HIGHLIGHTS

2012-2013

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THRUST AREAS OF RESEARCH

TUMOUR BIOLOGY

In Tumor Biology division, scientists are working mainly on breast, prostate, urinary bladder and brain cancers, lymphomas and leukemias and cancers in northeast region of India focusing mainly on identification of diagnostic, predictive and prognostic biomarkers, new drug targets and gene-environmental links.

1. STUDY ON GENE EXPRESSION AND HYPERMETHYLATION PROFILES IN EARLY ONSET BREAST CANCER

This study had been undertaken to examine the gene expression and methylation profiles of early onset breast cancer by using microarray analysis in Indian patients with the aim to elucidate the molecular pathogenesis and to identifying biological markers of clinical significance. Gene expression and methylation profiling was done in 20 early onset tumors (<40 years) and 20 late onset tumors (>55 years) using Human HT-12 v4 Expression BeadChip & Infinium Human Methylation 27 BeadChip. Differential methylation was found in 977 genes in early onset cases and 1396 genes in late onset breast cancer cases. Validation of expression of several differentially expressed genes such as ALDH1B1, ALDH2, CBX2, CCNJ, FOX (3 genes), JMJD2A, MATN, METTL11A, PRMT, SOX genes (3), TMEM41A, WNT5A, WNT3, , HMGN1, JMJD4, MCMs (2), SMARCAs (2 genes) is being done by real time PCR in breast cancer cases of both early and late onset groups.

2. STUDY ON MICRO RNA SIGNATURES ASSOCIATED WITH BREAST CANCER STEM LIKE CELLS (CSCS) AND THEIR ROLE IN DRUG RESPONSE

Tumor recurrence and treatment failure are well known in cancer therapy and recently linked with Cancer Stem Cells (CSCs). Breast CSCs can be sorted out based on the presence of Aldehyde Dehydrogenase (ALDH) enzyme or by using CD44 and CD24 markers. Cancer stem cells can be enriched and propagated in suspension cultures as mammospheres. The mammosphere system offers an *in vitro* model to study the effect of anti-cancer drugs on

breast cancer stem cells. The present study is aimed to identify and characterize microRNAs and genes that are differentially expressed in breast CSCs.

Human breast cancer cell lines, MCF7, SUM159 and HBL100 were grown as adherent cultures. The cells were then stained with aldefluor and sorted out using FACS ARIA II. The sorted ALDH+ cells were then cultured in non-adherent, non-serum conditions to form primary mammospheres (termed M1 mammospheres), which were dissociated both enzymatically and mechanically to obtain single cells which were then subjected to serial passaging every 10th day leading to the generation of M2, M3, M4 mammospheres and so on to test the functional definition of stem cells of self renewal. Also, the dissociated cells from these mammospheres were again analyzed for the presence of ALDH$^{+/bright}$ cells and were sorted using FACS at every passage.

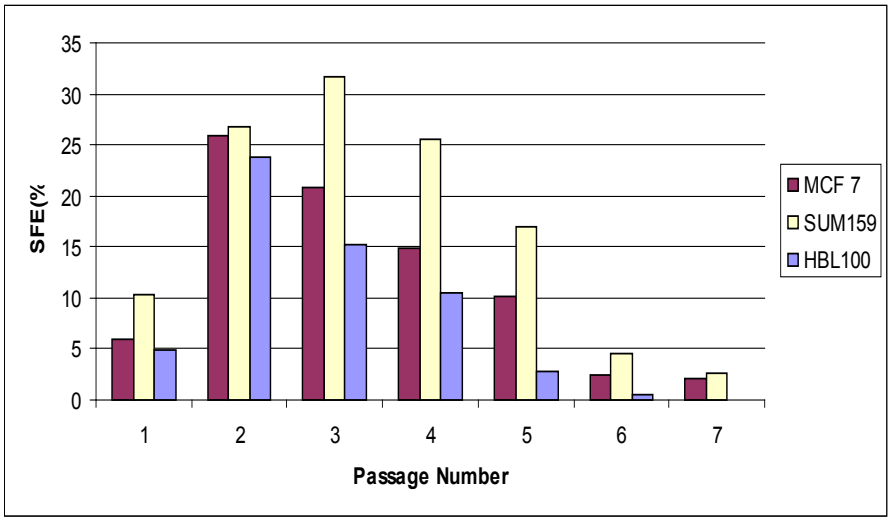


Fig. 1: Sphere Formation Efficiency (SFE) of various breast cancer/ normal cell lines

The study showed that primary mammospheres contain a distinct population that displays an ALDH$^{-/low}$ phenotype, but fails to generate mammospheres. Instead, the mammosphere-initiating potential rests within the ALDH$^{+/bright}$ cells, in keeping with the phenotype of breast cancer-initiating cells. With increasing passages, mammospheres showed a dynamic increase both in the number of mammosphere forming units and sphere forming efficiency (Fig. 1) until the second passage followed by a dramatic reduction. Also there is an increase in the number of smaller sized spheres relative to the larger ones over multiple generations of mammospheres. Treatment of ALDH-positive mammosphere (BCSC) with plant alkaloid

elipticine showed significant reduction in the formation of mammospheres while treatment with cytotoxic drug paclitaxel enhanced mammospheres formation. Interestingly treatment of mammospheres(BCSC) with combination of elipticine and paclitaxel also showed significant reduction in mammospheres formation.

Currently, we are in the process of standardization of isolation and propagation of cancer stem cells, by using CD44 and CD24 markers to select CSCs ($CD44^{+}/CD24^{-}$) by fluorescent activated cell sorting.

3. CHARACTERISATION OF HOST IMMUNE PROFILE ASSOCIATED WITH PROGRESSION OF SUPERFICIAL TCC OF URINARY BLADDER BY MICROARRAY ANALYSIS

The objective of the present study had been to characterize the immune dysregulation in urinary bladder cancer. Identification of defects in host anti-tumour immunity responsible for recurrences can help in predicting tumor behaviour. It would also help in understanding the immuno profile responsible both for recurrence and resistance to treatment.

During the year under report, quantitative real-time PCR was performed on 43 tumour (non-muscle invasive bladder cancer= 25 and muscle invasive bladder cancer= 18 patients) and 24 control (adjacent normal mucosa) tissue for IFNG, IL4, MYD88, REL, CTLA4 and COX2. qRT-PCR showed significantly increased expression of REL, CTLA4 and COX2 in tumour compared to adjacent normal mucosa. A comparison of the expression within the invasive and non-invasive groups showed significant upregulation of REL and downregulation of MYD88 in invasive tumours compared to non-muscle invasive tumours.

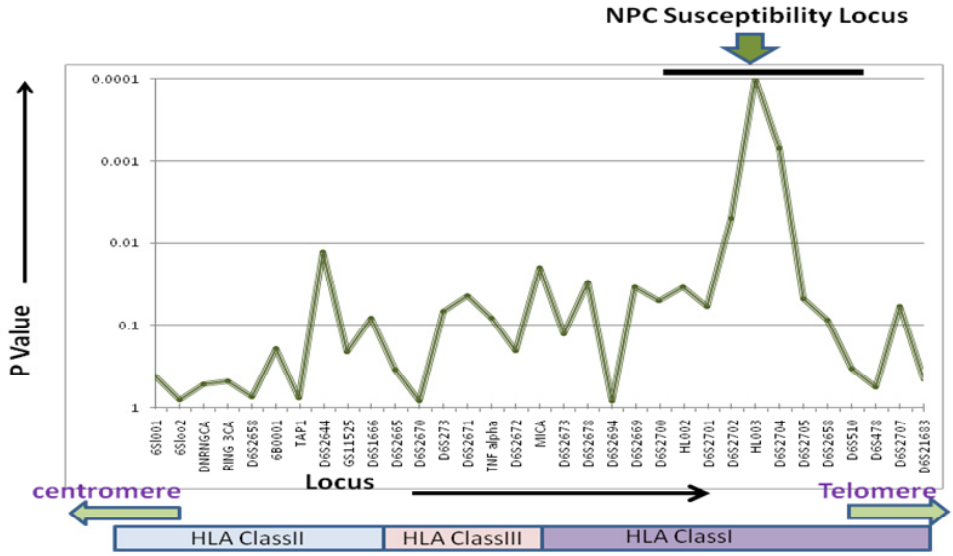
The histopathological examination of tumour tissue showed a mixed inflammatory cell infiltrate with predominantly lymphocytes in *MIUC* and plasma cells in *NMIUC*. Other inflammatory cells observed included macrophages and neutrophils and very occasionally eosinophils. Immunohistochemical staining for characterization of inflammatory cells revealed the mixed infiltrates of CD4+ and CD8+ lymphocytes, NK cells (CD56+) and macrophages (CD68+). NK cells and macrophages were found predominantly in invasive cancers compared to non-invasive cancers. A tissue microarray (100 cores) was constructed with 40 cases (*NMIUC*=26 and *MIUC*=14) and 10 normal mucosa in duplicate and immunohistochemistry was performed for TBX21, GATA3, PRKCE and SRC. TBX21 and GATA3 were both expressed in tumour and

inflammatory cells. The expression of TBX21 was significantly high in invasive bladder cancers (p value < 0.001) and PRKCE expression was significantly high in non-invasive cancers (p = 0.044). Both GATA3 and SRC were expressed in tumour cells but there was no statistically significant difference between invasive and non-invasive bladder cancers.

