HIGHLIGHT DOCUMENT
NATIONAL INSTITUTE OF PATHOLOGY
2015-2016

At National Institute of Pathology the thrust areas of research are tumor biology, infectious diseases including *leishmaniasis, tuberculosis, leprosy* and *chlamydiasis*, stem cell biology and environmental toxicology. The scientists conduct both basic as well as translational research leading to development of **Vaccines** for prevention and **Biomarkers** for screening, diagnosis, prognosis and prediction of drug response/resistance for various diseases **with mission to bring lab to bed**.

MAJOR PROJECTS UNDERTAKEN DURING THE YEAR

Tumor Biology

Study on micro RNA Signatures associated with Breast Cancer Stem like Cells(CSCs) and their role in Drug Response

The project is aimed to identify miRNA and gene expression signatures associated with breast cancer stem cells, to understand the molecular mechanisms there involved, and their contribution to response to chemotherapeutic agents. In the year under report in order to **evaluate the contribution of SOX family genes to formation of breast cancer stem cells**, we have evaluated the expression 8 SOX family genes in breast cancer stem cells derived T47D and MDA-MB-453 and compared them with their corresponding bulk cells. We found differential expression of *SOX1*, and *SOX3* in cancer stem cells compared to bulk cells suggesting their possible involvement formation of breast cancer stem cells. The expression of SOX family genes will be further extended to more number of cancer stem cells to confirm such involvement.

Targeted resequencing of breast cancer specific genes in early-onset breast carcinoma

This study is aimed to identify sequence variations and chromosomal rearrangements of deregulated genes in early onset breast cancers.

Whole exome sequencing has been done of 12 cases belonging to early and late onset tumors and analysis of data has been done to identify genetic variations associated with early onset and late onset tumors, using partek flow and partek genomics suite. We identified 2886 single nucleotide variations and 239 indels associated with early onset breast tumors, 5232 single nucleotide variations and 521 indels associated with late onset breast tumors. In addition 1991 single nucleotide variations and 137 indels were found common in both early and late onset tumors. On analysis of chromosome wise distribution of the variants, highest number of variants were found in chromosome 1 followed by chromosome 2 and 6. We have analysed variants of early and late onset tumors to identify pathways that are disrupted in these tumors. Pathways involved in early onset tumors include cAMP, axon guidance, ECM receptor signaling TNF signaling etc., while in late onset tumors regulation of endocytosis, regulation of keratins, Rap1 signaling pathways are uniquely disrupted. Further, we have analysed the whole exome data further for gene fusions, in these samples, three chromosomes, 1,7 and 17 were having more than 30 gene fusions, rest of the chromosomes have shown 4-8 fusions. No gene fusions were found in chromosoms 13, 18, and 21. Further whole exome sequencing in 22 breast tumors and 4 controls, including 9 early onset and 13 late onset tumors had also been carried out analysis of genetic variants is undergoing.

Differential Protein Profile for Identification of Markers in Recurrent Urothelial Cancer

The present study was planned to identify differentially expressed tumor proteins in tumour for use as biomarkers of recurrence.The differential protein profile for identification of markers in recurrent urothelial cancer was evaluated by processing tumour and normal mucosal samples (n=16). The cases included low grade non-invasive, high grade non-invasive and high grade invasive tumours. Proteins were extracted from tumor and normal tissue and quantitated by BCA

method. Proteins from tumour and normal tissue were labeled and subjected to liquid chromatography and mass spectrometry (MS/MS). Ratio of protein/peptide expression in tumor to paired normal was determined and fold change was calculated. A total of 3984 proteins were identified of which 1895 proteins (15381 peptides) were deregulated and included 1137 downregulated and 758 upregulated proteins in tumour tissue. Further data analysis showed 64 proteins common to all tumour samples with 100 proteins unique to low grade and 298 proteins unique to high grade. The gene ontology (GO) terms showed most of the proteins involved are involved in cell part (GO cellular component), catalytic activity (GO Biological process), and metabolic process (GO molecular function). Pathway analysis showed that the largest number of proteins was involved in integrin signalling pathway and the largest group of proteins belonged to the oxidoreductase class.

