphotek) described a 133-amino-acid polypeptide that can inhibit mismatch repair in bacteria, yeast, plants, or mammalian cells through genome-wide evolution. This technology is being used for numerous applications including the evolution of high-titer, high-specificity antibody producing cell lines. In selected applications, the mismatch repair defect is reversible.

Samir Hanash (Fred Hutchinson Cancer Research Center) spoke about the integrated Molecular Profiling Consortium and efforts to make an antibody repertoire that targets cancer proteins. He discussed antibody arrays that currently have a limited repertoire and limited sensitivity, as well as protein arrays which whereas labor intensive are sensitive, quantitative, and being applied to paraffin blocks. Dr. Hanash discussed serum and plasma profiling highlighting some of the challenges such as the fact there are between 5,000 and 10,000 proteins expressed at a range of 12 logs in concentration. He discussed strategies for fractionating the samples and using high-resolution mass spectrometry to deal with some of these limitations. A number of groups are exploring serum proteomics using various mouse models. Interestingly, most changes being found involve mouse host protein changes. Cystatin A was found to be produced by human tumor cells. FABP5 was also found to be produced.

Vivek Rangnekar discussed *Par-4*, a gene induced in a testosterone-regulated manner in prostate tissue and which is associated with cancer-selective apoptotic action. Experiments showed that Par-4 can act as a corepressor in collaboration WT1 and that IFN-treated cells have Par-4 in PML bodies. Endogenous Par-4 seems essential for apoptosis induced by diverse insults (such as TNF, TRAIL, ionizing radiation, growth factor withdrawal, thapsigargin, vincristine, taxol, and doxorubicin) that work via the intrinsic and extrinsic apoptotic pathways. Par-4 can induce apoptosis in *p53*- and *PTEN*-deficient backgrounds and even with Bcl-2 or Bcl-XL overexpression. Nuclear localization of Par-4 seems important for its mechanism of apoptosis. Dr. Rangnekar presented a model wherein Par-4 stimulates FasL/Fas trafficking to the cell membrane whereas inhibiting NF-κB transcription activity leading to reduced expression of XIAP proteins, and other results suggesting that Par-4–induced death is independent of the mitochondria. The domain and mechanism of the cancer-selective action of Par-4 was also presented (35).

Translating Knowledge from the Laboratory to Clinical Trials

Sessions 9 and 10 on translating knowledge from the lab to clinical trials were both chaired by Stephen Fesik (Abbott). Judith Sebolt-Leopold (Pfizer) discussed MEK inhibitors and CDK4 inhibitors. She discussed various strategies of approaching molecular targets, including (a) use of multitargeting agents such as kinase inhibitors, HSP90 inhibitors, or proteasome inhibitors; (b) combinations of signal transduction inhibitors and chemotherapeutics; and (c) selective inhibitor combinations. One inhibitor she discussed is PD0325901, which inhibits the step between MEK1/MEK2 and ERK1/ERK2 activation (36, 37). The reason for selectivity has to do with the fact the agent is non–ATP competitive and its use allows specific MEK inhibition after a single oral dose. PD0332991 is highly selective against CDK4/6. Preclinical combination of the two agents resulted in a significant increase in the number of complete responders and tumor-free survivors compared with the maximal response observed with either agent alone, despite better tolerability of higher doses in the single agent regimens. Colo205 was the most sensitive tumor model, whereas mice bearing BxPC-3 pancreatic tumors also showed benefit from the combination therapy. To get to the clinic more work is needed on mechanisms of synergy and more efforts are being directed at sequencing and dosing the two agents.