4. IMMUNOGENETIC PROFILE OF NASOPHARYNGEAL CANCER IN A HIGH-PREVALENCE REGION OF NORTH-EAST INDIA

Nasopharyngeal Carcinoma (NPC) is a rare tumour in most parts of the world, but occurs at relatively high rates in some geographic regions and among certain ethnic groups, with the highest incidence worldwide being reported from south-east Asia and southern China. High incidence of NPC in North-East (NE) region of India has been reported where it is the eighth most common cancer. The etiological factors of NPC include a complex interaction of genetic, viral, environmental and dietary factors. Antigenic presentation of EBV-derived peptides is suspected to be involved in the pathogenesis of EBV-associated diseases. In addition, polymorphisms in the HLA region, particularly in the class I region are also known to be associated with the occurrence of the disease. The current study has been undertaken to analyze if Epstein Barr viral sequences in the tumour tissue along with host imunogenetic factors can explain the high prevalence of nasopharyngeal carcinoma in different ethnic groups of NE States.

120 blood samples from newly diagnosed cases of NPC and 100 controls have been collected from two different centres in NE region, *i.e.* B. Barooah Cancer Institute, Guwahati, Assam and Regional Institute of Medical Science, Imphal, Manipur. Study of 33 markers selected from the HLA region (*Diepstra A. Lancet 2005*) was done by fragment length analysis and data analysis was done using Gene Mapper software. Allele frequencies were calculated by direct counting. The frequencies of the different alleles and genotypes were compared between patients and controls by use of Chi- Square test. When there was a significant difference, the specific allele or genotype causing this difference was assessed. Odds ratios and their 95% CI were calculated by logistic regression. Statistical significance was set at $p < 0.05$. The probability of an association was corrected with the Bonferroni inequality method, *i.e.* by multiplying the 'p' values obtained by the number of alleles compared (Pc).



Classic association analysis showed significant differences in allele frequency distribution for 2 microsatellite markers located in HLA class I region between patients and controls

Analysis of results showed significant difference in frequency of two microsatellite markers (HL003 and D6S2704) among patients and controls.($p \leq 0.05$).The study is continuing for further statistical analysis.

Detection of EBV in tissue/ blood samples was done using PCR and *in situ* hybridization. During the year, *in situ* hybridization conditions were standardized by using Epstein-Barr virus (EBER) PNA probe/ fluorescein, obtained from *Dako*. So far, 25 NPC cases and 15 non-NPC samples were processed for EBV detection by ISH, showing statistically significant differences in the presence of EBV sequences between the NPC patient group and the control group.

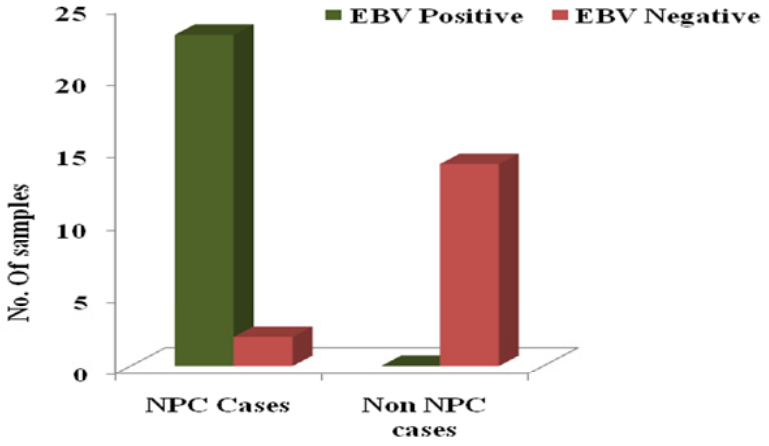


Figure: Bar graph showing frequency of EBV in NPC and non NPC patients

5. GENOME-WIDE ANALYSIS OF GENETIC ALTERATIONS IN PATIENTS WITH ESOPHAGEAL CANCER FROM NORTH-EAST INDIA USING SINGLE NUCLEOTIDE POLYMORPHISM ARRAYS

The current study aims to investigate a link between genetic variations including chromosomal changes, LOH and copy number alterations associated with ethnic variation in NE populaton of India showing familial clustering of esophageal cancer through Affymetrix® Genome-Wide Human SNP array. This would also help in identifying suitable biomarkers for pre-clinical screening, early diagnosis in familial cases and target for biological intervention.

15 tumour tissues and blood samples (germ-line DNA) from the same patients were analyzed using Affymetrix® Genome-Wide Human SNP Array 6.0 chip. Thirteen of 43 genes in amplified regions and 18 of 50 genes in deleted regions were found to be significantly associated with cancer-associated pathways. Of these 13 genes in amplified regions, 6 genes (*NTRK2, TPO, PLA2G5, PAK1, MAPK10, FGF12* and *FGF4)* were found to be significantly associated with MAPK signalling pathway. Of 18 genes found in deleted regions, several genes were found to be most significantly involved in MAPK signalling pathway (*MAPT, CACNA1D, TGFBR2, PPP3CC, CACNB2* and *FGF14*), cytokine-cytokine receptor interaction (*IL22, IL26, CACNA1D,*

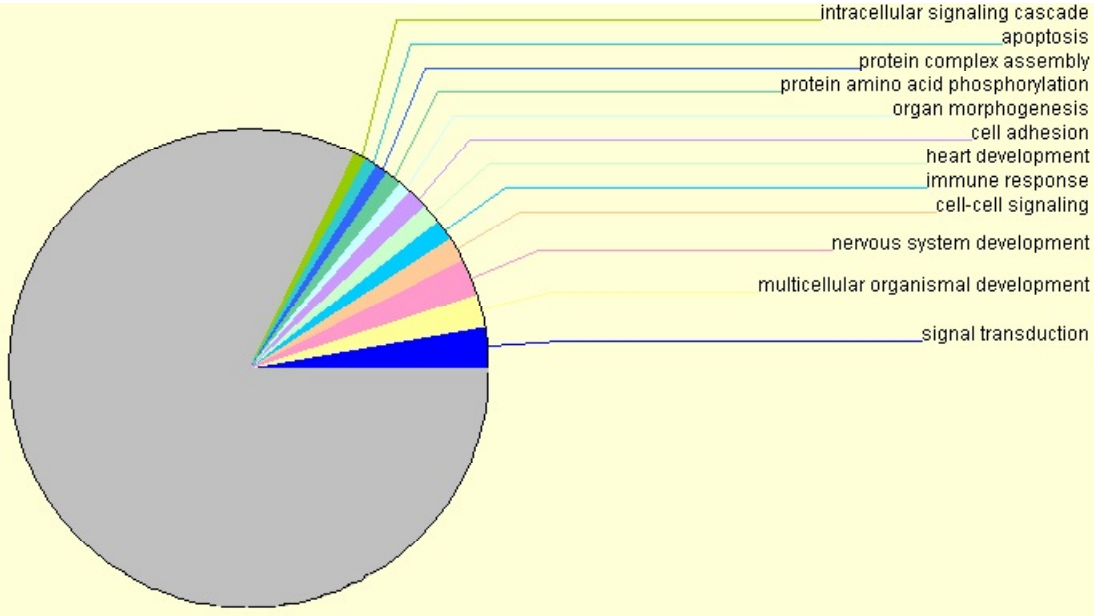


Fig. 1: Candidate genes involvement in biological function.