Genome-wide analysis of genetic alterations and gene expression profiles in hormone sensitive and hormone refractory prostate cancer

Study of genome wide analysis of genetic alterations in patients with prostate cancer of varying histological grades and aggressiveness was performed using array CGH based method. Array CGH gives a much higher resolution of closely spaced aberrations and allows linking of ratio changes to genomic/genetic markers.CGH+SNP based study was carried out on biopsy samples of prostate cancer tissues using Agilent Sureprint CGH+SNP arrays. The data was analyzed using Agilent Cytogenomics software. The results showed marked DNA sequence copy number changes in 75% of primary prostate cancer tissue samples. 75% of the cases showed loss on chromosome 8 (p23.3) and chromosome 10 (q11.22). Loss was also observed on chromosome 6 on both p and q arms, chromosome 15 (q11.2). Large chromosomal loss was found on chromosome 13 (q11-q34). Gain was seen in chromosome 16 (p11.2) in 50% of the cases.Array CGH results indicated that losses of several chromosomal regions were common genetic changes in primary tumors, suggesting that deletional inactivation of putative tumor suppressor genes in these chromosomal sites is likely to underlie development of prostate cancer. These chromosome aberrations may have prognostic utility as markers of prostate cancer progression. Study is being continued for analyzing more number of cases.

Understanding the role of chemokines in development of glioblastomamultiforme

Glioblastoma multiforme (GBM) is the most lethal neoplasm of Central Nervous System with 12-15 months survival . Chemokine signalling pathway are involved in myriad of biological processes and could directly influence the tumor growth by activating pathways related to cell survival and cell proliferation or indirectly by promoting angiogenesis. This study has been undertaken to identify important chemokine axis associated with GBM growth and development which could be used for therapeutic intervention. Chemokine genes involved in biological functions such as cell proliferation and cell cycle were selected. Those genes which were upregulated in GBM but not astrocytoma were sorted. These genes were *CXCR4, CCRL2, CCR5, PF4V1, CXCL6,* and *CXCL8* (*IL8).* For further validation at protein level IL8 and its receptors were selected. A tissue microarray of 55 DA and 91 primary GBM tissues stained with Anti-Human CXCL8 was analysed. In DA only 30.90% (17/55) cases were positive for CXCL8 while 67.03% (61/91) positivity was observed in GBM. Difference in immune positivity for CXCL8 between GBM and DA was found statistically significant with $p< 0.001$.Expression of CXCL8 was primarily found in tumor astrocytes confirmed by co-expression study done with the help of immuno- fluorescence. However, no significant difference was observed between GBM and DA for its receptors CXCR1 and CXCR2 . This project is ongoing and will continue further with validation on cell lines and targeting with antibodies and drugs *in- vitro*

Expression of gonadotropin releasing hormone receptor in glioblastoma cell line-derived exosomes and its potential as circulatory biomarker

The present study aimed at investigating the expression of GnRH receptor, belonging to the rhodopsin-like Gprotein coupled receptor (GPCR) family, in glioblastoma cell line, LN229, and cell line-derived exosomes to explore its potential as circulatory marker for post-treatment monitoring of GBM patients. GnRH receptor was observed to be expressed both at the gene and protein level in GBM cell line, LN229, and was also found to be expressed at protein level in **LN229 cell line-derived exosomes**. Interestingly, we observed significant enrichment of GnRH receptor protein in cell line-derived exosomes in comparison to cell lysate. **Expression of GnRH receptor protein in cell line-derived exosomes opens up**

the opportunity to explore the potential of GnRH receptor as circulatory marker for post-treatment monitoring in GnRH receptor positive glioblastoma patients.

Molecular regulation of mTOR signaling in acute lymphoblastic leukemia (ALL)

Despite major improvements in understanding of the molecular genetics of ALL, the mechanisms that lead to the abnormal proliferation and survival of T and B lymphoblasts remain largely unknown. Therefore, treatment of leukemia still remains a challenge for clinicians. Major efforts have been made to develop new compounds targeting signaling pathways implicated in ALL cell proliferation and survival. One such pathway is represented by the mammalian target of rapamycin (mTOR). This study has been undertaken with the objective of studying the expression of mTOR gene in acute lymphoblastic leukemia (ALL) samples using real time PCR and to identify subset of patients having high expression of mTOR and its association with response to chemotherapy. Peripheral blood samples from 50 patients of acute lymphoblastic leukemia (ALL) admitted to the Division of Haematology& Division of Paediatrics, Safdarjung Hospital New Delhi for induction chemotherapy, were collected during the current year. Expression of mTOR gene in response to induction chemotherapy was studied in 50 ALL samples and 20 Healthy control. Response to chemotherapy was determined at the end of completion of induction chemotherapy. Expression of mTOR was found to be significantly up regulated in non-responder patients of ALL as compared to responders. mTOR inhibitor in combination with conventional chemotherapeutic drugs used in ALL will be evaluated in leukemic cell lines to study the effect on cell cycle, apoptosis and mTOR signaling. Study is expected to give insight into incorporation of mTOR inhibitors into the treatment regimen of ALL.

