PLENARY ORAL PRESENTATIONS

PO-1
Convergence of Information Technology and Cancer Surveillance in the Descriptive epidemiology of cancer cervix in India

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The Indian Council of Medical Research initiated a network of cancer registries under the National Cancer Registry Programme (NCRP) in 1981 and data collection commenced in these registries from January 1982. Since then, the registries have provided information on incidence and patterns of cancer that in terms of quality and validity meet international standards. Thus, in India, for cancer, and perhaps for only this disease, we have a systematic programme of data collation so as to have reliable incidence and mortality rates, thereby laying a foundation for scientific research - whether that research be epidemiological, basic, clinical or in cancer control. However, India being a vast country, setting up of new registries throughout the country as in some Western countries would involve enormous cost in establishing and maintaining the same. Therefore, under a project, on 'Development of an Atlas of Cancer in India' a cost-effective design and plan using advances in modern electronic information technology, was conceived, to collate and process relevant data on cancer so as to fulfill the objective of obtaining an overview of patterns of cancer in different parts of the country; and, calculating estimates of cancer incidence wherever feasible. The Results presented are based on the data from both the cancer registries of the NCRP and that from the project on cancer atlas. Cancer of the cervix has been and continues to be the most important cancer in women in India. This is despite the decline in the incidence of this cancer, and in the absence of any organised screening programme. In the past two decades since the commencement of the NCRP, the Population Based Cancer Registry (PBCR) at Chennai (Madras) has shown cancer cervix as the leading site of cancer in women and continues to do so. Among the cancer registries in India, Chennai PBCR has always recorded the highest incidence rate. (Age Adjusted Incidence Rate (AAR): 28.8/100,000). This figure is somewhat lower than the highest incidence rates in the world. The most recent data from the report of the above project on 'cancer atlas' show that at least five districts have even higher incidence rates than that recorded at Chennai. Four of these five districts are concentrated in the north eastern region of Tamil Nadu state and Pondicherry. The atlas has further revealed that this area has also some of the highest incidence rates of penile cancer. There are reports in the literature that the prevalence of Human Papilloma Virus (HPV) is not only high among cancer cervix patients, but also high among patients with penile cancer. This part of Tamil Nadu state has also a high prevalence of Human Immunodeficiency Virus. Thus, this part of India is worthy of undertaking several research studies and control measures in cancer cervix.

PO-2
Targeting Transcription Factors for Prevention and Therapy of Cancer

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NF-kB, a transcription factor, is present normally in the cytoplasm as an inactive heterotrimer consisting of p50, p65 and IκBα subunits. When activated, NF-kB translocates to the as a p50-p65 heterodimer. This
factor regulates the expression of various genes that control apoptosis, viral replication, tumorigenesis, various autoimmune diseases, and inflammation. NF-kB has been linked to the development of carcinogenesis for several reasons. First, various carcinogens and tumor promoters have been shown to activate NF-kB. Second, activation of NF-kB has been shown to block apoptosis and promote proliferation. Third, the tumor microenvironment can induce NF-kB activation. Fourth, constitutive expression of NF-kB is frequently found in tumor cells. Fifth, NF-kB activation induces resistance to chemotherapeutic agents. Sixth, various genes involved in tumor initiation, promotion, and metastasis are regulated by NF-kB. All these observations suggest that NF-kB could mediate tumor progression and thus can be used as a target for chemoprevention and for the treatment of cancer. Besides NF-kB, we have also targeted AP-1 and STAT3, other transcription factors that mediate tumorigenesis. We will present the data which shows that phytochemicals are important inhibitors of NF-kB, AP-1 and STAT3 activation, and can suppress the expression of genes involved in carcinogenesis and tumorigenesis in vivo.

**PO-3**

**Understanding the De-Regulation of Cell Cycle in Tobacco Chewing Mediated Oral Cancer**

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The present abstract summarizes the salient observations made during our studies to comprehend the key molecular events leading towards cell cycle deregulation during the process of oral carcinogenesis due to tobacco chewing habit. The phase I of the study involved investigating the expression levels of different cell cycle regulatory proteins in oral squamous cell carcinoma (OSCC) as compared to premalignant lesions and normal oral mucosa. Cyclins A, B1, B2, D1, D2, D3 and E were screened at both RNA and protein levels (IHC as well as western blot analysis). Besides the screening of cell cycle regulators, the important cell death regulators like p53 and Bcl2 were also analyzed. It was observed that among cyclins, Cyclin A and D1 showed the over expression with cancer progression. Among the cyclin dependent kinases (Cdks) CDK4 (catalytic part of Cyclin D1) showed significant overexpression in oral tumors. Since CDK4 is inhibited by the INK4 family members like p14(ARF), p15(INK4B) and p16(INK4A), hence there expression levels was also evaluated. The expression levels of all three CDKI's showed the gradual decrease with progression from normal epithelium to premalignant lesions to OSCC. A high p53 immunoreactivity was observed. Surprisingly there was absence of mutation in p53 gene whereas frequent genomic rearrangement was seen in coding region of p53 as well as its promoter. Bcl-2 protein expression was observed more with higher-grade samples and oral cancer progression. In phase II, detailed promoter analysis of Cyclin D1 and CDK4 showed binding of STAT5 to CyclinD1 and binding of a novel transcription factor (named CDK4 Regulating Factor) to CDK4 promoter sequence. The present study shows that upregulation of CyclinD1 and CDK4 as well as down regulation of p16 contributes in a significant way towards the tobacco chewing mediated oral carcinogenesis.

Thus our studies led us to several first hand findings having implications in early and sensitive diagnosis, effective treatment planning and in predicting prognosis.

**PO-4**

**Involvement of Bax in the Regulation of Curcumin-induced Apoptosis**

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Deregulated expression of pro and antiapoptotic proteins and their regulators often has an important role in chemoresistance. Curcumin, a dietary compound from turmeric, is known to induce apoptosis in a variety of cancer cells. To understand the role of Bax, a proapoptotic protein, in curcumin-induced apoptosis we used HCT116 human colon cancer cells with one allele of Bax gene (Bax+/−) and Bax knockout HCT116 (Bax−/−) cells in which Bax gene is inactivated by homologous recombination. Cell viability decreased in a concentration-dependent manner in Bax+/− cells treated with curcumin (0-50 μM) whereas only minimal changes in viability were observed in Bax−/− cells upon curcumin treatment. In Bax−/− cells curcumin induced activation of caspases 9 and 3 was blocked and that of caspase 8 remained unaltered. Curcumin-induced release of cytochrome c, Smac and AIF was also blocked in Bax−/− cells and reintroduction of Bax, downregulation of the antiapoptotic protein Bcl-XL by anti sense DNA as well as overexpression of Smac highly sensitized the Bax−/− cells towards curcumin-induced apoptosis. There was no considerable difference in the percentage of apoptotic cells in Bak RNAi transfected Bax+/− or Bax−/− cells treated with curcumin when compared with their corresponding vector transfected cells treated with curcumin. The present study demonstrates the role of Bax but not Bak as a critical regulator of curcumin-induced apoptosis and implies the potential of targeting antiapoptotic proteins like Bcl-XL or over expression of proapoptotic proteins like Smac as interventional approaches to deal with Bax-deficient chemoresistant cancers for curcumin-based therapy.
Dlg translocates to the cell nucleus and that it is this fraction that is preferentially targeted by the E6 protein. Studies on the E6-dependent and independent regulation of Dlg stability demonstrate a complex pattern of phosphorylation that is induced by a series of converging kinase signalling pathways.

**PO-6**

**Recent Developments in HPV Prophylactic Vaccines**

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**PO-7**

**Modulation of Mitogenic Signaling Cascades in Mouse Liver by the Hepatitis B Virus X Protein**

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Transcriptional activation of diverse cellular genes by the X protein (HBx) of hepatitis B virus (HBV) has been suggested as one of the mechanisms for HBV-associated hepatocellular carcinoma (HCC). However, such functions of HBx have been studied using transformed cells in culture and have not been examined in the normal adult hepatocytes, a natural host of HBV. Using an efficient hepatocyte-specific viral-based gene delivery system developed in our laboratory earlier, we studied the mechanisms of HBx action in vivo. We show that HBx induces a significant increase in the activity of extracellular signal-regulated kinases (ERKs) in the liver of experimental mice. Inhibition of HBx-induced ERK activation following intravenous administration of PD98059, a MEK inhibitor, confirmed the requirement of MEK in the activation of ERKs by HBx. HBx induction of ERK activity was sustained up to 30 days. Interestingly, sustained activation of c-Jun N-terminal kinases (JNKs) up to 30 days was also noted. Such constitutive ERK and JNK activation by HBx also led to sustained stimulation of further downstream events such as increased levels of c-Jun and c-Fos proteins along with the persistent induction of AP-1 binding activity. The minimum domain of HBx responsible for such activation has been identified. Taken together, our data suggest a critical role of these molecules in HBx-mediated cell transformation.

**PO-8**

**Presence of Papillomavirus Sequences in Condylomatous Lesions of the Mamillae and in Invasive Carcinoma of the Breast**

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**Background:** Viruses including Epstein-Barr virus (EBV), a human equivalent of murine mammary tumour virus (MMTV) and human papillomavirus (HPV) have been implicated in the aetiology of human breast cancer. We report the presence of HPV DNA sequences in areolar tissue and tumour tissue samples from female patients with breast carcinoma. The presence of virus in the areolar-nipple complex suggests to us a potential pathogenic mechanism.

**Methods:** Polymerase chain reaction (PCR) was undertaken to amplify HPV types in areolar and tumour tissue from breast cancer cases. *In situ* hybridisation supported the PCR findings and localised the virus in nipple, areolar and tumour tissue.