PPP3CC and *FLT1*) and Wnt signalling pathway (*PPP2CB, WNT7A, PPP3CC* and *NFATC1*). The other predominant pathways found were focal adhesion, ECM-receptor interaction, Wnt signalling pathway, ErbB signalling pathway, cytokine-cytokine receptor interaction, Jak-STAT signalling pathway, VEGF signalling pathway and mTOR signalling pathway. All these pathways are reported to play a potential role in tumorigenesis.

Biological GO analysis showed amplified and deleted regions associated with cell adhesion, cell differentiation, cell signalling apoptosis, cell proliferation, cell migration and cellular morphogenesis, to be significantly associated with esophageal cancer.

Validation of candidate biomarkers by siRNA knockdown of key genes will be done in established esophageal cancer cell lines (KYSE30, KYSE70, KYSE180, KYSE410 and KYSE450). These cell lines are being maintained in a medium containing RPMI 1640 + Ham's F12 (1:1) with 2% Fetal Bovine Serum (FBS) at 37°C with 5% CO2. Doubling time of the cell lines is on an average 30 - 35 hours. At initial stage of functional analysis, we have selected one gene from our data, *i.e.* Fibroblast Growth Factor 12 *(FGF12)* and protocol for functional genomics is being standardized.

6. EPIGENETIC STUDIES IN ESOPHAGEAL CANCER IN HIGH RISK REGION OF NORTH-EAST INDIA

The etiology of esophageal cancer in NE Indian population is different from other populations in India due to wide variations in dietary habits or nutritional factors, tobacco/ betel quid chewing and alcohol habits. The key question however is: Are heritable epigenetic alterations, such as methylation and histones in DNA, responsible for the differential regulation of gene expression and gene silencing for high incidence of various cancers in NE Indians? The objective of the study is to find out the contribution of epigenetic modifications on the development or progression of esophageal cancer in high risk population from NE India.

Total 40 esophageal cancer patients were recruited at BBCI, Guwahati and biopsies were collected from tumour and adjacent normal esophageal tissue. Genomic DNA was extracted and purified from tissue samples using QIAamp DNA mini kits (*Qiagen*). Total RNA was extracted from tumour and normal samples using RNeasy mini kit (*Qiagen*) following the manufacturer's protocol. RNA was re-suspended in nuclease free water at a concentration of 100 - 150 ng/µl with the A_{260}/ A_{280} ratio between 2.0 - 2.2. The Human Tumor Suppressor

Genes EpiTect Methyl Complete PCR arrays (*Qiagen*) were used to profile the methylation status of 94 tumour suppressor gene promoters. The differential promoter methylation profiling of 94 tumour suppressor genes was done in 6 paired normal and tumour tissue samples. Additionally, the differential expression profiling of 84 histone modification enzymes was also done by real time PCR array (*Qiagen*) in 24 normal and 24 tumour tissue samples. Tumour suppressor genes promoter methylation and histone modification enzymes expression data was analyzed by online software freely available at *Qiagen* website.

7. DYNAMIC REGULATION OF LYMPHOCYTE SIGNALING IN ACUTE LEUKEMIA

In our earlier studies, cellular redox level in acute leukemia was found to be high but their role in signalling is not very clear. The aim of the present study is to explore the role of oxidative stress in the pathogenesis of acute leukemia. The goal is to find out dynamically regulated intracellular molecules and if these intracellular molecules can be used for targetted therapy of acute leukemia. For this, measurement of cell cycle analysis, T-cell age-related changes and calcium flux analysis will be done by flow cytometry using suitable antibodies.

Twenty samples from patients with acute leukemia have so far been collected. Of these, 5 were T-ALL, 5 were B-ALL and 10 were with AML. PBMCs were isolated from patients' samples and controls. PBMCs from 5 patients of B-ALL were cultured in 96-well culture plates

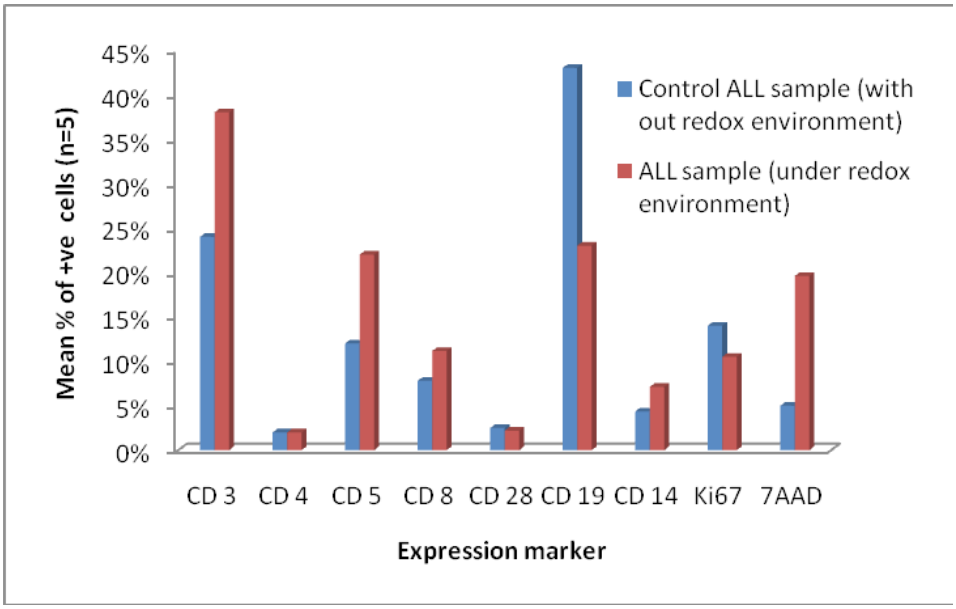


Fig. 1: Expression analyis of proliferation, apoptosis and signalling marker on B-cell acute lymphoblastic leukemia.

in RPMI-1640 for 72 hours and cells were treated with optimized concentration of hydrogen peroxide to generate redox environment. Expression of various cell signalling markers and proliferation marker Ki67 was analyzed. Cellular ROS level was analyzed in T- cells, B-cells and NK cells and it was found that these cells have dynamically changing ROS levels. The dynamic behaviour of cells due to intracellular redox environment was analyzed and significant changes in cell proliferation and regulation of apoptosis were found (Fig. 1).

8. MOLECULAR REGULATION OF MAMMALIAN TARGET OF RAPAMYCIN (MTOR) SIGNALLING IN ACUTE LYMPHOBLASTIC LEUKEMIA

Acute Lymphoblastic Leukemia (ALL) is caused by the uncontrolled clonal proliferation of immature lymphoid cells. mTOR is a serine/ threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis and transcription. The aim of the present study is to identify the regulatory molecules of mTOR signalling in ALL. Cell cycle analysis was done with PBMCs isolated from acute leukemia patient's sample using propidium iodide dye by flow cytometry. 2 x 10^6 cells were cultured per well in RPMI media supplemented with 10% FCS and incubated at 37^0C in a humidified CO_2 incubator. Optimized concentration of the mTOR inhibitor was added to the medium. Cells were harvested every 24 hours for cell cycle analysis to understand the effect of mTOR inhibition on cell cycle. Data was acquired on flow cytometer and ModFit LT (*Macintosh*) was used to fit the data to cell cycle models. The results are represented in Table 1.

Table 1: Table represents cell cycle analysis of PBMCs isolated from B-ALL patients. Mean percentages of total cells in G1/ G0, S and G2/ M phase.