Infectious Diseases

Leishmaniasis

Analysis of clinical efficacy of oral miltefosine in treatment of post kala azar dermal leishmaniasis (PKDL) in India

Recent studies have shown significant decline in the final cure rate after miltefosine treatment in visceral leishmaniasis. We evaluated the efficacy of miltefosine in the treatment of post kala-azar dermal leishmaniasis (PKDL) patients recruited over a period of 5 years with 18 months of follow-up. In this study 86 confirmed cases of PKDL were treated with two different dosage regimens of miltefosine (Regimen I- 50mg twice daily for 90 days and Regimen II- 50 mg thrice for 60 days) and the clinical outcome assessed monthly. Cure/relapse was ascertained by clinical and histopathological examination, and measuring parasite burden by quantitative real-time PCR. *In vitro* susceptibility of parasites towards miltefosine was estimated at both promastigote and amastigote stages. Seventy three of eighty six patients completed the treatment and achieved clinical cure. Approximately 4% (3/73) patients relapsed by the end of 12 months follow-up while a total of 15% (11/73) relapsed by the end of 18 months. Relapse rate was significantly higher in regimen II (31%) compared to regimen I (10.5%)($P<0.005$). Parasite load at the pre-treatment stage was significantly higher ($P<0.005$) in cases that relapsed compared to the cases that remained cured. *In vitro* susceptibility towards miltefosine of parasites isolated after relapse was significantly lower (>2 fold) in comparison with the pre-treatment isolates ($P<0.005$). Relapse rate in PKDL following miltefosine treatment has increased substantially, indicating the need of introducing alternate drugs/ combination therapy with miltefosine.

Studies on miltefosine resistance in visceral leishmaniasis:

Increasing incidence of relapse in VL cases treated with miltefosine raised the concern for its immediate surveillance in the field to safeguard efficacy. We investigated the parasitic factors apparently involved in miltefosine unresponsiveness in natural population of *Leishmania donovani* using isolates from pretreatment group LdPreTX (n=6), relapse cases after miltefosine treatment (VL and PKDL) LdRelapse (n=5) and in experimental MIL resistant (LdM30, n=2) parasites. LdRelapse

and LdM30 parasites exhibited significantly lower accumulation of miltefosine ($p<0.05$) compared to LdPre-TX parasites. MIL induced ROS levels were significantly low ($p<0.05$) in macrophages infected with LdM30 and LdRelapse parasites compared to LdPreTX parasites, also intracellular thiol content was significantly higher ($p<0.05$) in LdRelapse and in LdM30 indicating better tolerance for oxidative stress in unresponsive isolates. Transcriptome profiling revealed that several genes involved in antioxidant defense mechanism, metabolic process, transporters, cell component and cell motility are preferentially expressed in LdM30 and LdRelapse parasites than wild type *L. donovani* parasites. Several other genes mainly transporters like ABCF2, amino acid transporter, surface acylated putative protein, APH and mitochondrial precursor peptide, chaperon TCP20, clathrin coated assembly protein, C5 sterol desaturase, autophagy protein ATG10 were preferentially expressed in LdPreTX parasite compared to LdRelapse case and LdM30 parasites. The study provides the understanding of parasitic factors and pathways responsible for miltefosine unresponsiveness in VL and PKDL.

Mechanism of resistance towards paromomycin in *Leishmania donovani*

Paromomycin (PMM) is a new treatment option for VL control in India as a monotherapy and in combination therapy. Microarray was successfully exploited to analyze the genes showing modulated expression in PMM resistant parasites. We identified a total of 267 genes (approx. 2.9%) differentially modulated based on 2 fold cut off in PMM-R parasites. 174 genes were up-regulated and 93 genes were down-regulated in PMM-R isolates. The plot $\log_2$ transformed expression ratio of K133 PMMR (red line) compared to K133 WT (green line) as function of the chromosomal location of microarray probes is shown in **Fig. 1**. Up to 4 fold up- or down-regulation in drug resistant parasite was observed (**Fig. 1**).