**Results:** Papillomavirus DNA was present in 25 of 29 samples of breast carcinoma and in 20 of 29 samples from the corresponding mamilla. The most prevalent type in both carcinomas and nipples was HPV 11, followed by HPV 6. Other types detected were HPV 16, 23, 27 and 57 (nipples and carcinomas), HPV 20, 21, 32, 37, 38, 66 and GA3-1 (nipples only) and HPV 3, 15, 24, 87 and DL473 (carcinomas only). Multiple types were demonstrated in seven carcinomas and ten nipple samples.

**Conclusions:** The data demonstrate the occurrence of HPV in nipple and areolar tissues in patients with breast carcinoma. The authors postulate a retrograde ductular pattern of viral spread that may have pathogenic significance.

**PO-9**

Chromatin Meets Cancer - Histone Deacetylases as Targets in Cervical Cancer Therapy

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To elucidate the molecular effects of histone deacetylase (HDAC) inhibition in the context of HPV 16/18-induced carcinogenesis, we used the HPV 18-positive cervical carcinoma cells as well as primary human foreskin keratinocytes, which were separately immortalized with amphotropic retroviruses carrying the open reading frames of HPV 16 E6, E7 or E6/E7. Here we show that HDAC inhibition strongly inhibit G1 to S transition in HPV-positive cells, which was paralleled by an up-regulation of the cyclin-dependent kinase inhibitors (CKIs) p21cip1 and p27kip1 as well as the complete loss of cdk2 activity. Although HPV expression was hitherto thought to be required to maintain a proliferative phenotype, cdk2 suppression was achieved even in the presence of ongoing viral gene expression. HDAC inhibition also triggered an E7-dependent degradation of pRb and other pocket proteins, while the levels of E2F remained unaffected. The presence of free intracellular E2F and the concomitant up-regulation of CKIs during G1 arrest Results in a classical "conflicting growth situation", which finally renders the cells to undergo type II apoptosis through the mitochondrial pathway. Programmed cell death is mediated both by suppression of NF-kB and a strong activation of the E2F target gene p73. These data provide novel molecular insights into how the transforming potential of HPV can be circumvented. Furthermore, pretreatment with HDAC inhibitors highly sensitize formerly resistant HPV-positive cells to undergo TNF-α and TRAIL-mediated apoptosis. This may determine future therapeutic strategies in which HDAC inhibitors can effectively eliminate HPV-positive cells by an apoptotic route that does not rely on the reactivation of the "classical" p53 pathway through a preceding shut-off of viral gene expression.

**PO-10**

Osteopontin, a Chemokine like Protein Regulates Tumor Growth and uPA-dependent MMP-9 Activation through NIK/PI 3-kinase/MAPK Signaling Pathways

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Cancer progression depends on an accumulation of metastasis supporting cell signaling molecules that target signal transduction pathways and ultimately gene expression. Osteopontin (OPN) is one such chemokine like metastasis gene which plays key role in regulating the oncogenic potential of various cancers by controlling cell motility, invasiveness and tumor growth. We have recently reported that OPN stimulates NFkB-mediated pro-MMP-2 activation through IkBα/IKK signaling pathways. The molecular mechanism by which various upstream kinases regulate OPN-induced NF-kB/AP-1 activation and uPA secretion in human breast cancer cells is not well defined. We have shown that OPN induces \( \alpha_\beta_3 \) integrin-mediated PI 3'-kinase activity and Akt phosphorylation in both low and highly invasive breast cancer cells. OPN enhances NF B activation through phosphorylation and degradation of I\( \beta \) by inducing the IKK activity. OPN also enhances uPA secretion, cell motility and ECM-invasion. Moreover, the data also revealed that OPN stimulates \( \gamma_3 \) integrin-mediated c-Src kinase activity and c-Src-dependent EGF receptor transactivation in these cells. OPN also induces c-Src and EGF receptor-dependent ERK phosphorylation and AP-1 activation. Interestingly, dn c-Src also suppressed OPN-induced PI 3'-kinase activity in these cells indicating that c-Src acts as master switch in regulating OPN-induced MAPK and PI 3'-kinase signaling pathways. Furthermore, OPN induces NIK activation and NIK-dependent MAPK/I\( \gamma \) -mediated NF-kB activation in melanoma cells. OPN also enhances uPA secretion and uPA-mediated pro-MMP-9 activation in these cells. Taken together, these data demonstrated that OPN regulates NF-kB/AP-1-mediated uPA secretion and uPA dependent pro-MMP-9 activation through NIK/PI 3'-kinase/MAPK signaling pathways and all of these ultimately control the breast and melanoma cell motility, invasiveness and tumor growth.

**PO-11**

**Cancer Causation by Viruses**

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Several different virus families contain members involved in human carcinogenesis. Epstein-Barr virus (EBV) and human herpes virus type 8 (HHV-8), various types of papillomaviruses, hepatitis B virus (HBV), hepatitis C virus and human T-lymphotropic retrovirus type 1 (HTLV-1), all belong into different virus families. Whereas EBV, HHV-8, high risk human papillomaviruses (HPV), and HTLV-1 are considered as direct carcinogens, where the malignant phenotype of virus-positive tumors depends on persistence of viral genomes within the tumor cells, other infections contribute indirectly to human cancer development. Human immunodeficiency virus (HIV) infections frequently result in B cell lymphomas and Kaposi sarcomas as a consequence of prolonged immunosuppression. Prevention of apoptosis in skin exposed to intensive solar exposure emerges as a possible mechanism by which several cutaneous papillomavirus types seem to contribute indirectly to the development of squamous cell carcinomas of the skin. Amplification of persisting papillomavirus or polyomavirus DNA sequences by concurrent infections with herpes simplex or cytomegalovirus may also contribute to the emergence of malignant tumors. Several virus infections cause specific and/or random chromosomal aberrations within infected host cells. Specific changes have been reported for adenovirus type 12, herpes simplex and human cytomegalovirus infections. In view of multiple steps required for cancer development, it is difficult to assess the role of these modifications for subsequent proliferative events. A recently newly discovered virus family, *Anelloviridae*, contains a large number of different genotypes of TT viruses. These viruses are ubiquitous, present in the majority of human adults and persist in the human host probably for life time after primary infection. Although these viruses have yet been established as human pathogens, their remarkable variability, resulting to the isolation of up to 24 different genotypes from one single
tumor biopsy, should encourage the search for potential "high risk" types. The identification of viruses as causative agents for specific human cancers permits novel approaches for the prevention, diagnosis and therapy of virus-linked cancers. Clinical trials to prevent hepatitis B-linked hepatocellular carcinomas by hepatitis B vaccines, but also by preventing precursor lesions of cervical cancer by HPV vaccination provided evidence for the cancer-preventive potential of this approach. The global application of HBV and the respective HPV vaccines could significantly reduce the world-wide cancer burden. Attempts to develop therapeutic vaccines against virus-linked cancers or their precursor lesions, in contrast, thus far yielded mainly disappointing results.

PO-12
Immunotherapy for Cancer and Precancer Lesions from Cervical Cancer Trials in the Lab and the Clinic

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Vaccines to prevent human papillomavirus (HPV) infection, based on HPV virus like particles (VLPs), are in late stage clinical trials, having been shown "100% effective" at preventing persisting infection with HPV16 in at least two recent major phase II clinical trials. These HPV VLP vaccines are conventional vaccines, which work by inducing neutralizing antibody to the virus. They will therefore have to be given before infection with HPV has occurred. The critical question for such vaccines is to work out how long protection against HPV infection might last following immunization, and hence to work out the optimal target group for vaccination, and vaccine delivery strategy for preventing HPV associated disease including cervical cancer. Vaccines currently available include only two oncogenic HPV types (HPV16 and HPV18) and will therefore likely prevent about 70% of the HPV infections associated with cancer. They will therefore NOT replace conventional screening programs for HPV. Vaccines to treat existing HPV infection including HPV associated cancer are at a much earlier stage of development. Several are in trial but none have yet proven effective at eradicating HPV infection any more rapidly than would occur through natural processes. The reasons for this are many: optimal vaccine material and adjuvants are unknown, and there are no surrogate markers of efficacy to speed up clinical trials. There are underlying problems with poor presentation of HPV antigens by skin cells, such that even if good therapeutic vaccines are developed, the induced effector cells may not be able to find their target cells. More basic research will be required to sort out the key elements of an effective therapeutic vaccine, while ongoing early phase clinical trials will test possible candidate vaccines for efficacy and for induction of cytotoxic T cells with the likely characteristics for success.

PO-13
Chronic Inflammation, Stress Response Enzymes and DNA Damage

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Common pathways of chronic degenerative diseases involve biologically relevant reactive oxygen species (ROS) and reactive nitrogen species (RNS). These can be generated by biochemical redox reactions, phagocytes and up-regulation of response enzymes like cyclooxygenase-2 (COX-2), lipoxygenases (LOX) and inducible nitric oxide synthase (iNOS). The resulting oxidative stress is implicated in several human cancers where chronic inflammation and persistent infections are involved. We have developed ultrasensitive methods for measuring DNA damage induced by 4-hydroxynonenal (HNE) and malondialdehyde (MDA) primarily formed by lipid peroxidation of N-6 polyunsaturated fatty acids (N-6 PUFAs) such as arachidonic acid and linoleic acid. These are also metabolized by enzymes overexpressed during chronic inflammation such as COX-2, LOX and iNOS to form the above reactive aldehydes. We have reported an increased DNA damage caused by reactive aldehydes to occur in colonic polyps of familial adenomatous polyposis patients where COX-2 is overexpressed. In the DMBA-TPA multistage mouse skin carcinogenesis model, a strong positive correlation was observed between the formation of HNE-derived DNA adducts and the LOX-catalysed arachidonic acid metabolites, hydroxyeicosatetraenoic acids (HETE). In the SJL mouse model and in p53 knock-out mice, HNE-derived DNA adducts in affected tissues were elevated due to an increased iNOS and nitric oxide overproduction. Taken together our results clearly demonstrate an increased DNA damage to occur during chronic inflammation as a result of overexpressed stress response enzymes; the ensuing mutations and genetic instability may drive cells towards malignancy.