Steven Fesik spoke about the discovery of a Bcl-2 family inhibitor, ABT-737, that binds to Bcl-2, Bcl-xL, and Bcl-w with subnanomolar affinity, exhibits synergistic cytotoxicity with chemotherapy and radiation in a variety of different cell lines, and regresses SCLC as a single agent in xenograft tumor models. The compound was discovered using SAR by nuclear magnetic resonance (a nuclear magnetic resonance–based screening technique invented by Fesik), parallel synthesis, and structure-based drug design. In addition to using structures to improve potency, a structure-based approach was used to design out albumin binding (a severe limitation in earlier compounds). Based on extensive mechanistic studies, it was found that ABT-737 does not directly release cytochrome *c*–like Bid but acts more like Bad and displaces BH3 peptides and proteins from Bcl-2 and Bcl-xL. In preliminary studies aimed at identifying the genetic basis for sensitivity/resistance to ABT-737 treatment in SCLC cell lines, sensitivity was correlated to the expression of Bcl-2, whereas resistance was associated with high levels of Mcl-1. Current approaches to further define drug sensitivity include the use of microarrays and siRNA libraries.

Steven Grant (Medical College of Virginia) gave a general overview of targeting signal transduction pathways and current strategies involving HDAC inhibitors, STI571, TRAIL, HSP90 antagonists, proteasome inhibitors, flavopiridol, UCN-01, and MEK inhibitors. He also provided evidence that tumor cells may be particularly susceptible to simultaneous interruption of survival signaling and cell cycle regulatory pathways. As an example, he referred to recent work demonstrating that the Src kinase Lyn may be involved in some forms of Gleevec resistance and evidence that certain combination regimens (e.g., flavopiridol + velcade) are very active against Lyn(+) cells (38, 39). Velcade seems to act by inhibiting the degradation of IκB leading to NF-κB trapping in the cytoplasm, which lowers the threshold for CDK inhibitor–mediated lethality (40). In human leukemia cells, flavopiridol plus Velcade results in c-*jun* NH_2-terminal kinase activation and down-regulation of XIAP and Mcl-1. He also discussed the rationale for combining flavopiridol with the HDAC inhibitor SAHA, which leads to a dramatic increase in mitochondrial damage and apoptosis in human leukemia cells. Phase I trials involving flavopiridol/Velcade and SAHA/flavopiridol are either under way or scheduled to begin shortly.

Eugene Gerner (University of Arizona) discussed a strategy to reduce colon cancer mortality that included risk assessment, screening, prevention, and treatment strategies targeting genetic risk factors in this disease. He showed evidence that Myc expression is reduced after wild-type adenomatous polyposis coli (APC) expression, whereas Mad1 (but not Max) is increased after APC expression. He discussed the generation of a Myc intestine-specific knockout using FABP-Cre and LoxP-Myc crossed into the multiple intestinal neoplasia (*Min*) background resulting in an *APC*- and *Myc*-deficient mouse that has villous atrophy. This showed effects of Myc on development and the phenotype was patchy consistent with FABP expression. Preliminary results suggest that loss of Myc expression reduces APC-dependent intestinal tumor formation.

Dr. Gerner further discussed evidence indicating that promoters for *Myc* and several other genes contain a G-quadriplex, which is the basis for an anti–colon cancer agent that inhibits but is not selective for Myc signaling (41). Dr. Gerner mentioned a model showing effects of APC inhibiting Myc expression and stimulating Mad1 expression ultimately leading to reduced ODC expression, which leads to reduced levels of polyamines and decreased carcinogenesis. This pathway is currently being evaluated in clinical cancer prevention trials (42). He discussed the role of genetic variability in determining the progression of polyps and an ODC SNP associated with adenoma recurrence. The ODC genotype predicts adenoma risk reduction by aspirin and may form the basis of a clinical predictive test in future cancer prevention trials.

W. Gillies McKenna (University of Pennsylvania) presented the use of targeted therapy involving radiation as having met with much success because of imaging. He discussed radiation plus Cetuximab as useful for loco-regional control and overall survival. He also examined basic studies involving effects of ras in combination with Myc in radioresistance. In collaborative studies with Eric Stanbridge using knockout cell lines of K-*ras* or N-*ras*, his group found restoration of sensitivity to radiation upon reintroduction of activated *ras* genes. PI3K also conferred radioresistance. siRNA to K-*ras* in SW480 colon cancer cells conferred radiosensitivity associated with decreased phospho-Akt expression and decreased MAPK activity. His group also done siRNA to PI3K p85 and p110 subunits as well as Akt1, Akt2, and Akt3 to show radiosensitization effects. Combination of FTI plus radiation in T24 cells was done in the preclinical phase, whereas the clinical trial used a continuous infusion FTI protocol in lung and head and neck cancer. Some clinical responses were observed, although none of the NSCLC specimens had ras mutations raising the issue of whether ras was the target. He speculated that EGFR activation of ras without mutation might explain some of the clinical responses that prompted the use of phospho-Akt as a marker of ras inhibition in the absence of mutation. His group found a statistically significant correlation between response and inhibition of phospho-Akt. He emphasized that in targeting signaling pathways, it is important to understand how the pathways are networked and not to consider only which genes are mutated.