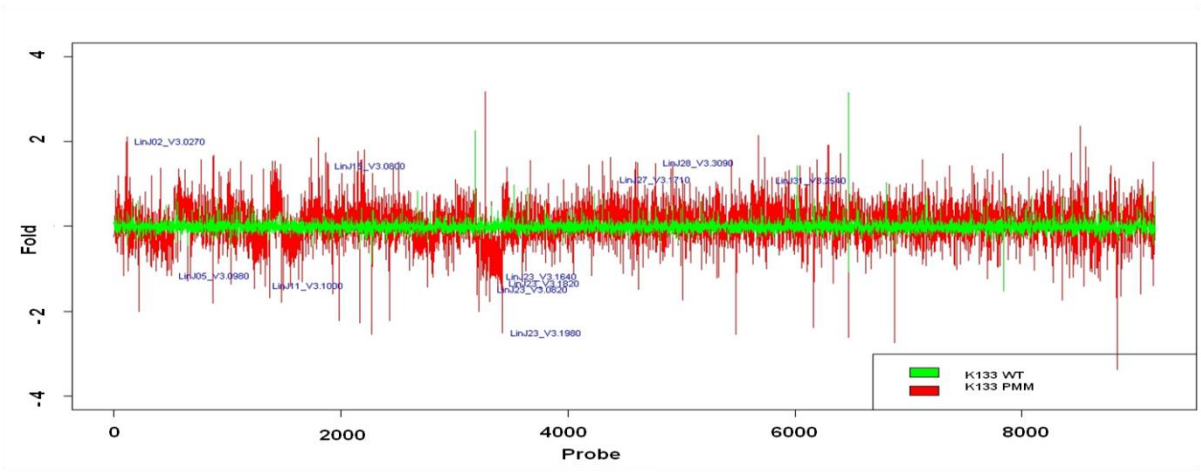

Fig 1: Comparative transcriptional responses following PMM adaptation in *L.donovani*. Overlap of log2 transformed PMM-R and PMM-S expression ratio plotted as a function of chromosomal location of probes represented the full genome microarray. The plot represents the average values of three independent hybridizations for each isolates.

To analyse gene expression level on genomic scale, chromosome map was generated using Custom R program. Analysis of chromosome map identified that higher number of up regulated genes are located on chromosome 6, 12, 32, 35 and 36. There were no up regulated genes on chromosome 3, 13, 20 and 23. Maximum numbers of down regulated genes were located on chromosome 23 in PMM-R isolates.

Tuberculosis:

One of the important facets in deciphering pathogenesis of M.tb is to understand the role of molecular three-dimensional structure of a protein and its function. Therefore, understanding protein structure and its function is very important. While proteins are often presented as solid, rigid bodies, they are in reality highly dynamic which is an important feature in terms of their function and recognition.

A B

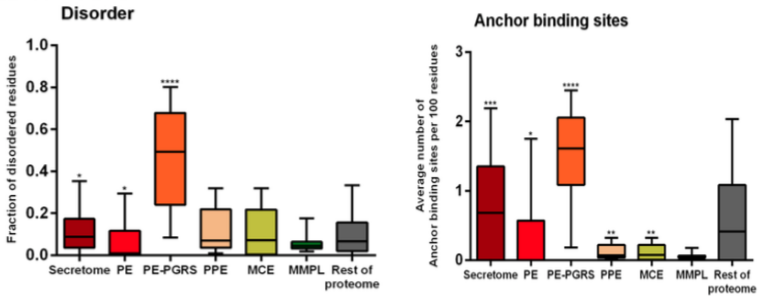

Figure 1. A) Boxplots shows Protein disorder content and average number of ANCHOR binding sites per 100 residues and B) Secretome of PE, PE_PGRS3, PPE, Mce and MmpL families with rest of the proteome. Disorder content and ANCHOR Binding sites of each group is plotted on Y-axis and Families on X-axis.

It is now established that fragments of some proteins and sometimes the entire protein do not usually have a well-defined structure in solution, but assume such structures only in a specific functional state. Such proteins are known as intrinsically disordered or unstructured. We performed the disordered protein analysis in the PE/PPE, Mce, MmPL and secretome of M.tb as well as prediction of protein binding sites and ELM search was also carried out (Figure 1). Experimental validation of *in-silico* analysis is important and it is being validated with one of the member of PE/PPE.