PO-14
Clinical Applications of HPV-DNA Testing in Cervical Cancer Control

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Human papillomavirus (HPV) DNA testing are increasingly being reported in clinical applications for the following conditions: (a) Triage of women with cytological determinations of atypical squamous cells of undetermined significance (ASC-US), (b) As a marker for test of cure post-treatment and, (c) Adjunct to cytology in routine cervical cancer screening programs. HPV-Testing methods were either the Hybrid Capture 2 (HC2) test or the polymerase chain reaction (PCR) test. a) Using The Bethesda System (TBS) 1991, HPV-DNA detection rate was reported in Negative 32%, ASCUS 49%, LSIL/HSIL 93%. On review using TBS 2001, HPV-DNA was detected in ASC-US 56% and ASC-H 71%. The major findings of the ASCUS-LSIL Triage Study (ALTS) was summarised by Schiffman et al. The prevalence of oncogenic HPV was too high to permit effective triage of LSIL using HPV DNA testing by HPV HC2. HPV triage is at least as sensitive as immediate colposcopy for detecting CIN 3 among women with ASCUS. A program of repeat cytology is also sensitive if an ASCUS threshold is maintained and loss to follow-up is minimal. The immediate colposcopy strategy is certainly the least specific, referring 100% of women to colposcopy. b) Systematic review of studies (1985 to March 2002) by Paraskevaidis et al indicates the sensitivity of HPV DNA testing in detecting treatment failures was quite good in most studies, reaching 100% in four of them whereas the specificity of the test differed across the studies, ranging from 44% to 95%. Among women in whom the treatment was considered to be successful, 84.2% had a negative postoperative HPV DNA test and 15.8% a positive one. The corresponding rates for cases with treatment failures were 17.2% and 82.8% respectively. In meta-analysis of 11 studies (published between 1996 and 2003) by Zielinski et al, the negative predictive value (NPV) for recurrent/residual disease of HPV-DNA testing was 98% and that of cervical cytology 93%. When HPV-DNA testing was performed in conjunction with cytology, the sensitivity was 96%, specificity 81%, the associated positive predictive value (PPV) 46% and NPV 99%. c) HPV-DNA testing is reported as more sensitive than
cytology in predicting high grade abnormality of cervix (HSIL). A combination of HPV-DNA and Papanicolaou testing had almost 100% sensitivity and negative predictive value. Women with persistent positive HPV-DNA tests and normal cytology are at risk of developing HSIL. There is suggestion that HPV-DNA test could be used for primary screening in women older than 30 years, with cytology used to triage HPV-DNA positive women. Research continues into approaches for improving the performance and cost-effectiveness of HPV detection methods via improved HPV typing capabilities and Rapid Capture machine allowing increased throughput. Combining this test with expression levels of other markers such as proliferative and cell cycle regulatory proteins will allow subdivision of HPV-DNA positive women into those who are at greater risk of cancer and those who can be safely followed by screening at longer intervals.

PO-15
Prophylactic HPV Vaccines to Prevent Cervical Cancer

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The establishment of sexually transmitted HPV infections as the central cause of cervical cancer provides an exceptional opportunity for cervical cancer prevention through vaccination. Prophylactic vaccines are based primarily on induction of virion neutralizing antibodies by non-infectious virus-like particles (VLPs) composed of assemblages of the L1 major capsid protein. VLP vaccines for the major oncogenic type HPV16 have consistently induced high titer of neutralizing antibodies with minimal side effects and induced 100% protection from persistent HPV16 infection in proof of concept efficacy trials. The ability of the VLPs to avidly bind and induce a variety of innate immune responses in systemic antigen presenting cells likely contributes to their induction of potent B and T cell responses after injection, even in the absence of adjuvant. Three large phase III prophylactic HPV VLP trials are now in progress and there is widespread optimism for the prospects of regulatory approval for general marketing of a VLP vaccine in 2-4 years. A licensed prophylactic HPV vaccine would raise a number of implementation issues. These include the general acceptance of a vaccine targeting a sexually transmitted infection, the logistics of administering a series of three injections to adolescents or preadolescent girls, and the relative benefits of also vaccinating males. The relatively high cost of VLP vaccine production and distribution, the expected type-specificity of their protection, and the unlikely prospects for therapeutic efficacy will be impediments of particular concern for vaccine implementation in developing countries, where 80% of cervical cancer occurs. Second generation vaccines that address the limitations of the current VLP vaccines are under development. Examples include mucosal deliver of VLPs, chimeric VLPs containing E7 polypeptides to function as combined prophylactic/therapeutic vaccines, and L2 minor capsid protein-based vaccines to induce protection against more HPV types. However, none of these strategies is sufficiently advanced to warrant large-scale efficacy trials at the present time.

PO-16
Cervical Cancer Prevention-Emerging Options for Developing Countries

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Introduction: Of the 490,000 cervical cancer cases and 270,000 deaths in women worldwide each year, over 85% are in the developing world. The majority of these cancers would be prevented in developing countries by once or twice in a lifetime screening of women between 30 to 50 years of age, followed by treatment of precancerous lesions, if the testing and treatment were effective. Objectives: To assess alternative screening methods to cervical cytology: visual methods and rapid tests for HPV and outpatient treatment with cryotherapy. Methods: Data were compared from methodologically rigorous studies in low-resource settings on the performance of visual inspection with the naked eye after washing the cervix with dilute acetic acid (VIA) or Lugol's iodine (VILI) with cytology; and the effectiveness of cryotherapy. Progress on the development of two rapid, accurate, simple, and affordable HPV screening tests was also assessed. Results: Studies from India, China, and African countries have shown that the sensitivity of VIA and VILI exceed that of cytology, the specificity of cytology is better, and the cost-effectiveness of VIA and VILI dominate cytology, especially when cryotherapy is used without prior confirmation by colposcopy and biopsy. Development is progressing on two HPV tests for low-resource settings: a batch-based, DNA detection assay based on hybrid capture 2 (Digene Corporation, Gaithersburg, MD) and a viral protein activity marker lateral flow strip assay (Arbor Vita Corporation, Sunnyvale, CA). Conclusion: VIA and VILI are promising alternatives to cytology, cryotherapy is effective, and progress is being made on development of HPV tests for screening in low-resource settings.

PO-17
Activator Protein 2 Alpha (AP-2) Status Determines the Chemosensitivity: Implications in Cancer Chemotherapy

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Inactivation of proapoptotic genes or activation of survival signaling leads to chemoresistance. Activator protein 2, a developmentally regulated sequence-specific DNA-binding transcription factor, has been shown to function like a tumor suppressor. While genetic alterations in AP-2α gene in cancer cells have not been reported, progressive loss of AP-2α expression with tumor progression has been reported in breast, colon, prostate cancer and melanoma. The loss of AP-2α expression in invasive breast cancer has been correlated with hypermethylation (of CpG island in the promoter of AP-2α gene) mediated silencing of AP-2α gene. We have shown previously that overexpression of AP-2 inhibits the growth of cancer cells by inducing cell cycle arrest and apoptosis. Here we show that controlled expression of AP-2α, using tetracycline inducible system, increased the chemosensitivity of cancer cells by several fold. Under these conditions, neither AP-2α expression nor drug treatment resulted in apoptosis induction while in combination, the cancer cells underwent massive apoptosis. We found endogenous AP-2α is induced by a variety of chemotherapeutic drugs. Blocking endogenous AP-2α by siRNA lead to chemoresistance of human cancer cells irrespective of their p53 status. This suggests that AP-2α induction by chemotherapeutic drugs plays a major role in determining the chemosensitivity. We further show that 5-aza-2′deoxycytidine (5aza2dC) induced re-expression of AP-2α in MDA-MB-231 breast cancer cells, wherein AP-2α expression is silenced by hypermethylation, resulted in
massive apoptosis induction, increased chemosensitivity and loss of tumorigenesis upon chemotherapy. However, in MDA-MB-231 cells transfected with AP-2a siRNA, 5aza2dC treatment failed to increase apoptosis and chemosensitivity upon chemotherapy. Considering the fact that 75% of invasive breast cancers have epigenetically silenced AP-2a, our approach of combined treatment of breast cancer with 5-aza-2’deoxycytidine and chemotherapy provides a novel way of modifying the chemosensitivity of breast cancer. These results establish an important role for AP-2a in cancer cell chemosensitivity and provide new insights for modifying the chemosensitivity of cancer cells by activating apoptotic pathways. Overall, our data provides both in vitro and in vivo validation for a strategy to reverse chemoresistance in human cancers, in particular breast cancer and underscores the value of tailoring cancer therapy on the basis of tumor genotype.

PO-18
HPV and Cancers at Non-Genital Sites

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The etiologic role of HPV infections in cancers of the lower genital tract is well established. The virus is responsible for almost all cases of squamous cell carcinoma and adenocarcinoma of the cervix and for significant fractions of vulvar, vaginal, perineal and penile cancers. While HPV sequences have been reported to be present in cancers at many sites other than the lower genital tract (e.g., body of uterus, ovary, esophagus, oral cavity, colon, lung), the evidence for an etiologic role of the virus is most compelling for some cancers of the oropharynx. The HPV-associated oropharyngeal cancers are differentiated from oropharyngeal cancers not associated with HPVs by virological, molecular and clinical criteria as follows. The HPV-associated cancers have the viral genome localized to the tumor cells, a lesser frequency of p53 mutations, more frequent basaloid pathology, and a better prognosis. The presence of HPV sequences (derived from the lysis of tumor cells) in the plasma of some of these patients may be indicative of more extensive disease and of the risk of recurrence. HPV type 16 accounts for an overwhelming majority of the HPV-associated cancers. The precursor lesion of these cancers has not yet been identified. Prophylactic HPV-based immunization against cervical cancer may be expected to also prevent HPV-associated oropharyngeal cancers.