Optical Imaging Applications in Cancer Research

The final oral session on optical imaging applications in cancer research was chaired by Bruce Tromberg (University of California at Irvine). This session was combined with a meeting of the Network for Translational Research in Optical Imaging and an NCI Steering Committee meeting held on November 9, 2004. Dr. Tromberg spoke

about the use of optical imaging techniques in breast cancer. One of the current challenges revolves around standardizing the technology. One of the goals is to improve on the weakness of mammography in women with high radiographic density, typically premenopausal and perimenopausal individuals. Mammography has a >20% false-negative rate in women ages <50 years, which translates into roughly 9 to 10,000 undiscovered cancers per year.

According to guidelines established by the NIH, mammography is not useful in women ages <40 years. Overall, up to 10% of breast cancers remain undiscovered which translates into ~20,000 cases per year in the United States. Diffuse optical imaging provides a quantitative spectral fingerprint of tissue composition. Several tissue constituents are quantitatively imaged in breast tissue, including the concentration of deoxyhemoglobin, oxyhemoglobin, lipid, water, and the relative density and size of light scattering particles. Composite "tissue optical indices" are developed from these measurements that report on cellular metabolism, angiogenesis, and matrix integrity. Data are acquired using either hand-held scanners or tomographic imagers. Results from 60 patients were shown showing that optical variables change significantly during the appearance and progression of malignant tumors. In addition, these variables can be rapidly monitored at the bedside of patients undergoing neoadjuvant chemotherapy. Preliminary data from 11 patients shows that chemotherapy-induced cell death leads to measurable reductions in deoxyhemoglobin, water, and total hemoglobin content. These changes can be used to predict responders (versus nonresponders) after only 1 week of a 9-week treatment. A key challenge is in differentiating between complete, partial, and nonresponders because research has shown that complete pathologic response is correlated with patient survival.

Irving Bigio (Boston University) discussed the localized determination of the absorption and scattering properties of tissue, using the methods of "optical pharmacokinetics" and "elastic-scattering spectroscopy," for the management of cancer/precancer treatment by photodynamic therapy. He and collaborators are using elastic scattering spectroscopy to monitor changes associated with Barrett's esophagus, by assessing the changes in scattering properties of organelles, and they employ optical pharmacokinetics to monitor drug concentrations in tissue noninvasively in real time (43). Dr. Bigio mentioned that, in addition to drug concentrations, other localized tissue properties in tumors are obtained, including hemoglobin saturation.

Christopher Contag (Stanford University) showed that bioluminescence imaging can be used to follow tumor responses to therapy and assess the minimal residual disease states that persist after therapy. The sensitivity of this imaging approach indicated that imaging of bioluminescent markers should be used to develop therapies that target minimal disease states and to investigate the mechanisms of immune surveillance with the goal of preventing relapse. Toward this end, he showed how this method can be used to monitor hematopoiesis from single stem cells in mice (44). He also showed how immune surveillance of malignancy can be monitored as a means of improving cell-based therapies for cancer.

The success of these developments in mouse models of human cancer suggested that new optical tools need to be developed and applied to monitoring human disease. Dr. Contag outlined the constraints on optical imaging in clinical environments and suggested some approaches where optics has strengths over other imaging modalities and described the approach being taken at Stanford for moving optical imaging tools into the oncology clinic.