The virulence mechanism of mycobacteria is very complex. Broadly, the virulence factors can be classified as secretion factors, cell surface components, enzymes involved in cellular metabolism, and transcriptional regulators. The mycobacteria have evolved several mechanisms to secrete its proteins. RipA, possessing peptidoglycan hydrolase activities is shown to be secreted by the TAT secretion pathway. Inhibition of this export system will prevent localization of peptidoglycan hydrolase and results in sensitivity to existing lactam antibiotics, opening up new candidates for drug repurposing. RipA is a secretory protein and has been shown to possess p60 domain that is capable of hydrolyzing dipeptide, D-glutamyl-meso-diaminopimelic acid. These properties of RipA protein such as virulence, invasion, secretion and cell-wall association make RipA an ideal candidate to evaluate the potential efficacy as a possible vaccine candidate. The physical interaction of RipA with MoxR1 protein, an AAA+ ATPase having chaperonic activity assists in proper folding of RipA in the cytoplasm prior to its secretion (Figure 2). Secreted RipA protein interacts with other proteins and gets cleaved to start its peptidoglycan hydrolase activity.

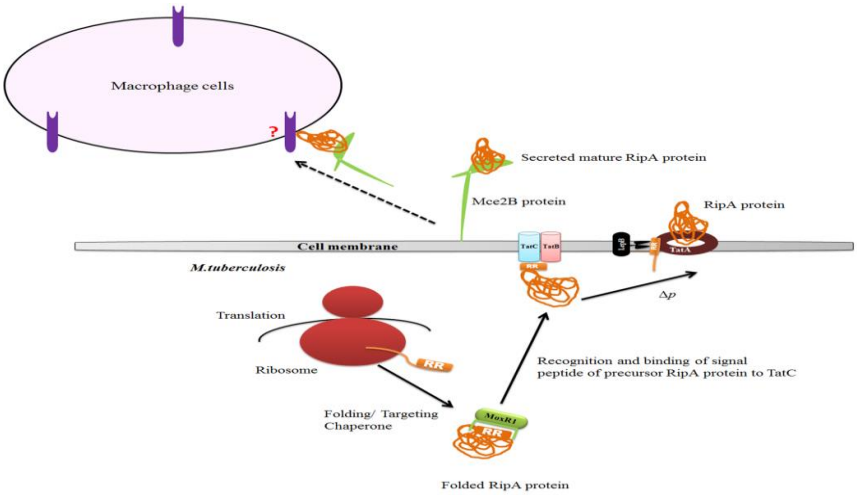

Figure 2. Proposed Model for RipA secretion through TAT pathway

M.tb has evolved mechanisms to survive in macrophages that represent one of the most stressful environments for bacteria. For successful colonization, M.tb forms a niche by establishing molecular interaction networks within the host system. M.tb has two cyclophilins, PpiA and PpiB. It is a secretory protein and interacts with several host proteins such as those involved in iron regulation, immune defense mechanism and signal transduction

Peptidyl-prolyl cis-trans isomerases (Ppiases), are ubiquitously expressed enzymes that assist in protein folding by isomerization of peptide bonds preceding prolyl residues. We have described chaperone-like activity of mycobacterial Ppiases. We have also demonstrated its role in responding to host generated stresses like hypoxia and oxidative stress by transiently expressing *M.tb* PpiA and PpiB in HEK293T cells. Presence of these proteins show increased survival as compared to control cells in response to oxidative stress and hypoxic conditions generated after treatment with H_2O_2 and $CoCl_2$ (Figure 3). *M.tb* Ppiases play role in modulating host immune responses. Sera of TB patients showed high levels of antibody to *M.tb* Ppiases in the patient sera as compared to the sera of healthy humans. Treatment of THP-1 cells induced secretion of pro-inflammatory cytokines as a direct function of concentration of rPpiA. Alternatively, treatment with rPpiB inhibited secretion of TNFα and induced secretion of IL-10. Furthermore, heterologous expression of *M.tb* PpiA and PpiB in *Mycobacterium smegmatis* increased its survival in THP-1 cells as compared to vector control (Figure 4).