PO-19
Recent Advances in Diagnosis and Management of Cancers - a Genetic Approach

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Introduction: The diagnosis, assessment of prognosis and minimal residual disease (MRD) of cancers depends on understanding of chromosomal and gene alterations using highly sensitive molecular techniques. Objective: The aim of the study was to evaluate cytogenetic and molecular anomalies at diagnosis and sequential follow-ups in leukemias and cancers. Methods: Conventional cytogenetic and Fluorescence In Situ Hybridization (FISH) analysis were done in 420 cases of leukemias of which 195 cases were follow-ups. Ten samples of urinary bladder cancer and 4 of breast cancer were analyzed using FISH to assess aneusomies and HER-2/neu
amplification status respectively. Status of other genes like p53 and N-myc were also evaluated in various cancers. Seventy cases of Retinoblastoma were analyzed using conventional cytogenetics and mutational analysis was conducted in 14 cases using denaturation high-performance liquid chromatography (DHPLC). Results: Sequential cytogenetic and FISH analysis in leukemias revealed appearance of additional anomalies that were later correlated with hematological and clinical findings. Most of these cases revealed disease progression and relapse in subsequent follow-ups that was preceded by cytogenetic/molecular relapse. In some cases of leukemias and other cancers, FISH analysis revealed molecular changes and MRD that were not evident using conventional cytogenetics. FISH analysis in urinary bladder and breast cancers reveals prognostic significance of gene alterations and recurrence up to 6 months sooner than cytoscopy and cytology. Novel mutations were identified in some Retinoblastoma cases using DHPLC. Conclusion: Importance of analysis of chromosomal and gene alterations using highly sensitive molecular techniques in management of cancers is highlighted.

PO-20
Haemopoietic Stem Cell Transplantation (HSCT): Newer Advances

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High dose chemotherapy (HDCT) followed by haemopoietic stem cell transplantation (HSCT) is now an established therapy for treatment of a no of non malignant and malignant conditions. Severe aplastic anemia, haemoglobinopathies (beta-thalassemia, sickle cell anemia), immunodeficiency diseases (SCID, WAS etc), genetic metabolic disorders (mucopolysaccharidosis) are common non- malignant conditions. Among malignant conditions, acute leukemia, chronic myeloid leukemia, myelodysplastic syndrome, multiple myeloma, Hodgkins and non Hodgkin's lymphoma and high risk neuroblastoma are important indications for HSCT. HDCT with HSCT is also being considered in the treatment of poor risk germ cell tumors, childhood tumors and auto-immune disease (rheumatoid arthritis, systemic lupus erythematosus, scleroderma) especially for the patients who have failed after standard therapy. Haemopoietic stem cells can be obtained either from a genetically identical twin (syngeneic) or from an HLA-identical matched sibling or unrelated donor (allogeneic) or from patient's own (autologous) BM or peripheral blood (PB). Accurate HLA typing is essential for patients receiving allogeneic transplants. In addition to standard serology currently, DNA based techniques such as as PCR- with sequence specific oligonucleotide probes are used (for class II regions) for HLA typing. The probability of finding a HLA match in the family is about 25-35%. For the remaining, either family members other than HLA-identical siblings or matched voluntary unrelated donors (MUD) could be alternative donors. The later can be identified through the help of bone marrow donor registry programme which are already in place in the developed countries. In India, a beginning has been made but the progress is very slow. Traditionally, BM has been used as a source of stem cells for the purpose of transplantation but during the past 15 years, peripheral blood (PB) & umbilical cord has become an important source. PB stem cells are mobilized using inj G-CSF with or without chemotherapy with the help of apheresis machine. Practically today, all the autologous stem cell transplantations are being done using PB stem cells. Even for the allogeneic HSCT, use of PB stem cells have increased over the past decade. PB stem cells have an advantage of early recovery (engraftment), with no increased risk of acute GVHD. However, there is slight higher risk of chronic GVHD. Umbilical cord (UC) blood is a rich source of most primitive (stem) cells that are able to produce 'in vivo' long term repopulating haemopoietic stem cells compared to adult stem cells. Since, the total yield of stem cells from a single cord blood is limited, presently, UC blood is being used for children weighing up to 25 Kg. More than 6000 transplants have been done worldwide using UC blood. With improved supportive care and experience, more and more
allogeneic HSCT are being done now for patients of higher age (>45 years) which was earlier limited to younger patients (<40 to 45 years). Another development in the past few years is the use of more immunosuppressive regimens (called non-myeloablative or less intensive) for conditioning rather than myeloablative chemotherapy. Drugs such as Fludarabine, antithymocyte globulin (ATG) or 2 CDA are commonly used for this purpose. Use of non-myeloablative regimens is associated with decreased risk of infectious complications and reduced frequency & intensity of acute GVHD. Currently such transplants are being done for patients with low grade lymphomas, myelodysplastic syndrome, chronic lymphocytic leukemia etc. Recent understanding of the phenomenon of 'stem cell plasticity' has led to exploration of use of embryonic stem cells / BM stem cells in the repair of myocardium post myocardial infarction, treatment of neurological disorders such as stroke, Parkinson's disease, spinal cord injury, duchene muscular dystrophy etc. Most of this work is in experimental stage at present but it appears that during coming years, stem cell therapy will be used for the treatment of many of these diseases.

PO-21

The Nutrigenomics of Cervical Cancer

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India has one third of the world's cervical cancer burden. This, together with continuing unacceptably low treatment response rates begs for novel and innovative forms of diagnosis and therapy for both prevention and treatment of locally advanced and metastatic disease. Human papillomavirus (HPV) has been shown to be the principle etiological factor in the pathogenesis of this cancer although studies by others and us demonstrate the requirement for additional co-factors. Nutrigenomics encompasses the fields of biotechnology, genomics, molecular medicine and human nutrition, enabling examination of diet and nutrition in a whole new light. The aim of nutrigenomics is to scrutinize and comprehend an individual's response to micro and macronutrients through the analysis of their unique genomic make up, which can consequently lay the foundation for the cultivation of safe and effective dietary treatments for the individual. Recent data for Indian women have shown a high incidence of folate deficiency. Our preliminary epidemiological studies have shown an association between HPV infection and low folate levels. It was observed that folate deficiency co-existing with HPV, increased the risk of developing Cervical Intraepithelial Neoplasia (CIN) by seven times. Hence folic acid deficiency may be a precursor or transmission stage of HPV in cervical cells and enhance its progression. Folate metabolism is also influenced by Single Nucleotide Polymorphisms (SNPs) of methylene tetrahydrofolate reductase (MTHFR) gene, observed at 677 (A-C) and 1298 (C-T) nucleotides in the gene sequence. Both these polymorphisms greatly impair folate metabolism. Folate deficiency either due to a genetic reason or dietary deficiency will result in accumulation of homocysteine. This accumulation will be compounded when there is deficiency of Vitamin B12 and B6. We will present data and a working hypothesis to explain how accumulation of homocysteine can influence tumor progression in HPV initiated cells. This involves the modulation of the NF kappa B inhibitor I kappa B by homocysteine and consequent activation of NF kappa B system. A second important consequence of folate and homocysteine involves the up-regulation of folate receptors (FR). Work on the regulation of FR in cervical cancer cells has led to an intimate study of hnRNP-E1, which can specifically bind to the sense strand of the HPV-16 L2 viral capsid protein mRNA to inhibit its synthesis in vitro. There is substantial experimental evidence to support a model for translational up-regulation of the FR in folate deficiency. This is based on a critical role for accumulated intracellular homocysteine which promotes the interaction of hnRNP E1 with an
18-base cis-element located in the 5' untranslated region of FR mRNA which leads to increased biosynthesis of FR at the translational level. Therefore information on hnRNP-E1 expression in the cervix is valuable in assessing its possible functional role in FR synthesis in vivo. In addition, because hnRNP-E1 expression can potentially modulate HPV viral proliferation in the cervix, knowledge of hnRNP-E1 expression patterns could also provide insights into the possible biological constraint(s) exerted by these proteins on HPV proliferation. This would be particularly informative in women with HPV-mediated transformation of cervical tissue to cancer.

PO-22
p16INK4a, A Novel Biomarker for Early Detection and Prevention of Cervical Cancer

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Extensive research over the past 20 years provided strong evidence that persistent infections with high-risk type human papillomaviruses (HR-HPVs) cause cervical cancer. However, depending on their age, more than 20% of normal women are infected with these viruses and only very few develop clinically relevant dysplastic lesions or even cancer. During an acute HPV infection, expression of viral genes, in particular the viral E6 and E7 oncogenes is restricted to differentiated epithelial cells, which lost the capability to replicate their genomes and are therefore at no further risk for acquiring functionally relevant mutations upon genotoxic damage. High grade cervical dysplasia, however, is initiated by deregulated expression of viral oncogenes in replicating basal or parabasal cells, where the E6-E7 genes submerge control of the cell cycle and mitotic spindle pole formation and induce severe chromosomal instability. Expression of HR-HPV E7 oncogene products in basal or parabasal cells uniformly results in strong over-expression of the cyclin dependent kinase inhibitor p16INK4a. This can be used to identify dysplastic cells in histological slides, cytological smears or samples taken for biochemical analyses with very high sensitivity and specificity and suggests that novel, more precise and thus less costly cervical cancer screening algorithms will be established soon. Clinical studies, that confirm this concept will be reviewed in detail at the conference. Moreover, HPV-mediated over-expression of p16INK4a also elicits an immune response against cervical cancer cells and their precursors that may play an important role in the immune surveillance of HPV-transformed cells.