Dr. Contag showed developments in *in vivo* confocal microscopy using fiber-based miniaturized microscopes that have utility in the detection and typing of malignant lesions in the clinic. These tools will be able to reveal, at the cellular level, both the structural and functional changes that mark malignancy (45). In particular, Dr. Contag showed that ratios of nuclear to cytoplasmic volume could be calculated using a dual-axis confocal microscope that operates at 1,340 nm, and that fluorescent markers of disease could be detected with a dual axes instrument operating at 488 nm. The target tissue for the first instruments is the esophagus and other hollow organs in the body where endoscopic approaches are already being used. The use of these tools will continue to increase as the capabilities of these instruments are advanced and as new fluorescent reagents are codeveloped with these powerful mini-microscopes.

The objectives for optical imaging in the clinic are to capitalize on the strengths of optics to complement other imaging modalities and to address the unmet needs of monitoring disease where the use of more expensive and less mobile modalities is not practical.

Wafik El-Deiry (University of Pennsylvania) discussed recent progress in the use of bioluminescence imaging to monitor tumor development or growth in mice in response to oncogene expression in normal human cells or in response to gene silencing of tumor suppressors in tumor xenografts. He gave several examples of the use of gene silencing of tumor growth inhibitors or apoptotic genes such as *p53*, *DR5*, or *Bnip3L* to show a selective growth advantage for the genetically altered tumors *in vivo* (46, 47). He provided examples of imaging transcriptional events within tumor xenografts *in vivo* following systemic chemotherapy, experimental therapy, or local gene therapy. He showed efforts to develop molecular beacons to monitor events within tumor cells noninvasively to eventually be able to predict response to cancer therapy. Finally, he showed use of bioluminescence imaging in high-throughput drug screening as well as monitoring response to specific systemic therapeutic agents such as TRAIL or novel small molecule therapeutics that appear to modulate the p53 pathway.

Acknowledgments

Received 2/24/2005; accepted 4/6/2005.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

References

1. Hutchinson LM, Chang EL, Becker CM, et al. Use of thymosin β15 as a urinary biomarker in human prostate cancer. Prostate. In press 2005.
2. Everley PA, Krijgsveld J, Zetter BR, Gygi SP. Quantitative cancer proteomics: stable isotope labeling with amino acids in cell culture (SILAC) as a tool for prostate cancer research. Mol Cell Proteomics 2004;3:729–35.
3. Hu MC-T, Lee D-F, Xia W, et al. IkB kinase promotes tumourigenesis through inhibition of Forkhead transcription factor FOXO3a. Cell 2004;117:225–37.
4. Li YM, Pan Y, Wei Y, et al. Upregulation of CXCR4 is essential for HER2-mediated tumor metastasis. Cancer Cell 2004;6:459–69.
5. Zhou BP, Deng J, Xia W, et al. Dual regulation of Snail by GSK-3-mediated phosphorylation in control of epithelial-mesenchymal transition. Nature Cell Biol 2004;6:931–40.
6. Wang H, Fu W, Im JH, et al. Tumor cell α3β1 integrin and vascular laminin-5 mediate pulmonary arrest and metastasis. J Cell Biol 2004;164:935–41.
7. Im JH, Fu W, Wang H, et al. Coagulation facilitates tumor cell spreading in the pulmonary vasculature during early metastatic colony formation. Cancer Res 2004;64:8613–9.
8. Allinen M, Beroukhim R, Cai L, et al. Molecular characterization of the tumor microenvironment in breast cancer. Cancer Cell 2004;6:17–32.

9. Kerbel RS, Kamen BA. The anti-angiogenic basis of metronomic chemotherapy. Nat Rev Cancer 2004;4: 423–36.

10. Shaked Y, Bertolini F, Man S, et al. Genetic heterogeneity of the vasculogenic phenotype parallels angiogenesis; implications for cellular surrogate marker analysis of antiangiogenesis. Cancer Cell 2005;7: 101–11.

11. Bertwistle D, Sugimoto M, Sherr CJ. Physical and functional interactions of the Arf tumor suppressor protein with nucleophosmin/B23. Mol Cell Biol 2004; 24:985–96.