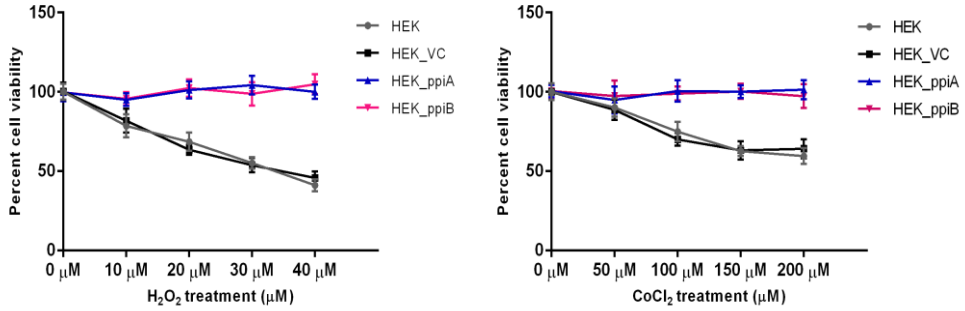

Figure 3. Mycobacterial Ppiases impart resistance to the HEK293T cells against Oxidative and Hypoxic stress. HEK cells transiently expressing PpiA and PpiB could resist oxidative stress and hypoxia caused by H_2O_2 and $CoCl_2$ as compared to the wild type and vector control.

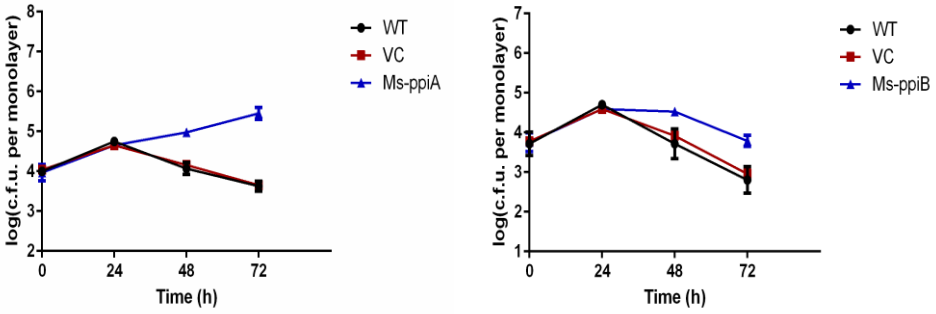

Figure 4. *M.tb* Ppiases aid in intraphagosomal survival of the pathogen. *M. smegmatis* strain expressing PpiA and PpiB of *M.tb* exhibit increased survival in THP cell lines as compared to wild type *M. smegmatis* and vector control.

These results demonstrated that *M.tb* Ppiases are immunogenic proteins that can possibly modulate host immune response and enhance persistence of the pathogen within the host by subverting host cell generated stresses.

Chlamydiasis

Chlamydia trachomatis infects human host and tries to alter the host genomic environment in such a manner that favours its survival and successive proliferation. This is an important strategy employed by *C. trachomatis* to ensure its survival at genomic and proteomic level. It has been argued that Th1 and Th2 cytokines or blunting of initial cytokine response might also be important in the disease manifestation and its maintenance in *C. trachomatis*-induced Reactive Arthritis (ReA). Based on intra-articular and circulatory profile of key Th1/ Th2/ Th17 cytokines, *viz.:* IFN-gamma, IL-4, IL-6, IL-17, it was concluded that *C. trachomatis*-induced ReA patients have a Th-1 dominant profile. IFN-gamma levels

were synergistically enhanced in both synovial fluid and serum in the infected group while IL-6 appeared to be the key player for this proinflammatory and protective response. Upregulated level of IFN-gamma inhibit *C. trachomatis* multiplication and control the disease progression. Apparently, the role of IL-6 is important in regulation of Th1 /Th2 /Th17 cytokine pathway in *C. trachomatis*-induced ReA.

ADULT STEM CELL BIOLOGY

A new cell culture process for growing cultured epidermis for application in burns was earlier standardized at our laboratory. The technique involved culture of human epidermal keratinocytes in the presence of a specific sub-set of SWISS 3T3 feeders cells (Chugh et al 2015) which were growth arrested with low concentration of Mitomycin C using an innovative dose derivation (Chugh et al 2016). A Prototype has been prepared (Yerneni and Chug 2014). As part of the Quality Control and Quality assurance issues, earlier we found no detectable residues of mitomycin C in the final product. We have now completed the pre-clinical testing of the cultured keratinocytes for tumorigenesis in nude mice and Karyotyping by G-Banding in cultures established from human skin Biopsy.