PO-23
HPV Vaccines: Prospects for Eradicating Ano-genital Neoplasia

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The ability to generate human papillomavirus (HPV) virus like particles (VLPs) by the synthesis and self-assembly in vitro of the major virus capsid protein L1 has transformed our prospects for preventing cervical carcinoma in women. Immunisation with L1 VLPs provides type specific protection in all the animal infections so far tested. In Phase I trials in humans HPV L1 VLP vaccines are safe and highly immunogenic stimulating robust B and T cell responses and generating high titres of neutralising antibody. Phase II and Phase III trials are in progress with preliminary data suggesting that antibody levels persist at measurable levels for at least 30
months post vaccination. Phase II proof of principle efficacy trials are immensely encouraging with evidence that these vaccines protect against infection. However it must be recognised that VLP vaccines are type specific, likely to be expensive, require a cold chain and medical or para medical personnel for delivery. Furthermore L1 vaccines must be delivered before the sexual debut to prepubertal females (or males) and social and cultural issues may be important in determining vaccine take up. L1 based vaccines are not protective after exposure and in post exposure HPV infection, cell mediated immunity is critical. Studies in animal papillomavirus infections suggest that immunisation with specific early proteins, particularly E1 and E2, could be effective and immunotherapies for established lesions such as ano-genital warts and low-grade intra-epithelial lesions are realistic. Such vaccines are likely to be combined with immunomodulators such as cytokines in order to maximise the response. Prime/boost strategies combining DNA and/or protein and/or recombinant viruses look to have significant potential as immunotherapies for benign or low grade HPV induced disease. Immunotherapies for HPV associated high-grade pre-cancers and invasive cancers are problematic. Almost every vaccine delivery system known has been used to deliver HPV E6 and E7 oncoantigens in transplantable tumour models in rodents. HPV specific cytotoxic T cells are generated and antigen specific killing of HPV expressing tumour cells can be demonstrated. Clinical trials with any of these modalities have been minimal and to date no regression of any cervical cancer in response to the various immunotherapies has been shown but partial responses in high grade pre-cancers have been demonstrated. Tumour evasion mechanisms, such as down regulation of MHC Class I, remain a tough barrier for successful cancer immunotherapies.

PO-24
Screening with HPV-test for Cervical Cancer

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Several studies have addressed on the problem of low sensitivity of the traditional screening test for cervical cancer. The most promising new technology to improve the test is based on the etiology of cervical cancer resulting in HPV-test. Evidence, so far stems mainly from the developed countries and with a design where the same woman is subjected to the two tests compared for sensitivity. This is a valid approach if there is no overdiagnosis, i.e. if all the lesions confirmed will progress to cancer. This is not true for the preinvasive lesions detected by screening for cervical cancer. In India there are going on one of the very few randomised trials where each woman is randomly allocated for one screening test only. In Finland the same approach is applied in the routine screening that is run as a public health policy since 1960's. In Finland about 200 000 women are annually invited and 150 000 women attend the routine organised programme of screening for cervical cancer. Individual municipalities decide on joining and financing the programme but the policy is regulated by a national by-law. There is a mass screening registry within the Finnish Cancer Registry that designs, collects data and analyses the performance and outcome of the screening programme. Municipalities have their own screening laboratory or make an agreement with an external one. Within this infrastructure the HPV-test was introduced in 2003. Several municipalities served by the Cancer Society of Finland laboratory joined an individually randomised trial with one third screened with HPV-test one third screened with automation assisted test and one third with traditional papsmear. The objective is to assess the sensitivity of each test and to evaluate the effectiveness of routine screening with each test compared to the two other tests. The activity falls in the area of health services research as routine public health policy is evaluated but the design is that of an scientific experiment. In 2003 altogether 15 000 women were randomised and the target population will get increased year by year. In fact, the automation assisted screening was run since 1999 with more than 500 000 tests at present. As the screening interval is 5 years in Finland the final evaluation for the HPV-test takes place in 2008 earliest. The cost of each test is the same for the municipality and the difference to the automation assisted screening
and HPV-test screening is covered by the Cancer Society of Finland and by research grants. The cost can be directly evaluated only for the same infrastructure without allowing for variation as to ages of target population and for screening interval. Automation assisted screening seems to result in equal detection of cervical cancer and precancerous lesions as the conventional screening (Nieminen et al. Int J Cancer 2003; 103:422-426), data on interval cancer rates are not yet available. Preliminary experience on HPV-screening in a pilot study predicts HPV-test to be more sensitive by detecting severe and moderate dysplasias (Nieminen et al. BJOG 2004; in press) at higher rate than the conventional pap test.

PO-25
Molecular Epidemiology, Prevention and Therapeutic strategies in the Management of Hepatocellular Carcinoma

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Hepatocellular Carcinoma (HCC) is the most frequent primary tumor of the liver in adults. It ranks as the fifth most common cancer in the world and the third most common cause of cancer related deaths. Most of the HCC develop on underlying cirrhotic liver. The major etiologic risk factors for HCC development include HBV and HCV infection, toxins (alcohol, Aflatoxin B1) and various inherited metabolic liver diseases, such as Hemochromatosis and alpha-1-antitrypsin deficiency. The most frequently altered human genes in HCC are tumor suppressor genes such as p53, RB1, p16, and IGF2R genes in which Loss of Heterozygocity has been reported, with a frequency higher than 20%. Other genes, like GST and CYP450 (detoxifying genes) are also altered, but the frequency of individual gene mutations is low. Early detection of HCC remains the best strategy for reducing tumor related mortality. FNAC or biopsy is the gold standard for HCC diagnosis. Ultrasound and AFP assessment every six month is the recommended procedure for surveillance in patients with cirrhosis. A multidisciplinary approach comprising of interventional radiologists, medical oncologists, radiation oncologist and surgeons are required for optimal care of patients with HCC. The options includes resection of liver segment, tumor ablation by injecting with absolute alcohol or acetic acid or by using radiofrequency probe, liver transplantation, chemoembolisation, systemic chemotherapy, certain experimental drugs, hormones and cytokines. The ineffectiveness of conventional chemotherapeutic strategies in this condition has prompted studies of novel systemic strategies, including Antioestrogen therapy, Thalidomide, long acting Somatostatin, Interferon and Interleukin 2 therapy. Preventive measures should have a major impact on the incidence of HCC. Further, the prevention of a local recurrence or the development of new HCC lesions in patients after successful surgical or non-surgical HCC treatment (secondary prevention) is of paramount importance. Based on rapid scientific advances, molecular diagnosis, gene therapy and molecular prevention are becoming increasingly part of our patient management and will eventually complement and in part replace existing diagnostic, therapeutic and preventive strategies. Overall, this should result in reduction of the incidence of HCC, one of the most devastating malignancies worldwide.

PO-26
Host immune Response to Human Papilloma Virus (HPV) as Predictive Marker for Persistence or Progression of Cervical Neoplasia

The immune response to HPV and HPV-induced cervical neoplasia appears to be best understood in the context of T helper cells 1 and 2 (Th-1 and Th-2) subsets that can determine susceptibility or resistance to the disease. The Th-1 (inflammatory) response is associated with cell-mediated immunity and an IgG2 isotype antibody response. The Th-2 (anti-inflammatory) response is associated with humoral immunity and an IgG1 isotype antibody response. Deviation from a balanced Th-1:Th-2 response to a Th-2 predominant response may reflect the capacity of Th-2 cytokines to down-regulate or cross-regulate cytokines associated with Th-1. Alternatively, pre-existing Th-2 cytokines can redirect a Th-1 response to a Th-2 response by immune deviation. Most cancers appear during a Th-2 response, ineffective for viral clearance. HPV onco-proteins E6 and E7 are continually expressed in cervical cancers and high-grade intraepithelial neoplasias. Detection of IgG isotype reactivity with the E7 oncoproteins of HPV appears to reflect the effectiveness of the immune response against cervical cancers and their precursors. Till date there is no efficient marker to differentiate the women with progressive disease from the women whose CIN lesions will regress or remain stationary. From immuno-biologic point of view, women having predominant Th1 response (high serum IgG2 level) are more likely to clear the infection/lesion compared to those women having predominant Th2 response (high serum IgG1 level). Estimation of titers of IgG1, IgG2 and their relative proportion in the serum can serve as a predictive marker for progression of CIN lesions to higher grades and invasive disease. Serial estimation of IgG1 and IgG2 levels during follow up of cervical cancer patients may help to diagnose the recurrent disease earlier as a predominant humoral response is likely to be associated with reappearance of neoplasia. An ELISA test has been developed and optimized at James Graham Brown Cancer Center to estimate the serum IgG1 and IgG2 against E7 protein of HPV 16 and 18. The assay is being done in the sera collected from women having normal cervix, precancer of cervix and cancer of cervix. The updated results will be presented.

PO-27
The Role of HPV Testing in the Early Detection of Cervical Neoplasia in Low-Resource Settings

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The knowledge that cervical neoplasia are caused by persistent infection with high-risk types of human papillomaviruses (HPV) has led to the evaluation of vaccination and the detection high-risk HPV types as a cervical screening strategy. The accuracy of HPV testing in primary screening for cervical neoplasia has been evaluated in several cross-sectional studies. The second-generation Hybrid Capture II (HC II) probe B (which is a pool of full-length RNA probes for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 microtitre assay) has been widely used for HPV testing in a number of studies. Polymerase chain reaction (PCR) assays have also been evaluated. The sensitivity of HPV testing by HC II in detecting CIN 2,3 lesions and invasive cancer varied from 62-100% and the specificity varied from 41-96% in different developed and developing country settings. The sensitivity of HPV testing when specimens have been taken and/or analyzed in developing country settings has generally been lower than that where the entire specimen chain (collection/testing) was completed in a developed country. In studies from China, Mexico, South Africa and Zimbabwe, the sensitivity and specificity varied from 62 to 97% and 41 to 92%. In a pooled analysis of 4 cross-sectional studies with a common protocol in India, involving 18 085 women aged 25-65 years, the sensitivity and specificity to detect
CIN 2.3 lesions and invasive cancer were 68% (95% CI: 61-74%), and 94% (95% CI: 61-74%) respectively. The sensitivity varied from 50 to 80% in the individual studies. Results from a large randomized intervention trial Maharashtra, India, comparing HPV testing, cytology and visual screening with acetic acid (VIA), indicate that all the tests had similar detection rates of CIN 2-3 lesions. The sensitivity of HPV testing in vaginal self-sampling studies was generally lower than that of cervical direct sampling by clinicians or nurses. The lower sensitivity in self-sampling studies as compared to clinician sampled studies indicate that adequacy of specimen collection is an important determinant of the success of HPV testing. Recently an international working group of the International Agency for Research on Cancer (IARC) concluded that there is sufficient evidence that HPV testing can reduce mortality from cervical cancer. HPV testing is a promising approach with the highest reproducibility among all cervical screening tests. However, it is costlier (20-30 US$) than other screening tests and requires sophisticated laboratory infrastructure including testing equipment, trained technicians and storage facilities for samples. Future developments such as less expensive, and faster testing are essential for HPV testing to be feasible in low-resource settings.