12. Kuo M-L, den Besten W, Bertwistle D, Roussel MF, Sherr CJ. N-terminal polyubiquitination and degradation of the Arf tumor suppressor. Genes Dev 2004;18: 1862–74.

13. Calin GA, Sevignani C, Dumitru CD, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A 2004;101:2999–3004.

14. Calin GA, Liu CG, Sevignani C, et al. MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. Proc Natl Acad Sci U S A 2004; 101:11755–60.

15. Saal LH, Holm K, Maurer M, et al. *PIK3CA* mutations correlate with hormone receptors, node metastasis, ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. Cancer Res 2005;65:2554–9.

16. Puc J, Keniry M, Li HS, et al. Lack of PTEN sequesters CHK1 and initiates genetic instability. Cancer Cell 2005;7:193–204.

17. Schimmer AD, Welsh K, Pinilla C, et al. Small-molecule antagonists of apoptosis suppressor XIAP exhibit broad antitumor activity. Cancer Cell 2004;5: 25–35.

18. Wang Z, Cuddy M, Samuel T, et al. Cellular, biochemical, and genetic analysis of mechanism of small molecule IAP inhibitors. J Biol Chem 2004;279: 48168–76.

19. Carter BZ, Gronda M, Wang Z, et al. Small-molecule XIAP inhibitors derepress downstream effector caspases and induce apoptosis of acute myeloid leukemia cells. Blood. In press 2005.

20. Zong WX, Ditsworth D, Bauer DE, Wang ZQ, Thompson CB. Alkylating DNA damage stimulates a regulated form of necrotic cell death. Genes Dev 2004; 18:1272–82.

21. Goulet AC, Chigbrow M, Frisk P, Nelson MA. Selenomethionine induces sustained ERK phosphorylation lead to cell cycle arrest in human colon cancer cells. Carcinogenesis 2005;26:109–17.

22. Baker GL, Landis MW, Hinds PW. Multiple functions of D-type cyclins can antagonize pRb-mediated suppression of proliferation. Cell Cycle 2005;4:330–8.

23. Olive KP, Tuveson DA, Ruhe ZC, et al. Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. Cell 2004;119:847–60.

24. Clapper ML, Coudry J, Chang WC. β-Catenin-mediated signaling: a molecular target for early chemopreventive intervention. Mutat Res 2004;555:97–105.

25. Hensley HH, Chang WC, Clapper ML. Detection and volume determination of colonic tumors in Min mice by magnetic resonance micro-imaging. Magn Reson Med 2004;52:524–9.

26. Johnson S, Grosshans H, Shingar J, et al. *RAS* is regulated by the *let-7* microRNA family. Cell 2005; 120:635–47.

27. Tian X, Aruva MR, Qin W, et al. External imaging of *CCND1* cancer gene activity in experimental human breast cancer xenografts with Tc- 99 m-peptide-PNA-peptide chimeras. J Nuclear Med 2004;45:2070–82.

28. Tian X, Aruva MR, Qin W, et al. Noninvasive molecular imaging of *MYC* mRNA expression in human breast cancer xenografts with a [99mTc]peptide-peptide nucleic acid-peptide chimera. Bioconjugate Chemistry 2005;16:70–9.

29. Wu HI, Brown JA, Dorie MJ, Lazzeroni L, Brown JM. Genome-wide identification of genes conferring resistance to the anticancer agents cisplatin, oxaliplatin, and mitomycin C. Cancer Res 2004;64:3940–8.

30. Meng G, Zhao Y, Nag A, et al. Human ADA3 binds to estrogen receptor (ER) and functions as a coactivator for ER-mediated transactivation. J Biol Chem 2004;279: 54230–40.

31. Huyen Y, Zgheib O. Ditullio RA Jr, et al. Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. Nature 2004;432:406–11.

32. Komarova EA, Kondratov RV, Wang K, et al. Dual effect of p53 on radiation sensitivity *in vivo*: p53 promotes hematopoietic injury, but protects from gastro-intestinal syndrome in mice. Oncogene 2004;23: 3265–71.