Time of incubation	mTOR inhibiting drug	G1/ G0		S		G2/ M	
		Control	B-ALL	Control	B-ALL	Control	B-ALL
0 hours	Without	98	99	0	0	0	0
	With	98	99	0	0	0	0
24 hours	Without	98	97	0	0	1	1
	with	97	97	2	1	1	1
48 hours	Without	94	96	4	2	1	1
	with	82	72	12	20	6	7
72 hours	Without	92	92	5	6	3	4
	With	81	70	15	21	4	6

INFECTIOUS DISEASES

LEISHMANIASIS

1. TRANSCRIPTOME PROFILING OF MILTEFOSINE RESISTANT VS MILTEFOSINE SENSITIVE LEISHMANIA PARASITE

Comparative transcriptome profiling of miltefosine-resistant and sensitive *L. donovani* using genomic microarray analysis revealed differential expression of ~3.9% of the total *Leishmania* genome in miltefosine-resistant parasite. In comparison to wild type parasite, miltefosine resistant parasite showed an upregulated DNA synthesis, transporter activities and downregulated protein metabolic process as determined by BLAST2GO, AmiGO and KEGG pathway analysis. Current results suggest several probable mechanisms by which the parasite sustains miltefosine pressure including (i) increased ABC 1 mediated drug efflux (ii) reduced protein synthesis and degradation (iii) altered energy utilization *via* increased lipid degradation and (iv) increased antioxidant defence mechanism *via* elevated trypanothione metabolism and reduced oxidative phosphorylation.

2. DRUG SUSCEPTIBILITY OF CLINICAL ISOLATES OF LEISHMANIA DONOVANI TOWARDS PAROMOMYCIN

Paromomycin (PMM) has been recently introduced to treat VL in mono- as well as in combination therapy. At NIP, we evaluated PMM susceptibility in VL and PKDL clinical isolates, including miltefosine pre-treatment isolates and MIL post-treatment isolates including relapses. The PMM IC_{50} ranged from 3.41 ± 0.29 to 10.70 ± 1.12μM with mean IC_{50}= 7.05 ± 2.24μM. Furthermore, the PMM sensitivity was similar ($p > 0.05$) in parasites non-exposed (mean IC_{50} = 7.73 ± 2.25μM) or exposed (mean IC_{50} = 6.79 ± 2.25μM) to MIL. The inherent PMM susceptibility of 8 PKDL isolates ranged from 4.92 ± 0.34 to 8.62 ± 1.82μM. The PMM IC_{50} of PKDL isolates was similar ($p > 0.05$) in parasites non-exposed (n= 5, mean IC_{50} = 6.12 ± 1.40μM) or exposed (n= 3; mean IC_{50} = 6.29 ± 2.02μM) to MIL (Fig. 1). All VL/ PKDL field isolates examined in the study were found susceptible to PMM suggesting its potential efficacy in VL and PKDL therapy.

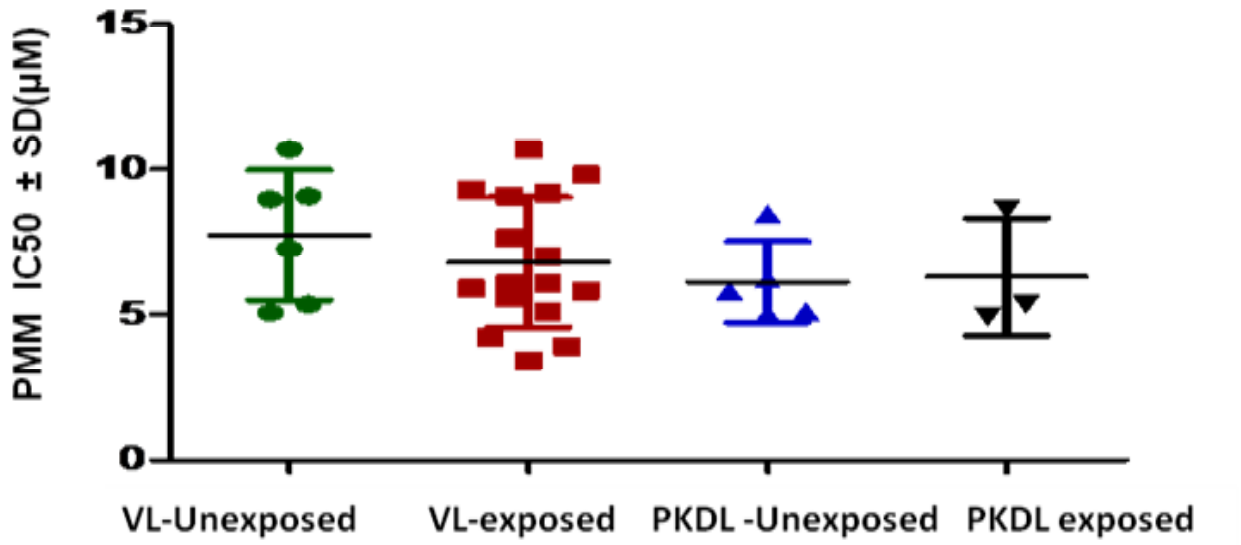


Fig. 1: **In vitro miltefosine susceptibility of VL and PKDL isolates towards paromomycin.** Sensitivity of VL and PKDL isolates at intracellular amastigote stage were determined by infection in murine macrophage cell line J774A.1. Each individual value represents mean IC50 ± SD of the results from two separate assays.

2. STUDIES ON IMMUNOLOGICAL RESPONSES TO RECOMBINANT PSA (PARASITE SURFACE ANTIGEN)- A POTENTIAL VACCINE CANDIDATE

The PSA is an excreted secreted protein belonging to the family of promastigote surface antigens and has been identified as the active constituent of recently developed canine vaccine, CaniLeish. We investigated immune responses to PSA in comparison with Total Soluble *Leishmania* Antigen (TSLA), in order to evaluate its potential as an immunoprophyactic antigen for human leishmaniasis.

Generalized cellular mediated immunity was analyzed in terms of lymphoproliferative responses to Parasite Surface Antigen (PSA) and TSLA with Phytohemagglutinin (PHA) as positive control. VL (n = 12) cases showed hardly any proliferation (SI Mean ± SD, 1.329 ± 0.539), that was comparable to the naïve group (n = 19) (SI Mean ± SD, 1.190 ± 0.28). Healed VL (n = 16) (SI Mean ± SD, 5.99 ± 1.987) and PKDL (n = 18) (SI Mean ± SD, 3.353 ± 3.972) groups showed significantly high stimulation compared to naive group. PSA induced poor lymphoproliferation in VL and PKDL and marginally significant ($p = 0.047$) in HVL group (Fig. 2). In PKDL and healed VL cases, the CMI responses (as judged from the levels of Th-1 cytokines, IFN-γ and TNF-α) was found significantly higher ($p < 0.001$) than healthy control subjects upon TSLA stimulation.

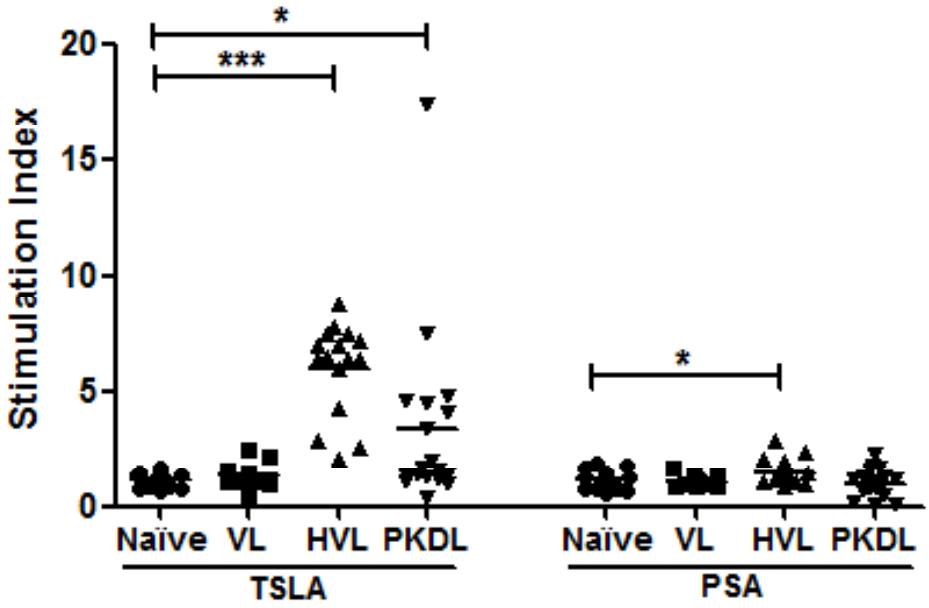


Fig. 2: **Proliferative responses of lymphocytes to TSLA and PSA.** Lymphocytes from patients with PKDL (n=18), VL (n=12), healed VL (n=16) and from healthy group (n=19) were incubated with PSA (10µg/ml), TSLA (10µg/ml) or PHA (10µg/ml) for 5 days and lymphoproliferation was measured by BrdU incorporation for the last 12-14 hrs using Biotrak cell proliferation ELISA system. Data were analyzed with respect to naïve group by the nonparametric Mann Whitney test. *P<0.05* is considered statistically significant. **P< 0.05, **P<0.01, *** P<0.001*

For IL-10 cytokine, the measured values for PKDL, healed VL and VL groups were low and comparable to naïve group. PSA failed to stimulate IFN-γ, TNF-α or IL-10 in any of the study groups. Analysis of *Leishmania*-specific cell mediated cytotoxicity based on the measure of granzyme B, upon stimulation of PBMCs with TSLA and PSA *in vitro* revealed significantly high granzyme B level in response to TSLA compared to PSA among the different study, indicating that PSA failed to show any significant cell mediated cytotoxicity even in *Leishmania* exposed individuals. Besides, the strong correlation between stimulation index and the granzyme B level against TSLA in healed VL individuals provides evidence that *Leishmania*-specific cell mediated cytotoxicity is part of the acquired immune response developed against the parasite.