PO-28
Genomic Gains and Losses in Diffuse Large B-Cell Lymphoma by Array Comparative Genomic Hybridization: Clinical Outcome Correlations and Target Gene Discovery

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Conventional karyotype and chromosomal comparative genomic hybridization (CGH) studies of diffuse large B-cell lymphoma (DLBCL) have revealed few chromosomal abnormalities associated with outcome. In order to evaluate the association between genomic copy number changes in DLBCL and clinical outcome at a higher resolution, we assayed a panel of 64 newly-diagnosed DLBCL specimens by array-CGH. For each specimen, the respective patient had a known response to anthracycline-based therapy, and the median follow-up was 5-years. Forward and reverse hybridizations were performed for all specimens to avoid dye-bias, using BAC/PAC arrays with 1-4 Mb resolution coverage of the genome (Spectral Genomics). After customized normalization, the circular binary segmentation (CBS) algorithm was used to identify regional copy number changes along each chromosome in each specimen. Of the 64 specimens, 60 (93.7%) displayed regional copy number changes. The frequency of gain/loss of each clone for all 64 specimens was then calculated based on the CBS results, and, additionally, based on singleton clone changes if they were outside of CBS-defined regions. Clones displaying change in 10% of specimens for at least two contiguous clones were further considered as recurrent sites for clinical correlations. 54 sites of recurrent gain and 38 sites of recurrent loss were identified. Association between loss or gain of sites and International Prognostic Index (IPI) were evaluated using Fisher's exact test, and associations between loss or gain of sites and time to treatment failure (TTF) and overall survival (OS) were tested using the log-rank test. In a univariate analysis, 16 chromosomal regions (indicated in parentheses as Mb intervals from the p telomeric end) showed significant ($p \leq 0.05$) correlation with clinical outcome. Among these, 4 predicted adverse outcome whereas 12 predicted favorable outcome. Gain of chromosome 13 (85-91.9) and loss of chromosome 16 (33.8-35.6) were associated with both adverse TTF and OS. Gain of chromosome 6 (0.1-5.9) and loss of chromosome 2 (2.4-4.1) were associated with adverse TTF and OS, respectively. Favorable TTF was associated with gain of chromosomes 3 (138.4-188.7), 9 (122.1-132.8), and 19 (43.1-63.7), and loss of chromosomes 1 (78.2-79.1), 4 (24.9-34.7), 6 (62.2-170.5), 7 (18.8-19.2), 9 (8.3-12.5), 10 (107-120), and 15 (41.2-45.5). Favorable OS was associated with gain of chromosomes 3 (0.2-204.6), 9 (122.1-132.8), 19 (43.1-63.7), and 20 (22.5-63.6), and loss of chromosomes 1 (78.2-79.1) and 4 (24.9-
Gain of chromosome 3 (138.4-188.7) was the only marker significantly associated with lower IPI. In a multivariate analysis after stratifying by IPI, using the stratified log-rank test, loss of chromosomes 7 (18.8-19.2), 1 (78.2-79.1), 2 (2.4-4.1), and 16 (33.8-35.6) and gain of chromosome 9 (122.1-132.8) provided significant extra contributions in predicting clinical outcome. Notably, 7 of the sites were £5 Mb, facilitating the identification of target genes. In summary, array-CGH has lead to the identification of gain or loss at several novel chromosomal regions with prognostic significance in DLBCL, which in some cases are of a size amenable for target gene identification.

PO-29
Molecular Stratagies for Cancer Drug Development

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PO-30
Understanding Human Gliomas by Proteomics Approach

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Gliomas are the most common primary brain tumors. We have studied protein profiles of astrocytomas - a histological sub type, by 2-DE/MS approach and examined differentially expressed proteins as useful molecular indicators to understand these tumors. We identified 72 distinct, differentially expressed proteins belonging to various functional groups, 29 of which were short listed for consistent differential expression and may have a role in their pathology. Some were found to be differentially expressed in both Gr III and IV astrocytomas, while others were associated with a particular grade. These proteins can be further explored as individual markers or as a set of markers for astrocytoma. Some notable observations were, under expression of Prohibitin - a potential tumor suppressor protein, Rho-GDP dissociation inhibitor, (Rho-GDI) - a regulator of Rho GTPases and heat shock proteins (HSPs) as well as destabilization of glial fibrillary acidic protein, GFAP - a major protein of the glial filaments, in Gr these malignant tumors. We attempt to explain glioma malignancy and progression in terms of their combined role. The molecular species pattern of GFAP can be considered as a useful indicator for differentiating the types of gliomas and grades of astrocytomas. Further, destabilization of GFAP may be associated with its phosphorylation at hitherto unknown Thr sites and we implicate the role for a specific kinase and a protease in generating GFAP molecular forms in these tumors.

PO-31
Preliminary Investigations on HPV and its Association with Esophageal Cancer

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HPV association has been documented in esophageal squamous cell carcinoma [EC] by several studies in high-risk areas for the occurrence of the said malignancy. In this study we are trying to evaluate i) incidence of high-risk oncogenic variants of HPV in EC biopsies, ii) the anti-HPV status in those patients, specifically high-risk oncogenic variant HPV-16 antibody, and correlate the serology with molecular studies to profile HPV association with EC in India. In the preliminary investigations using multiple PCR on endoscopic EC biopsies, a significant prevalence of the highly oncogenic HPV-16 was documented. To simultaneously assay the viral DNA and anti-HPV-16 antibody in a larger sample size, an in house ELISA is being developed. Towards this end, immunoreactive rHPV-16 L1 major capsid protein has been expressed in yeast, VLP made and ELISA carried out. We also expressed immunoreactive His-tagged rHPV-16 L1 protein derived capsomeres and carried out ELISA using the same. Significance of this study, on small sample number, will be discussed.

PO-32
E-cadherin/β-catenin: Their Role in Intercellular Adhesion of Metastatic Prostate Cancer Cells in Bone

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Background: The development of strategies for the treatment of patients with metastatic prostate cancer requires the understanding of mechanisms of cellular adhesion and growth of cancer cells in bone, which is the most prevalent site of prostate metastasis. In normal prostate, the cytoplasmic domain of a critical intercellular adhesion molecule, E-cadherin, is linked to the actin cytoskeleton via its interaction with α-, β-catenins and p120 for the maintenance of cellular polarity and differentiation of normal epithelial cells. In contrast, lost or reduced membranous expression of E-cad./β-cat. protein with concurrent hypermethylation of E-cad. gene has been reported to be associated with invasive prostate cancer cells, thereby allowing their detachment and migration from the primary site in prostate. However, the role of these interrelated cellular adhesion proteins in cellular adhesion of metastatic cancer cells in bone remains poorly understood. Aims and Methods: The aim of this study was to evaluate the E-cad./β-cat. protein expression by immunohistochemical (IHC) staining method and the methylation status of E-cad. gene by methylation specific-PCR in prostate cancer cells at the primary site in prostate and metastatic site in bone or lymph node. Results: In benign prostate hyperplasia (BPH), the high percent (>50%) of cells with membranous expression of E-cad. and β-cat. protein was observed in 91% and 82% of cases respectively, whereas the rest of the cases exhibited low percent (6-50%) of expression of both proteins. In contrast, the frequency of high percent of membranous expression of E-cad. and β-cat. protein reduced to 18% and 27%, respectively, while a low percent of expression was found in the remaining cases of patients with primary prostate cancer. There were statistically significant differences between BPH and primary prostate cancer in terms of the E-cad./β-cat.-positive cells (Fischer's Exact p<0.001 for E-cad. and p=0.008 for β-cat.). Moreover, a statistically significant association was observed between low membranous expression of E-cad. protein and hypermethylated E-cad. gene in the primary prostate cancer (Fischer's Exact p=0.005). Surprisingly, metastatic prostate cancer cells in bone exhibited high membranous expression of E-cad. and β-cat. protein in 88% and 82%, respectively, and low membranous expression in the remaining cases. The results showed a statistically significant difference from that of the primary site (Fischer's Exact p<0.001 for E-cad. and p=0.001 for β-cat.). Furthermore, E-cad. gene was found to be unmethylated in all of the case of metastasis, revealing its significant association with high expression of E-cad. protein (Fischer's Exact
p<0.001). **Conclusions:** The consistent membranous expression of both E-cad. and β-cat. proteins and concurrent unmethylated E-cad. gene in metastatic prostate cancer cells in bone suggest their functional role in the maintenance of cellular adhesion of cancer cells in bone. Indeed, this is a paradoxical observation that challenges the present paradigm that E-cad. protein is a metastatic suppressor gene's protein.