33. Nagata Y, Lan KH, Zhou X, et al. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell 2004;6:117–27.

34. Gumireddy K, Baker SJ, Cosenza SC, et al. A non-ATP-competitive inhibitor of BCR-ABL overrides imatinib resistance. Proc Natl Acad Sci U S A 2005;102: 1992–7.

35. Gurumurthy S, Goswami A, Vasudevan KM, Rangnekar VM. Phosphorylation of Par-4 by protein kinase A is critical for apoptosis. Mol Cell Biol 2005; 25:1146–61.

36. Rinehart J, Adjei AA, Lorusso PM, et al. Multicenter phase II study of the oral MEK inhibitor, CI-1040, in patients with advanced non-small-cell lung, breast, colon, and pancreatic cancer. J Clin Oncol 2004;22: 4456–62.

37. Sebolt-Leopold JS, Herrera R. Targeting the mitogen-activated protein kinase cascade to treat cancer. Nat Rev Cancer 2004;4:937–47.

38. Dai Y, Rahmani M, Pei XY, Dent P, Grant S. Bortezomib and flavopiridol interact synergistically to induce apoptosis in chronic myeloid leukemia cells resistant to imatinib mesylate through both Bcr/Abl-dependent and -independent mechanisms. Blood 2004; 104:509–18.

39. Dai Y, Rahmani M, Corey SJ, Dent P, Grant S. A Bcr/Abl-independent, Lyn-dependent form of imatinib mesylate (STI-571) resistance is associated with altered expression of Bcl-2. J Biol Chem 2004;279: 34227–39.

40. Gao N, Dai Y, Rahmani M, Dent P, Grant S. Contribution of disruption of the nuclear factor-κB pathway to induction of apoptosis in human leukemia cells by histone deacetylase inhibitors and flavopiridol. Mol Pharmacol 2004;66:956–63.

41. Seenisamy J, Rezler EM, Powell TJ, et al. The dynamic character of the G-quadruplex element in the c-MYC promoter and modification by TMPyP4. J Am Chem Soc 2004;126:8702–9.

42. Gerner EW, Meyskens FL Jr. Polyamines and cancer: old molecules, new understanding. Nat Rev Cancer 2004;4:781–92.

43. Bigio IJ, Bown SG. Spectroscopic sensing of cancer and cancer chemotherapy: current status of translational research. Cancer Biol Ther 2004;3:259–67.

44. Cao Y, Wagers A, Beilhack A, et al. Shifting foci of hematopoeisis during reconstitution from single stem cells. Proc Natl Acad Sci U S A 2004;101:221–6.

45. Wang, TD, Mandella MJ, Contag CH, Kino GS. Dual axes confocal fluorescence microscope for *in vivo* molecular and cellular imaging. J Biomed Optics 2004; 9:735–42.

46. Wang S, El-Deiry WS. Inducible silencing of KILLER/DR5 *in vivo* promotes bioluminescent colon tumor xenograft growth and confers resistance to chemotherapeutic agent 5-fluorouracil. Cancer Res 2004;64: 6666–72.

47. Fei P, Wang W, Kim SH, et al. Bnip3L is induced by p53 under hypoxia, and its knockdown promotes tumor growth. Cancer Cell 2004;6:597–609.

Supplementary Information

An intensive two-day meeting was held recently in Philadelphia, Pennsylvania from Sunday, November 7 to Tuesday, November 9, 2004 to bring together leading experts to discuss the molecular basis of tumor progression and to explore opportunities for therapeutic development when drug resistance occurs. Over 250 participants attended 14 different sessions featuring 3-5 speakers in the oral sessions and included were poster sessions and social events. Introductory remarks were made by Karen Antman, NCI and the keynote address was delivered by Robert Horvitz, MIT. The meeting included a working lunch with NCI program directors discussing important aspects of NIH grant programs as well as funding opportunities. A number of awards were given for outstanding submitted abstracts. The final session was jointly held with the Network for Translational Research in Optical Imaging in conjunction with an NCI Steering Committee meeting. Written feedback from conference attendees was very positive but also included some helpful suggestions for improvement such as more discussion time and more structure to the poster sessions.