3. IMMUNE RESPONSES AGAINST CENTRIN KNOCK-OUT LIVE ATTENUATED LEISHMANIA PARASITES IN THE HUMAN CELLS

Currently, no vaccine is available for leishmaniasis and only treatment option available is chemotherapy which is expensive, toxic and associated with high relapse and resistance rates. Deletion of Centrin 1 gene in *L. donovani* (*LdCen1*$^{-/-}$) results in attenuation of mammalian infecting, amastigote form with no effect on growth of promastigotes form of the parasite. Evidence from studies in mouse models indicates that live attenuated *Leishmania* parasite *LdCen1*$^{-/-}$ developed through gene knock-out approach could be an ideal vaccine candidate as it possesses genetically defined, irreversible gene defect. This study aims to evaluate immune responses generated by *LdCen1*$^{-/-}$ *Leishmania* parasite in comparison to the wild type in human PBMCs cells. PBMCs showed predominant Th-1 response with stimulation of IL-2, TNF-α and IFN-γ after infection with wild type as well as with both knock-out parasites in comparison to control uninfected cells. Cytokines that play important role in Th-2 response (IL-4, IL-10) did not show any significant stimulation except IL-6, which showed significant stimulation after infection. Immune response induced by live attenuated parasites *LdCen1*$^{-/-}$ was similar to that of wild type. Th-1 and Th-17 responses stimulated by *LdCen1*$^{-/-}$ could provide protective response leading to host resistance to infection.

4. APPLICATION OF LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY FOR DIAGNOSIS OF VL AND PKDL

More recently, Loop-Mediated Isothermal Amplification (LAMP) was developed as a novel method to amplify DNA with rapidity and high specificity under isothermal conditions. At NIP, we have applied LAMP assay based on kDNA sequence amplification using SYBR Green for clear-cut naked eye detection of *Leishmania donovani* in 200 clinical samples for VL and PKDL diagnosis. All negatives remained orange while positive samples produced a green colour almost immediately (Fig. 3). The assay was positive in 53 of 55 VL blood samples (sensitivity, 96.4%), 15 of 15 VL BMA samples (sensitivity, 100%), 60 of 62 PKDL tissue biopsy samples (sensitivity, 96.48%). The LAMP assay was negative for 67 out of 68 control samples (blood, n=44; tissue biopsy, n=24), giving specificity of 98.5% for *L. donovani* DNA. The assay was *L. donovani* species-specific (responsible for VL in Indian sub-continent) and negative for *L. infantum, L. tropica* and *L. major*.

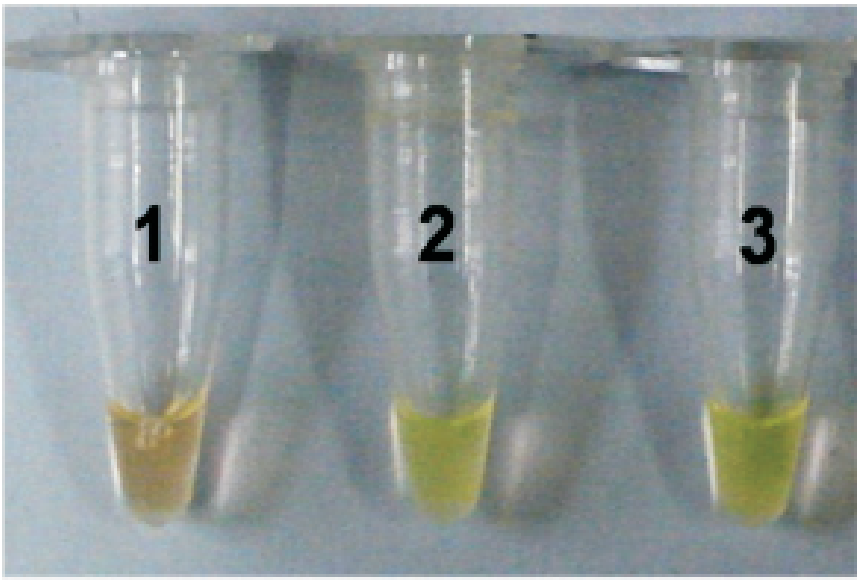


Fig 3: Visual Appearance of LAMP reactions after the addition of 1µl of 1/10 dilution of SYBR Green I. Negatives remained orange (tube 1, no template control) while positive samples produced a green colour almost immediately (tubes 2 and 3 containing 1pg and 1ng of Leishmania donovani DNA respectively).

CHLAMYDIASIS

1. IMMUNOPATHOGENESIS OF REACTIVE ARTHRITIS INDUCED BY CHLAMYDIA TRACHOMATIS

The sequelae of primary genital *Chlamydia trachomatis* infection can be severe; the sequelae might include not only chronic Reactive Arthritis (ReA), but also temporomandibular joint dysfunction. Reports indicate that ~5% of those with genital chlamydial infection develop acute ReA, and about half of these latter may proceed to chronicity. Further, published data indicate that 20–40% of individuals with inflammatory arthritis but no documented prior genital infection with *C. trachomatis*, and who are given a diagnosis of undifferentiated Spondyloarthropathy (uSpA), oligo- or mono-arthritis, are PCR-positive in Synovial Fluid (SF) and/ or tissue for *C. trachomatis* DNA. Research also indicates that the organism is present in the synovial tissue of patients with chronic disease in a persistent, rather than an actively growing, form. This study is aimed to find the prevalence of *C. trachomatis* in arthritic patients, *viz.*: ReA and uSpA (a *forme fruste* of ReA). Another focus of the study has been to understand the mechanism of chlamydial immunopathogenesis in the synovium in these patients.

85 patients (age range = 18 - 45 years) clinically diagnosed with genitourinary- induced

ReA/ uSpA (n=50) and inflammatory/ non-inflammatory controls, *viz.:* rheumatoid arthritis/ osteoarthritis (n=35) were enrolled in the study. PCR assays were performed for the diagnosis of *C. trachomatis* in the SF of ReA/ uSpA (n = 46) and RA/ OA (n = 35) patients. Intra-articular *C. trachomatis* infection was found in 19.5% (9/46) ReA/ uSpA patients by nested PCR (nPCR), while 15.2% (7/46) patients were *Chlamydia*-positive by semi-nested PCR (snPCR). Considering nPCR as gold standard, snPCR was found to be 75% sensitive and 100% specific. Overall, the presence of *C. trachomatis* in the joint fluid of ReA/ uSpA patients was significant ('p' value <0.05) in comparison to RA/ OA (1/35; 2.8%).

Investigation of the Th-1/ Th-2 cytokine pattern in the serum of *C. trachomatis*-induced ReA/ uSpA patients showed significantly high IFN-gamma (48.7 $\pm$ 19.6 pg/ml; p value= 0.005) and IL-4 levels (21 $\pm$ 7.5 pg/ml; p value= 0.02) in *C. trachomatis*-positive ReA/ uSpA patients in comparison to the controls. IFN-gamma was also significantly increased (p value <0.05) in *C. trachomatis*-positive ReA/ uSpA patients (mean $\pm$ S.D. 54.5 $\pm$ 20.9 pg/ ml) in comparison to *Chlamydia*-negative ReA/ uSpA patients (18.6 $\pm$ 12.7 pg/ ml).