After obtaining the ethics committee approval, a small unused piece of autograft skin from a 26 years old male patient was obtained from the burns OT of Safdarjung Hospital, New Delhi to the lab and epidermal cells were isolated and plated over Swiss 3T3 feeder cells of 4-150 group prepared by our in-house technique. The cultures were serially passaged and epidermal sheets were prepared by keratinocytes until six passages. The expansion logically proved that epidermal sheets to the tune of 60-100% coverage could be supplied in 22 to 28 days. The P5 cells were subjected to G-banding which showed no detectable Karyotype abnormalities in keratinocytes.

For preparing the injectable stocks of epidermal keratinocytes for 8 mice, the P4 cells in Matrigel were delivered to 8 nude mice in the range of 5 – 7 million per mouse by subcutaneous injections. A human embryonic kidney cell line, HEK293 at 90th passage, was similarly injected to 6 mice to serve as positive control. All six mice receiving HEK293 cells formed tumors at the site of injection at different post-injection time points. In the keratinocyte group, two mice showed resolution of the nodule caused by primary injection in 49 days while in the other two mice it resolved after 59 and 91 days, respectively. In the remaining four mice, the nodule showed initial rapid regression but a small and soft nodule remained unresolved. The dissection of the injection site showed only the residual Matrigel and histopathology of the nodule and the internal organs revealed no tumor cells.

Future course of action:

Clinical trial with the cultured epidermis will be undertaken. Coordinated efforts are being made to simultaneously obtain grants and cGMP construction to facilitate such trial. Additionally, efforts will also be made to identify a suitable industry.

Human environmental biomonitoring of Polynuclear Aromatic Hydrocarbons (PAHs) in urban megalopolis of NCR Delhi and investigate the association between PAH exposure and intrauterine growth restriction (IUGR)

Polyaromatic hydrocarbons are a ubiquitous group of environmental pollutants that have been shown to cause carcinogenic and mutagenic effects and are potent immuno-suppressants. Animal studies have shown that PAHs can cause harmful effects on the skin, body fluids, and ability to fight disease after both short- and long-term exposure. Mice that were fed high levels of one PAH during pregnancy had difficulty reproducing and so did their offspring. These offspring also had higher rates of birth defects and lower body weights. This study was designed to examine the association between IUGR and PAH exposure in expectant women. HPLC analysis of the extracts obtained from placental tissue and bloods for presence of the PAHs residues revealed presence of Acenaphthylene Phenanthrene and Pyrene in significant quantity in IUGR cases in comparison to control. The study revealed a positive correlation between presence of PAH in human placenta and/or blood and intrauterine growth restriction and low birth weight delivery.

Biomedical Informatics Centre's of ICMR (Phase-II) at NIP, New Delhi

Biomedical informatics centre, NIP, has identified its major research focus as implementation /development of biomedical informatics techniques for assisting disease diagnosis and therapies at the point of patient care, which is one of the major objectives sketched by ICMR for the taskforce project. Accordingly it has initiated research and training facility for biomedical scientists, research scholars and students to promote and support informatics in medical research.

During the year under report, the activities of BIC include:

- Development of Psoriasis Associated Gene database: The database includes comparative genomic tool to assist biomedical scientist in detecting known psoriasis SNPs in an individuals' genome/gene sequence.
- Developed TiD: a standalone software for identification of putative drug targets from whole proteome of pathogenic bacteria.
- In silico design of IL6, IL23 and TNF alpha antibodies for biologic based therapy development against autoimmune and inflammatory diseases.TNF-alpha, JAK-2 and PDE4b inhibitor design.
- e-pharmacophore based inhibitor design for cytoplasmic tyrosine kinase.
- Organized National level workshops: 20 candidates (MDs, PhD and M.Sc.) selected from 11 states of India were training in five-day workshop on National workshop on Next Generation Sequencing in disease diagnosis and therapeutic target discovery.