**PO-33**

**Screening in Breast Cancer and Cervical Cancer - Is it Cost Effective?**

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Breast cancer is the most frequently diagnosed cancer and is the second leading cause of cancer death among women in India. Mammography is perhaps the best screening tool for detecting early breast cancer. Estimates of mammography sensitivity range from 75% to 90% with specificity from 90% to 95%. The positive predictive value of mammography for breast cancer ranges from 20% in women under age 50 to 60% to 80% in women age 50-69. Randomized clinical trials (RCTs) have demonstrated a 30% reduction in breast cancer mortality in women 50-69 years who are screened annually or biennially with mammograms. The data on women under age 50 are less clear. Cost-effectiveness estimates of mammography screening vary widely. Recommendation for women age 40-49 is every 1-2 years and annually after age 50. Though majority of the patients with cervical cancer are young, 25% of the cancers occur in women over age 65. The Papanicolaou (Pap) smear is used to screen for cervical cancer. The lead time to develop invasive cancer is estimated at 8-9 years and early detection could be highly beneficial. Regular triennial screening would achieve 91%-96% of the benefit of annual screening. Screening is more cost-effective for women over age 65 with a history of inadequate screening. Efforts should be made to test women who have not undergone regular testing. No randomized controlled trials to test the effectiveness of Pap smears for prevention of cervical cancer have been conducted, however, case-control studies have clearly demonstrated that women with invasive cervical cancer were less likely to have been screened.

**PO-34**

**Innate Immune Response to Tumors: Role of γδT and NKT Cells**

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The importance of innate immune mechanisms in controlling viral infections, cancer and autoimmunity is currently an area of intense research. Recently, innate immune lymphocytes NK, natural killer T (NKT) and γδT cells have garnered much attention and their biological significance in the tumor immunity, allergic diseases and infectious diseases is being extensively exploited. These cells help participate in bridging the innate immunity with antigen specific acquired immune responses. γδT cells and NKT cells differ from conventional αβ T cells with respect to their tissue localization, TCR gene usage and antigen recognition. In addition to intact proteins and peptides, soluble nonpeptidic antigens and glycolipids are recognized by γδT cells and NKT cells, respectively. Substantial evidence suggests that γδ T cells and NKT cells represent important players in the immune system
arsenal of effector cells with potential anti tumor activity. CD1d reactive NKT cells that express the invariant Vα24-Vβ11 T cell receptor were enriched from peripheral blood of patients with cervical cancer after stimulation with PBS-14 pulsed dendritic cells. The immunomagnetically purified NKT cells showed potent tumor directed cytotoxicity. Similarly we investigated how Vγ9/Vδ2 T cells enriched from peripheral blood of patients with oral squamous cell carcinoma and stimulated with phosphoantigens can effectively lyse tumor cells. The presentation would focus on dissecting the events leading to activation, regulation, migration and death of these effector cells and their significance in tumor immunity. Potential strategies for immunotherapy of tumors, based on the activation of these effector cells will be discussed.

PO-35
Microarray Analysis of Gene Expression Profiles in Human Papillomavirus-Associated Cervical and Oral Cancers

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A causative role of HPVs in cervical intraepithelial lesions, including cervical carcinoma has been firmly established. DNA of high-risk HPVs such as types 16 and 18 is usually present in an episomal state in benign and premalignant lesions, but is frequently integrated into the genome in cervical carcinomas and in cell lines derived from such lesions. HPVs also appear to be one of the factors that contribute to the development of squamous cell carcinoma of the head and neck (SCCHN) in approximately 25% of the cases, particularly in the oropharynx. The E6 and E7 oncoproteins of high-risk HPVs are involved in cellular transformation. The E6 protein promotes polyubiquitination and proteasomal degradation of the cellular tumor suppressor proteins p53 and DLG. E6 is also known to interact with a number of other cellular proteins and activates telomerase. The E7 protein acts in concert by binding and inactivating the function of the pRB and related p107 and p130 proteins. E7 also interacts with additional cellular proteins such as TBP, histone H1 kinase, cyclin E, etc. The E6 and E7 proteins are also known to significantly alter cellular gene expression and promote chromosomal destabilization, foreign DNA integration and other mutagenic events in the cell. These events, possibly in combination with other cofactors or co-carcinogens, lead to the development of HPV-associated cancers. In order to better understand the early steps in HPV-associated carcinogenesis, we have studied global changes in cellular gene expression profiles in different HPV 16 and 18 positive cell lines (harboring extrachromosomal or integrated viral genomes) using the high density oligonucleotide U133A GeneChip® (Affymetrix) that contains approximately 22,000 human genes. Data analysis using various statistical tests showed that approximately 1600 genes were differentially expressed in all HPV-positive cell lines as compared to the normal cervix, and approximately 1200 genes were differentially expressed in all the HPV-positive cell lines as compared to the HPV-negative cell line C-33A. The above analysis also identified 140 cellular genes whose expression was specifically altered in the presence of HPVs. The results of the microarray analysis were validated by quantitative RT-PCR analysis of a representative number of differentially expressed genes. Using microarray analysis, we also identified 200 up-regulated and 130 down-regulated genes that showed ≥ 3-fold change in expression in HPV-positive oropharyngeal tumors as compared to the normal oral mucosa. The up-regulated genes included many that are involved in DNA replication, DNA repair, cell cycle progression and signal transduction. The down-regulated genes included those involved in DNA repair, adhesion, immune response, and apoptosis. The ultimate goal of our studies is to identify tumor markers and classify HPV-positive cancers by molecular profiling that can be of diagnostic and prognostic value. Furthermore, such studies may also identify potential new targets for anti-cancer therapy.
The Potential Impact of HPV Prevention Worldwide

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Human papillomavirus (HPV) infections are associated with a wide spectrum of mucocutaneous diseases ranging from benign skin warts through different grades of pre-cancer to invasive cervical cancer, which is the second most common cancer among women worldwide. While over 90% of genital HPV infections are transient and clear spontaneously without treatment, causing no clinical symptoms, invasive cervical cancer is an uncommon consequence of HPV infection in women. Nevertheless, cancer of the cervix, with an estimated 493,000 new cases and 274,000 deaths in the year 2002 is the major cause of cancer mortality among women in developing countries. Cervical cancer is preventable for two main reasons: First, because it is caused by a infectious agent, which can be averted. Secondly, it develops over many years offering several opportunities for interventions aimed at preventing progression to disease. Thus, effective prevention can be divided into two categories: primary and secondary prevention strategies. Primary prevention strategies are interventions aimed at avoiding or reducing the exposure to the infectious agent(s) and risk factor(s) causing the disease. These are generally based on behavioural, environmental or biological changes, including prophylactic vaccination, that renders the infection innocuous. Secondary prevention strategies are aimed at early detection and timely treatment or removal of recognized early clinical signs of disease, before it comes to a malignant or invasive stage, and these are mostly based on advancements in screening tests, diagnostics and health technology. Combined strategies for primary and secondary prevention are likely to offer the most effective approach for cervical cancer control over the next decade. With the advancements in vaccine development better primary prevention strategies can now be designed and implemented. Defining delivery strategies and finding the best combination of primary and secondary prevention programs will be the challenge.

Aurora Kinases in Chromosomal Instability and Cancer

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Aurora kinases representing a novel family of serine/threonine kinases have been identified as key regulators of the mitotic cell division process that are frequently over expressed in many human cancers. The three members of this kinase family, Aurora-A, Aurora-B and Aurora-C kinases, expressed and activated at highest levels during G2-M phase, are known to be involved in the regulation of centrosome function, bipolar spindle assembly and chromosome segregation processes. Elevated expressions of these kinases have been correlated with chromosomal instability and clinically distinct grades and stages of human cancers. Studies from our laboratory and those of others have revealed that while Aurora-A appears to play critical roles in the early as well as late stages of mitosis through its interactions with several key oncogenic and tumor suppressor proteins, Aurora-B and -C, on the other hand, are chromosomal passenger proteins that are involved in the regulation of chromosome congression, segregation and cytokinesis. Additionally, Aurora-A kinase influences DNA damage
response checkpoint pathway proteins while Aurora-B and -C kinases influence the spindle checkpoint pathway proteins, thus revealing that the Aurora kinase family not only regulates mitotic cell proliferation but also the two major cellular damage response pathways. Recent findings further demonstrate that Aurora kinases in addition to modifying their individual substrates also cross talk among themselves to regulate mitosis regulatory pathways. Therefore in addition to elucidating vertical regulatory cascade of each Aurora kinase, it would be important, in the future, to understand how cross talking among Aurora kinases are coordinated during normal cell cycle. This would provide useful knowledge for developing therapeutic strategies for human cancers associated with over expression of Aurora kinases. In this presentation, we will discuss the cellular pathways involving Aurora kinases critical in the development of chromosomal instability and malignant transformation in human cells.

**PO-38**
**Integrating Notch and EGFR/ ErbB2 Signaling in Human Cervical Cancer Progression**

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**Introduction:** We are interested in understanding the relationship between Notch and EGFR pathways in the context of human cervical cancer. **Objectives:** We examine the role of EGFR signaling in mediating the pro-oncogenic phenotypes mediated by Notch signaling in the context of human cervical tumors. **Methods:** This study principally combines an immunocytochemistry of human pre-neoplastic and neoplastic lesions with an analysis of human cervical tumor derived cell lines. In human cervical tumor derived lines, the levels of various EGFR family members was estimated along with the phosphorylation status of EGFR. In parallel, we determined the status of phosphorylated Akt, STAT3 and ERK with and without specific inhibitors of EGFR, src and ErbB2. Additionally, immunoprecipitates and co-localization experiments of EGFR, src and ErbB2 were undertaken. Following introduction of activated Notch1 alleles (AcN1) in HaCaT cells, these cells were analysed for levels of ErbB2. **Results:** In a limited analysis, human cervical cancers in parallel show upregulated Notch, ErbB2, STAT3 with widespread EGFR expression. Dominant negatives or chemical inhibitors of src block EGFR phosphorylation at tyrosine 84 but not PI3Kinase in CaSki cells. However an ErbB2 inhibitor blocks both STAT3 and PI3kinase. ErbB2 expression is blocked by gamma secretase inhibitors in CaSki cells and induced by AcN1 in HaCaT cells. **Conclusion:** These experiments define a linkage between Notch and EGFR/ErbB2 signaling in human epithelial cells. This information is being integrated with studies undertaken in Apurva Sarin’s laboratory on T cells and our data showing a role for deltex in AcN1-P3K activation.