Table I: Serum cytokine concentrations in arthritic patients. All values expressed as median (range).

	ReA/ uSpA CT-positive	RA/ OA CT-negative
IFN-gamma (pg/ ml)	45.53 (13.7 – 77)*	21.11 (9 – 77.7)
IL-4 (pg/ ml)	19.54 (11.9 – 33)**	14.70 (8 – 27)

* p= 0.005 versus RA/ OA; ** p= 0.02 versus RA/ OA patients.
Abbreviations: ReA: reactive arthritis; uSpA: undifferentiated spondyloarthropathy; RA: rheumatoid arthritis; OA: osteoarthritis; CT: *Chlamydia trachomatis*

TUBERCULOSIS

1. BIOMARKER FOR TREATMENT END POINT DETERMINANTS FOR TUBERCULOSIS

Tuberculosis (TB), a progressive disease taking one human life every 15 s, globally, is caused by invasion of *Mycobacterium tuberculosis* (M. tb) into macrophage. Once inside the macrophage, the fate of the pathogen is decided by the strength of the immune system of the host. Poor nutritional status is known to increase susceptibility to infectious diseases,

delay in recovery by suppressing immune functions and, therefore, is more common in people with active tuberculosis than in people without tuberculosis. Although, recently there has been considerable progress, little is known about the impact of sub-clinical micronutrient deficiencies on the immune system, susceptibility to various infectious pathogens and clinical manifestations. We are examining the underlying mechanism of functional modulation of macrophages, considered as the first line of host immune defence, by micronutrient.

There has been considerable interest about the host defence in *M. tuberculosis* infection and about the mechanisms and correlates of bacterial clearance leading to clinical recovery from TB. While the WHO's Directly Observed Treatment, Short Course (DOTS) anti-tubercular treatment regime has been giving positive results, the problem of sustained treatment without knowing the actual end point for treatment termination remains a serious issue. The classical microbiological and clinical markers for response to ATT have their limitations. So are problems with other markers which need *in vitro* stimulation with antigens. There is a serious need, therefore, to develop even surrogate markers for TB treatment efficacy early during ATT.

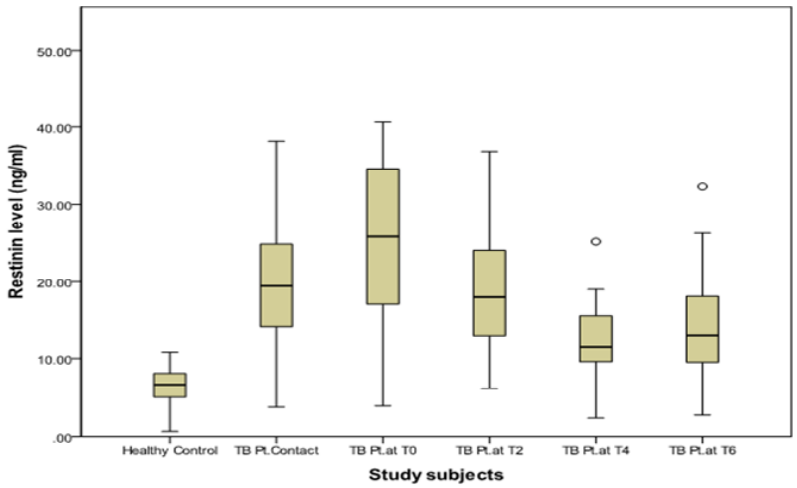


Fig: Resistin levels show significant decline as a function of anti-tuberculosis treatment (ATT) in TB patients. Comparison of the circulating resistin levels in TB Pt at T0 = serum sample taken before the start of treatment; TB Pt at T2 = serum sample taken after 2 months; TB Pt at T4 = serum sample taken after 4 month; TB Pt at T6 = serum sample taken after 6 months after start of treatment; healthy control and TB patients' contacts using the box plot analysis.

Such markers will be extremely useful in: a) designing new therapeutic strategies, b) reducing drug resistance due to non-adherence, c) validating new candidate drugs and vaccines for clinical trial, and d) developing new diagnostics. We investigated and identified resistin, a cysteine-rich secretory protein, positively associated with inflammation as a surrogate marker for TB treatment end point.

ADULT STEM CELL BIOLOGY

1. A NOVEL ARITHMETIC APPROACH FOR FOOL-PROOF PRODUCTION OF GROWTH ARREST IN 3T3 CELLS SUITABLE FOR HUMAN EPIDERMAL CULTURE

Optimal *in vitro* growth of stem cells requires feeder cells growth arrested either by irradiation or Mitomycin C (MMC). The objective of the study is to test a hypothesis that feeder effectiveness depends on calculated exposure availability of MMC and Swiss 3T3 cells are used as feeders aimed at producing human epidermis for application in burns. The growth stimulatory influence of feeders produced by minimal, medial and maximal inhibitory concentrations and the respective dose per cell combinations of MMC were verified by growth patterns of epidermal keratinocytes at various passages and at varying feeder-keratinocyte ratios with γ-irradiated feeders as controls. It was demonstrated that feeders inhibited by the medial MMC concentrations produced significant growth stimulation than the minimal and highly toxic concentrations whose influence was comparable to γ-irradiation. BrdU incorporation studies and colony forming efficiencies revealed similar influence. The medial MMC concentrations further sub-divided into dose per cell yielded significantly varying keratinocyte growth stimulation which was inversely proportional to the feeder extinctions. The present approach identified a cost-effective and optimized growth of epidermal keratinocytes.

2. STUDIES ON EPITHELIAL-MESENCHYMAL INTERACTIONS USING HUMAN EPIDERMAL KERATINOCYTE STEM CELLS AND INNOVATIVELY GROWTH-ARRESTED FIBROBLAST FEEDERS IN 3-D COLLAGEN MODEL

In view of the observed varied extent of growth arrest of 3T3 fibroblasts through manipulation of arithmetically derived doses of Mitomycin C (MMC) resulting in differential stimulation of epidermal keratinocyte proliferation in 2D cultures, experiments are designed to assess qualitative and quantitative differences in the morphogenesis of epidermis using *in vitro* 3-D skin model. The minimal, medial and maximal concentrations of MMC treatments resulted in significantly varied feeder cell extinctions in collagen synonymous to 2-D experiments. The basic technique of constructing whole skin model consisting of 3T3 fibroblasts in Type-I collagen gel as Dermal Equivalent and epithelialization by human epidermal keratinocytes followed by complete stratification of the epidermis at air-liquid interface were accomplished. This model will be used to undertake a future study on the role of differentially attenuated feeders towards healing of experimentally produced wounds in such 3-D models.

ENVIRONMENTAL TOXICOLOGY

1. 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

Due to high density of population in metropolitan cities such as Delhi/ NCR, the risk associated with the human exposure to atmospheric PAHs is the highest. Exposure to PAHs during pregnancy due to place of residence, road traffic, dietary and cooking habits may cause IUGR with delivery of low birth weight babies and increase a woman's risk of giving birth to children with congenital anomalies, such as limb defects, nervous system, musculoskeletal or cardiovascular defects, oral clefts, etc. Therefore, this study has been designed to examine the association between IUGR and PAH exposure in expectant women.

A detailed performa including information about the patient, residence, obstetrics, clinical and gynaecologic history, dietary and smoking habits and possibilities of exposure to pollution has been prepared. Samples of urine, maternal blood and placenta have been collected from pregnant women- those diagnosed as IUGR and admitted in Obstetrics and Gynaecological Department of Safdarjung hospital, New Delhi. Sample collection, storage conditions and extraction procedures have been standardized. According to EPA priority list, the following PAHs are considered as risk group: Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b] fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo-[a,h]anthracene and benzo[g,h,i]perylene. These compounds have been procured and standardization is under process for analytical technique like HPLC.

MAJOR ACHIEVEMENTS

1. The thrust areas of research at National Institute of Pathology are TUMOR BIOLOGY, LEISHMANIASIS, CHLAMYDIASIS, MYCOBACTERIAL DISEASES, ENVIRONMENTAL TOXICOLOGY and ADULT STEM CELL BIOLOGY.