Major sponsors of this GTCbio conference (http://www.gtcbio.com/) included Pfizer, Inc, The Abramson Cancer Center at the University of Pennsylvania, Landes Bioscience, the Fels Institute, Onconova, *Cancer Cell*, the Wistar Institute, the Kimmel Cancer Center at Thomas Jefferson University, *Nature Medicine*, and the New York Academy of Sciences. The Organizing Committee included Houston Baker, Vimla Band, Larry Clarke, Carlo Croce, Bimal Dasmahapatra, Stephen Fesik, John Glick, Andrei Gudkov, Meenhard Herlyn, Mien-Chie Hung, Russel Kaufman, Robert Kerbel, Tak Mak, Gillies McKenna, Suresh Mohla, Mark Nelson, Allen Oliff, Martin Padarathsingh, Ramon Parsons, Kornelia Polyak, Vivek Rangnekar, E. Prem Reddy, John Reed, Edward Sausville, Li-Kuo Su, Craig Thompson, Bert Vogelstein, Bin-Bing Zhou, and Wafik S. El-Deiry (Conference Chair).

In her introductory remarks, **Karen Antman** showed data that the 5-year survival from cancer in 2004 was 64% as compared to 50% in 1971 and 20% in 1935. While mortality per 100,000 has declined since 1992 (http://seer.cancer.gov/), we have an aging population and more progress is needed in prediction and prevention. The 2005 NCI budget is 4.87 billion dollars, which is a 2.8% increase over 2004. Nevertheless after cost of living and other mandated adjustments, available dollars are less than in 2004. Dr. Antman discussed various strategic priorities including: 1. Integrative Cancer Biology which addresses complexities of gene and protein networks, 2. Molecular Epidemiology including early detection, prevention and prediction. She mentioned various expected and unexpected geographic distributions for cancer incidence including lung cancer in tobacco areas, breast cancer in the Northern U.S. and prostate cancer in the northwest. 3. Cancer Interventions. The NCI is currently evaluating its clinical research programs. 4. Technology Development. Prospects for nanotechnology including nanoparticles in diagnostics and therapeutics and the potential of proteomics were discussed. 5. Clinical Trials. 6. Disparities, and 7. Molecular Imaging. The historical sequence of optical, functional and now molecular imaging is enhancing diagnosis and monitoring of response to therapy. NCI relationships with the FDA and CMS have resulted in collaboration in drug development and pilot programs of CMS funding for clinical research studies.

The meeting included a working lunch moderated by **Harriet Isom** with NCI program directors from Cancer Biology and Cancer Imaging discussing reorganization of the Study Sections within the Oncological Sciences Integrated Review Group, NIH Roadmap Initiatives in Cancer, and grant funding mechanisms. This session was included to provide information as well as answer questions of interest to

investigators at all stages of their careers. **Larry Clarke** and **Houston Baker** discussed the Cancer Imaging program and many available opportunities for collaboration with existing networks as well as investigator-initiated programs. **Martin Padarathsingh** and **Syed Quadri** spoke about the Cancer Biology programs and answered questions from young investigators in the audience interested in better understanding the grant submission and review process.

Outstanding poster awards were given to **Ching Ching Leow** (1st prize) for "Hath1, downregulated in colon adenocarcinomas, inhibits proliferation and tumorigenesis of colon cancer cells," **Andrei Thomas-Tikhonenko** (1st prize) for "Reactivation of p53 and deactivation of Myc in murine two-hit B-lymphomas result, respectively, in rapid tumor regression and the conversion to CD20-positive neoplasms," **Jussuf Kaifi** (2nd prize) for "Homing of metastatic esophageal cancer cells to lymph nodes and bone marrow – the role of CXCR4 expression," **Liang Xu** (2nd prize) for "Discovery and therapeutic potential of novel Bcl-2/Bcl-XL small-moleculae inhibitors in human breast and prostatic cancer," **Thomas Sayers** (2nd prize) for "The proteasome inhibitor VELCADE™ sensitizes tumor cells to TRAIL-mediated apoptosis," and **Magdalena Karbowniczek** (2nd prize) for "Rapamycin insensitive regulation of B-Raf kinase activity by Tuberin and Rheb."