**PO-39**
**Early Onset Breast cancer and Genetic susceptibility in Indian Women**

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Breast cancer is one of the leading causes of cancer death among Indian women. One in fifty eight women are affected by breast cancer in the age group of 30-70 years and are mainly from urban areas. Although the over all incidence rate of breast cancer in Indian women is not as high as in western countries (23.5 vs 90.7), the incidence
of early onset breast cancer cases (< 40 years) does not show significant variation as compared to population worldwide (12-33 per 100,000 women); suggesting that a greater proportion of all breast cancers is due to early-onset disease in Indian population. Since familial cancer cases often present at an early age in contrast to that of sporadic cancer, genetic factors are considered to be playing far greater role in conferring cancer susceptibility. Following the identification of breast and ovarian cancer susceptibility genes BRCA1 (MIM 113705) and BRCA2 (MIM600185), the frequency and spectrum of disease related mutations have been investigated in North Indian population. We present family history data and molecular analysis from high-risk group of patients for breast cancer. Screening for mutations in coding and intron and exon boundaries of BRCA1/2 genes has been studied in 204 breast cancer patients and 50 controls. The study group included 155 (75%) early onset cancer cases (<45 yrs); 48 (23.5%) familial cases, 11 (5.3%) cases with bilateral breast cancer and 8 (3.9%) cases having both breast and ovarian cancer. Total 21 sequence variants were noticed in 25 patients including 3 frame shift (FS), 5 missense (MS), 3 Splice Sites (SS) and 2 Nonsense (NS) in BRCA1 and 1 FS, 5 SS, 1 MS and 1 NS in BRCA2 genes. 66.6% BRCA2 variants were associated with early onset condition while 22.2% with family history. In case of BRCA1 variants again association with the early onset condition was found significantly high (87.5%) as compared to that of family history (18.7%). Genetic susceptibility to cancer is triggered in several ways. The most common mechanism involves uncontrolled cell division due to germline mutations in tumor suppressor genes and DNA repair genes, which ultimately lead to accumulation of mutations in major oncogenes. Breast cancer arising in women with and without a germ line mutation in BRCA1 and BRCA2 gene display different molecular characteristics suggesting unique mechanism of molecular pathogenesis. Molecular pathological analysis of these tumors has been done to define the genetic abnormalities relevant to this specific pathogenesis. Tumor material has been studied from 138 women with breast cancer, 61 having early onset, 36 familial cases and 41 having late onset and 15 showing germ line BRCA mutations. The expression of p53, p- glycoprotein, and E-cadherin revealed statistical significant differences among the various groups of patients. Expression of C-erb2 oncoprotein was also found different in early and late onset Familial cases with Odd ratio of the order of 4.5.

PO-40
Protykin: A Future Medicine for Chemotherapy in Patients with Advanced Breast Cancer

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Protykin® is an extract of Polygonum cuspidatum roots, an herb used in traditional Chinese medicine. It is a phytoestrogen (natural plant estrogen) and contains more than 1,000-times the amount of the same antioxidant ingredient in red wine that is believed to promote cardiovascular health and reduce the effects of premature aging. Protykin binds with estrogen receptor- alpha (ER-α) and enhances estrogen-like activity in the body, without causing any side effects, which are generally associated with synthetic hormone replacement therapy (HRT). Protykin extract also contains 50% trans-resveratrol, a compound found largely in the skins of red grapes and an oriental medicine used to treat diseases of the blood vessels, heart and liver. Resveratrol may be a powerful cancer-fighting ally. These unique features tempted us to Protykin can be considered as a novel chemopreventive agent in women with high risk of breast cancer and has promising implications for the use of Protykin as a treatment in women with this disease. To test the hypothesis we: 1. determined whether Protykin is able to modulate the breast tumor cell proliferation in vitro, and 2. determined if Protykin-riched diets suppress the tumor growth in nude mice and if so, what molecular events would be involved in this process. BrdU-ELISA assay showed that like other chemopreventive agents Protykin inhibited cellular proliferation both ER-positive (MCF-7) and ER-negative (MDA-MB-231) breast
tumor cell with a maximum inhibitory effect at concentrations close to 50 mg/ml. However, the effect was vigorous in ER-negative MDA-MB-231 metastatic cells. These in vitro results substantiate the in vivo studies and indicate that the growth of MDA-MD 231 breast tumor cell xenografted into athymic nude mice can be suppressed by Protykin when fed Protykin-riched (50-100mg/kg body wt) liquid diet. Moreover, the studies also indicate that protykin-induced suppression of tumor growth is mediated through the inhibition of angiogenic switch via modulation of positive and negative regulators of angiogenesis. Together these studies suggest that Protykin can be used as a potent anticancer drug. Further studies are warranted.

PO-41
Transcription Regulation through Chromatin- A New Target for Anti-Cancer Therapeutics

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Human genes are organized into a highly compact and dynamic nucleoprotein complex called chromatin, which consists of histones and associated non-histone proteins. Though apparently repressive, the precise organization of chromatin is essential for all the DNA-templated phenomenon inside the cell. Alteration in chromatin organization modulates the expression of underlying genes. The dynamic changes in chromatin structure are brought about by post-translational modifications of chromatin proteins (both histones and non-histones), ATP-dependent chromatin remodeling and histone chaperones. Dysfunction of any of these machineries are causally linked to several diseases, predominantly cancer. Therefore, chromatin-regulated/modulated gene expression is a new target for anti-neoplastic therapeutics. We focus on understanding the mechanism of chromatin transcription in humans and also searching for the small molecule modulators of histone modifying enzymes (HAT, HDAC and HMTases), which may serve as lead compounds to design novel anti-neoplastic therapeutics. We found that the multi-functional human transcriptional coactivator PC4 enhances p53 function by enhancing its DNA binding and thereby inducing the expression of Bax, a p53 responsive pro-apoptotic gene. Our recent findings suggest that the human histone chaperone nucleophosmin, whose expression dramatically increases in several cancers and upon DNA damage, enhances the acetylation-dependent chromatin transcription and also p53-driven gene expression in vivo. Interestingly, both PC4 and B23 get acetylated and presumably their function is regulated by this post-translational modification. We have discovered several small molecule modulators (activators and inhibitors) of HATs, HDACs and HMTases and their effect on gene expression is being studied in vitro and in vivo. These modulators are also capable of altering the acetylation of non-histone proteins like PC4 and B23. These molecules may serve as lead compounds in the synthesis of anti-neoplastic therapeutics.

PO-42
ATM, Telomeres and Cancer

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Signal transduction pathways activated by DNA damage are critical determinants of cell survival and cellular transformation. A number of genes encode proteins that may be capable of sensing DNA damage.
One of these genes, ATM (ataxia-telangiectasia mutated), appears to be a major regulator of cellular responses to ionizing radiation (IR). ATM is a protein kinase that is activated by IR and phosphorylates a number of different substrates following activation. We have shown that the ATM gene product influences telomere metabolism. A hypothesis explaining such results is that defective telomere maintenance in A-T cells could be due to altered interactions between the telomeres and the nuclear matrix. Consistent with this hypothesis is that telomere nuclear matrix interactions and nucleosomal periodicity are altered in A-T cells. However, the precise mechanism by which ATM regulates the structure and function of telomeres is not known. Some of the common metabolic abnormalities, such as poor growth and IR sensitivity, have been linked with lack of ATM as well as loss of telomeres. The restoration of the telomere length by ectopic expression of catalytic subunit of telomerase (hTERT) in cells deficient for ATM function does not correct the telomere chromatin defect and other cellular phenotypes of the A-T cells, suggesting that ATM is essential for the signaling of telomere mediated functions. The ATM protein is associated with chromatin and telomeres are packaged in telomere specific chromatin. Since the chromatin defect in A-T cells is well pronounced at telomeres, which are heterochromatic, we, therefore, attempted to search for the ATM interacting proteins that have chromodomain region(s). Chromodomain proteins appear to be structural components of a macromolecular chromatin complex and are also involved in remodeling chromatin structure. We will discuss the role of ATM interacting chromatinmodifying factors in genomic stability, telomere metabolisms and oncogenic transformation.

PO-43
Genetic Pathways in Cervical Cancer Progression

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Carcinoma of the cervix uteri (Cervical Cancer; CC) exhibits a multitude of complex karyotypic alterations suggesting deregulation of numerous genes critical in the tumor formation and progression. The molecular basis of this genomic instability is poorly understood. Our goal is to characterize these genetic changes in invasive CC and through various stages of precancerous lesions using high-throughput methods. We identified a number of chromosomal and genetic changes including a) dosage alterations of loss, gain, or amplifications; b) specific patterns of gene expression profiles; and c) identification of epigenetic signatures. We have also shown that a number of pathways such as FANC/BRCA, SLIT-ROBO, RARB, and MMP play a role in CC tumorigenesis. The importance of these observations will be discussed in relation to tumor progression and identification of biomarkers in the management of cervical cancer.

PO-44
Induction of Caspase-9 Expression Co-operating with p53-Induced Apoptosis in Human Lung Cancer Cells

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Low-dose of 5-aza-deoxycytidine (DAC) caused an accumulation of procaspase-9 through mRNA up-regulation, but the cells did not undergo apoptosis. However, when cells were treated with DAC and infected with a low dose of a recombinant wild-type p53 adenovirus vector (Ad-p53), a synergistic growth inhibitory effect was observed. Combination treatment induced Apaf-1 and procaspase-9 expression in which cytochrome c releases by Ad-p53 triggered the mitochondrial pathway of apoptosis. DAC sensitized lung cancer cells to cisplatin and paclitaxel. DAC treatment may have clinical implications when combined with chemotherapy or apoptosis-inducing gene therapy.

PO-45
Prognostic and Predictive Markers in Carcinoma Cervix

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PO-46
Clinical Development of Merck's Quadrivalent

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PO-47
Early detection of cancer cervix in resource-poor country

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