2. Study on molecular signature associated with breast carcinogenesis in early breast cancer patients identified differential expression of 578 genes specific and differential methylation of 977 genes in early onset cases Validation of expression of several differentially expressed genes such as ALDH1B1, ALDH2, CBX2, CCNJ, FOX (3 genes), JMJD2A, MATN, METTL11A, PRMT, SOX genes (3), TMEM41A, WNT5A, WNT3, , HMGN1, JMJD4, MCMs (2), SMARCAs (2 genes) is being done by real time PCR in breast cancer cases of both early and late onset groups.

3. ALDH-positive breast cancer stem cells (BCSC) sorted from MCF7, SUM159 and HBL100 cell lines showed self-renewal potential by showing mammosphere formation on repeated passages. Treatment of ALDH-positive mammosphere(BCSC) with plant alkaloid elipticine showed significant reduction in the formation of mammospheres while treatment with cytotoxic drug paclitaxel enhanced mammospheres formation. Interestingly treatment of mammospheres(BCSC) with combination of elipticine and paclitaxel also showed significant reduction in mammospheres formation.

4. Non-invasive urothelial bladder cancer was found associated with MYD88-dependent activation of NFκB pathway and a Th-2 dominant status while Th-1 polarization was observed in muscle-invasive urinary bladder carcinomas along with upregulation of TLR7/8 and NFκB activation. Immunohistochemistry was performed on Tissue microarray for TBX21, GATA3, PRKCE and SRC. The expression of TBX21 was significantly high in invasive bladder cancers (p value < 0.001) and PRKCE expression was significantly high in non-invasive cancers ($p = 0.044$). Both GATA3 and SRC were expressed in tumour cells but there was no statistically significant difference between invasive and non-invasive bladder cancers.

5. Study of 33 microsatellite markers from the HLA region by fragment length analysis in 120 cases of Nasopharyngeal carcinoma and 100 controls from North east region of India showed significant difference in frequency of two microsatellite markers (HL003

and D6S2704) among patients and contros.($p \leq 0.05$).Detection of EBV in tissue/ blood samples by PCR and *in situ* hybridization showed statistically significant differences in the presence of EBV sequences between the NPC patient group and the control group.

6. SNP analysis in esophageal cancer in NE region showed genes in amplified regions (*NTRK2, TPO, PLA2G5, PAK1, MAPK10, FGF12* and *FGF4)* were found to be significantly associated with MAPK signalling pathway. Genes found in deleted regions, several genes were found to be most significantly involved in MAPK signalling pathway (*MAPT, CACNA1D, TGFBR2, PPP3CC, CACNB2* and *FGF14*), cytokine-cytokine receptor interaction (*IL22, IL26, CACNA1D, PPP3CC* and *FLT1*) and Wnt signalling pathway (*PPP2CB, WNT7A, PPP3CC* and *NFATC1*). The other predominant pathways found were focal adhesion, ECM-receptor interaction, Wnt signalling pathway, ErbB signalling pathway, cytokine-cytokine receptor interaction, Jak-STAT signalling pathway, VEGF signalling pathway and mTOR signalling pathway. These candidate genes have been selected for further functional genomics studies.

7. In patients with CLL, Reactive Oxygen Species (ROS) modulates regulation of B-cell activation, proliferation and survival of the cells for prolonged period without apoptosis and the cross-talk between B- and T-cells that may lead to clonal proliferation of B-cells and dysfunctional T-cells. Cellular ROS level was analyzed in T- cells, B-cells and NK cells and it was found that these cells have dynamically changing ROS levels. The dynamic behaviour of cells due to intracellular redox environment was analyzed and significant changes in cell proliferation and regulation of apoptosis were found

8. First comprehensive insight into the underlying mechanism of miltefosine resistance in *L. donovani.*showed showed an upregulated DNA synthesis, transporter activities and downregulated protein metabolic process as determined by BLAST2GO, AmiGO and KEGG pathway analysis in miltefosine resistant parasite compared to wild type parasite. Current results suggest several probable mechanisms by which the parasite sustains miltefosine pressure including (i) increased ABC 1 mediated drug efflux (ii) reduced protein synthesis and degradation (iii) altered energy utilization *via* increased lipid degradation and (iv) increased antioxidant defence mechanism *via* elevated trypanothione metabolism and reduced oxidative phosphorylation.

9. Evaluation of paromomycin(PMM) susceptibility in VL and PKDL clinical isolates, including miltefosine pre-treatment isolates and MIL post-treatment isolates including relapses.

showed all VL/ PKDL field isolates susceptible to PMM suggesting its potential efficacy in VL and PKDL therapy.

10. Studies on immunological responses to recombinant PSA (Parasite Surface Antigen) revealed that PSA failed to generate significant cellular and humoral responses in healed VL and PKDL. PSA induced poor lymphoproliferation in VL and PKDL and failed to stimulate IFN-γ, TNF-α or IL-10 in any of the study groups. Analysis of *Leishmania*-specific cell mediated cytotoxicity based on the measure of granzyme B, upon stimulation of PBMCs with TSLA and PSA *in vitro* revealed significantly high granzyme B level in response to TSLA compared to PSA among the different study, indicating that PSA failed to show any significant cell mediated cytotoxicity even in *Leishmania* exposed individuals.

11. Centrin knock-out live attenuated *Leishmania* parasite in human cells suggested protective immunogenicity. Evaluation of immune responses generated by *LdCen1*$^{-/-}$ *Leishmania* parasite e in human PBMCs cells showed predominant Th-1 response with stimulation of IL-2, TNF-α and IFN-γ after infection with wild type as well as with both knock-out parasites in comparison to control uninfected cells. Cytokines that play important role in Th-2 response (IL-4, IL-10) did not show any significant stimulation except IL-6, which showed significant stimulation after infection. Immune response induced by live attenuated parasites *LdCen1*$^{-/-}$ was similar to that of wild type. Th-1 and Th-17 responses stimulated by *LdCen1*$^{-/-}$ could provide protective response leading to host resistance to infection.

12. Development of Loop-mediated Isothermal Amplification (LAMP) assay based on kDNA sequence of *Leishmania donovani* for diagnosis of VL and PKDL.by naked eye detection. The assay has shown sensitivity of 96.4% in VL, 100% in BMA and 96.48% in PKDL samples. The assay was *L. donovani* species-specific (responsible for VL in Indian sub-continent) and negative for *L. infantum, L. tropica* and *L. major*.

13. The basic technique of constructing whole skin model consisting of 3T3 fibroblasts in Type-I collagen gel as Dermal Equivalent and epithelialization by human epidermal keratinocytes followed by complete stratification of the epidermis at air-liquid interface were accomplished.

EXTRAMURAL PROJECTS 2012-13

NEW PROJECTS

1. **Study on micro-RNA signatures associated with Breast Cancer Stem like Cells (CSCs) and their role in drug response.**
   Dr. Sunita Saxena, Dr. BSA Raju- ICMR (2013-15)

2. **Understanding the role of micronutrients in *Mycobacterium tuberculosis* infection using guinea pig as a model.**
   Dr. Nasreen Ehtesham- ICMR (2013-17)

3. **Second phase of biomedical informatics centers of ICMR.**
   Dr. Sujala Kapur, Dr. AK Jain- ICMR (2013-17)

4. **Understanding the role of androgen receptor signalling in breast cancer.**
   Dr. Sunita Saxena, Dr. Jatin Mehta- ICMR (2013-15)

5. **Molecular mechanism of drug resistance in Acute Myeloid Leukemia (AML): Role of ATP-Binding Cassette (ABC) transporters.**
   Dr. Sujala Kapur, Dr. Pradeep Chauhan- ICMR (2013-15)

ONGOING PROJECTS

6. **Characterization of host immune factors associated with progression of superficial TCC of bladder by microarray analysis.**
   Dr. Sunita Saxena, Dr. Usha Agrawal- ICMR (2009-12)

7. **Epigenetic studies in esophageal cancer in high risk region of north-east India.**
   Dr. Sunita Saxena, Dr. Sujala Kapur- Twining Program of DBT (2011-14)

8. **Genome wide analysis of genetic alterations in patients with esophageal cancer from north-east India using single nucleotide polymorphism array.**
   Dr. Sujala Kapur, Dr. Sunita Saxena- ICMR (2011-14)

9. **Immunogenetic profile of nasopharyngeal cancer.**
   Dr. Sujala Kapur, Dr. Sunita Saxena- DBT (2010-13)