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3. Usha Agrawal**,** Nitu Kumari, Ashwani K. Mishra, Pawan Vasudeva,Anup Kumar, Nayan K Mohanti, Sunita Saxena **"Immune signature of urothelial cancer associated with grade, recurrence, and invasion."** Urologic Oncology: Seminars and Original Investigations (Accepted)

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Awards and Honours

1. **Dr. Poonam Salotra** invited delegate at "Kala-azar Elimination Program" partners' consultative meeting held at WHO, Geneva, Switzerland in Feb, 2015.
2. **Dr. Poonam Salotra** conferred TWAS fellowship at the 26th General Meeting of The World Academy of Sciences (TWAS) at Vienna, Austria, in Nov 2015.
3. **Dr. Ruchi Singh** received "BILL & MILINDA GATES foundation award for Young Investigator from India and Southeast Asia" presented by the International Society for Infectious Diseases at 17th International Congress on Infectious Diseases, Hyderabad, India March 2-5 (2016).
4. **Dr. Avninder P Singh** awarded Bishnupriya Devi award 2015 for best original article published in IJDVL titled "Histopathological characteristics in post kala-azar dermal leishmaniasis - a series of 88 cases" by Indian academy of dermatology**.**

Patent:

LK Yerneni and RM Chugh (2015). A method for processing of feeder cells suitable for adult stem cell proliferation. PCT WO2016067306, File Number PCT/IN 2015/000404, dated 29th October 2015

MAJOR ACHIEVEMENTS HAVING PUBLIC HEALTH IMPORTANCE

- Androgen receptor has been identified as independent predictive marker for response to neoadjuvant chemotherapy in locally advanced breast cancer cases. Study proposes to use anti androgens like bicalutamide along with other anti cancer drugs as novel therapy for the treatment of Triple Negative Breast Cancers, which are positive for AR.
- The methylation and expression status of circulatory proteins involved in immunoregulation (IL22RA2 and TNFSF13B), extra cellular matrix remodeling (SERPINA4) and contraction of the

circular muscle of human esophagous (TAC3) could be further explored as non-invasive biomarker for esophageal cancer.

- Next-generation sequencing (NGS) of HLA super locus (3.8 Mb regions) showed high association of five SNP located in HLA region with the Nasopharyngeal cancer. Of these five SNPs two SNPs were novel present in genes namely COL11A2 and MUC22 whereas three SNPs present in genes HLA DRB5, HLA-DPA1 and TAP2 were already known. Further the validation of these SNPs is undergoing in large sample size.
- Clinical efficacy of oral miltefosine in treatment of post kala azar dermal leishmaniasis (PKDL) in India was established.
- Reduced drug uptake and increased tolerance to oxidative stress are major parasitic factors associated with increasing relapses in miltefosine treated VL cases.
- Identified novel targets of Mycobacterium tuberculosis for its use in diagnostic and as potential vaccine candidate.
- Studies on the cytokine pathway in *Chlamydia trachomatis*-induced reactive arthritis revealed the role of IL-6 in regulation of Th1 /Th2 /Th17 cytokine pathway in *C. trachomatis*-induced ReA.

- The characterized "cultured epidermis" produced by our novel processing technology is qualitatively comparable to the international product "Epicel" of **Vericell Corporation, USA (**http://vcel.com**)** and is now ready for clinical trial-commercialization for burns.

FUTURE PLAN OF THE INSTITUTE

- Establishment of Tumour Tissue bank at NIOP
- Clinical trial with the cultured epidermis will be undertaken. Coordinated efforts are being made to simultaneously obtain grants and cGMP construction to facilitate such trial. Additionally, efforts are being made to identify a suitable industry.
- LAMP-based Diagnostic kits for Leishmania infection
- Toxicological and other pre-clinical studies to be pursued for the vaccine against kala azar
- Evaluation of combination therapy regimens based on miltefosine and Liposomal amphotericinB for treatment of PKDL
- Explore initial leads on tuberculosis: a) Specific *M.tuberculosis* proteins in boosting memory response of BCG vaccine for TB prevention and b) Large scale clinical validation of TB biomarkers

HIGHLY SIGNIFICANT ACHIEVEMENTS (NOT MORE THAN ONE PAGE IN BULLETTED FORM) FOR THE EXECUTIVE SUMMARY

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- An improved understanding of the cytokine pathway in *Chlamydia trachomatis*-induced reactive arthritis may lead to better patient management and treatment options.
- The characterized "cultured epidermis" produced by our novel processing technology was found qualitatively comparable to the international product "Epicel" of Vericell Corporation, USA (http://vcel.com) and is now ready for clinical trial-commercialization for burns.
- Biomonitoring of Polynuclear Aromatic Hydrocarbons (PAHs) in urban megalopolis of NCR Delhi revealed a positive correlation between presence of PAH in human placenta and/or blood and intrauterine growth restriction and low birth weight delivery.