10. **Study on the effect of siRNA mediated androgen receptor gene silencing on androgen signaling pathway in breast carcinoma.**
    Dr. Anurupa Chakraborty- Post-Doctoral Fellowship, ICMR (2010-12)

11. **Study on effects of anti-cancer drugs on breast cancer stem cells.**
    Mrs. Shanti Lata- ICMR-SRF (2011-13)

12. **Virtual centre of excellence on multidisciplinary approaches aimed at interventions against *Mycobacterium tuberculosis*.**
    Dr. Nasreen Ehtesham DBT (2008-13)

13. **Understanding the functional role of PHLPP1 in IFNγ-mediated innate immune responses of macrophages.**
    Dr. Nasreen Ehtesham DBT (2011-14)

14. **Detection of *Chlamydia trachomatis* in synovial samples from patients with undifferentiated spondyloarthropathy/ reactive arthritis.**
    Dr. Sangita Rastogi- ICMR-SRF (2010-14)

15. **Development of new live attenuated vaccine candidates for kala-azar.**
    Dr. Poonam Salotra- DBT (2011-14)

16. **Protective immunogenicity of centrin KO live attenuated *Leishmania* parasite in the animal models and in the human cells.**
    Dr. Poonam Salotra- DBT (2011-14)

17. **New tools for monitoring drug resistance and treatment response in visceral leishmaniasis in the Indian subcontinent.**
    Dr. Poonam Salotra- European Commission (2009-13)

18. **Pre-clinical studies of a PSA-based human vaccine candidate targeting visceral, cutaneous and muco-cutaneous leishmaniasis and development of the associated procedures for further clinical trials.**
    Dr. Poonam Salotra- European Commission (2009-12)

19. **Transcriptome profiling for identification and characterization of miltefosine resistance associated genes of *Leishmania donovani*.**
Dr. Poonam Salotra- ICMR (2009-12)

20. **A novel arithmetic approach for fool-proof production of growth arrest in 3T3 cells suitable for human epidermal culture.**
Dr. LK Yerneni- ICMR (2010-13)

21. **Studies on epithelial-mesenchymal interactions using human epidermal keratinocyte stem cells and innovatively growth-arrested fibroblast feeders in 3-D collagen model.**
Dr. LK Yerneni- ICMR-SRF (2010-13)

22. **Clinicopathological determinants in leprosy type 1 reactions.**
Dr.Avninder Pal Singh- ICMR Leprosy Task Force project (2010-12)

COMPLETED PROJECTS

1. **Study on gene expression and hypermethylation profiles in early onset breast cancer.**
Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. BSA Raju- DBT (2008-12)

2. **Transcriptome and proteome analyses of ALR2 and its involvement in the pathogenesis of diabetic retinopathy.**
Dr. Nasreen Ehtesham- DST (2009-12)

3. **Anti-inflammatory potential of n-3 polyunsaturated fatty acids in experimental ulcerative colitis: Biochemical and molecular mechanisms.**
Dr. Nasreen Ehtesham- DBT (2009-12)

DNB/ Ph.D. PROGRAMME

DNB

The Institute has been accredited for training in DNB Pathology course for the last 16 years.

PH.D.

The Institute has been recognized by the following universities:

- BITS, Pilani
- Indraprastha University, New Delhi.
- Jamia Hamdard, New Delhi.
- Symbiosis International University, Pune.

WHO TRAINING PROGRAM

- Basic Level Training Programme for Laboratory Technicians
- Advanced Level Training for Laboratory Technicians
- Training for Pathologists

	2012-13
Research Associate	6
Senior Research Fellow	20
Junior Research Fellow	3
CSIR – JRF & SRF	5
UGC - JRF & SRF	3
Total	**37**

PUBLICATIONS

1. **Bansal A**, Soni A, Rao P, **Singh LC,** Mishra A, Mohanty NK, **Saxena S**. Implication of DNA repair genes in prostate carcinogenesis in Indian men. ***Indian J Med Res,*** 136: 622-632 (2012).

2. **Bansal A,** Bhatnagar A, **Saxena S.** Metastasizing granular cell ameloblastoma. ***J Oral Maxillofac Pathol,*** 16: 122-4 (2012).

3. Sony A, **Bansal A,** Mishra AK, Batra J, **Singh LC,** Chakraborty A, Yadav DS, Mohanty NK, Saxena S. Association of androgen receptor, prostate specific antigen and CYP19 gene polymorphisms to prostate carcinoma and benign prostatic hyperplasia in north Indian population. ***Genetic Testing and Molecular Biomarker***, 16(8): 835-840 (2012).

4. Chauhan PS, Ihsan R, Mishra AK, Yadav DS, Saluja S, Mittal V, **Saxena S, Kapur S.** High order interactions of xenobiotic metabolizing genes and P53 Codon 72 polymorphisms in acute leukemia. ***Environmental and Molecular Mutagenesis,*** 53(8): 619-30 (2012).

5. Kaushal M, Mishra AK, Sharma J, Zomawia E, Kataki A, **Kapur S, Saxena S.** Genomic alterations in breast cancer patients in betel quid and non-betel quid chewers. ***PLoS One,*** 7(8): e43789 (2012).

6. Mishra AK, **Agrawal U,** Negi S, **Bansal A,** Mohil R, Chintamani C, Bhatnagar A, Bhatnagar D, **Saxena S.** Expression of androgen receptor in breast cancer and its correlation with other steroid receptors and growth factors. ***Indian J Med Res,*** 135(6): 843-52 (2012).

7. Chauhan PS, Bhushan B, **Singh LC,** Kumar AK, Saluja S, Mittal V, Gupta DK, **Kapur S.** Expression of genes related to multiple drug resistance and apoptosis in acute leukemia: Response to induction chemotherapy. ***Exp Mol Pathol,*** 92(1): 44-9 (2012).

8. **Singh LC,** Chakraborty A, **Kapur S, Saxena S.** Study on predictive role of AR and EGFR family genes with response to neo-adjuvant chemotherpy in locally advanced breast cancer in Indian women. ***Med Oncol,*** 29(2): 539-46 (2012).

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10. Tomar R, Mishra AK, Mohanty NK, **Jain AK.** Altered expression of succinic dehydrogenase in asthenozoospermia infertile male. ***Am J Reprod Immunol,*** 68: 486-90 (2012).

11. Kohli S, Singh Y, Sharma K, Mittal A, **Ehtesham NZ,** Hasnain SE. Comparative genomic and proteomic analyses of PE/ PPE multigene family of *Mycobacterium tuberculosis* H37Rv and H37Ra reveal novel and interesting differences with implications in virulence. ***Nucleic Acids Res,*** 40: 7113-7122 (2012).

12. Tyagi A, Kumar U, Reddy S, Santosh VS, Mohammed SB, **Ehtesham NZ,** Ibrahim A. Attenuation of colonic inflammation by partial replacement of dietary linoleic acid with α-linolenic acid in a rat model of inflammatory bowel disease. ***Br J Nut,*** 108: 1612-1622 (2012).

13. Sachdeva R, Bhardwaj N, Huhtaniemi I, **Agrawal U,** Jain SK, *et al.* Transgenesis-mediated reproductive dysfunction and tumorigenesis: Effects of immunological neutralization. ***PLoS One,*** 7(11): e51125. doi:10.1371/journal.pone.0051125 (2012).

14. Jairajpuri ZS, **Agrawal U.** Tumor associated macrophages- Friends turned foe? A case report and review of literature. ***Bangladesh Journal of Medical Science,*** 11(2): 139-142 (2012).

15. Agrawal BK, Deswal V, **Agrawal U.** "Aluminium phosphide" toxicity is not due to "aluminium". ***Am J Emerg Med,*** 30(7): 1306. doi: 10.1016/j.ajem.2012.04.026 (2012).

16. Kumar P, Bhakuni DS, Mullick G, Kartik S, **Rastogi S.** Diagnosis of *Chlamydia trachomatis* in joint fluid of chronic reactive arthritis and undifferentiated spondyloarthropathy patients. ***Indian Journal of Rheumatology,*** 7: S1-S2 (2012).

17. Kumar P, Khanna G, Batra S, Sharma VK, **Rastogi S.** A pilot study for detection of intra-articular chromosomal and extra-chromosomal genes of *Chlamydia trachomatis* among genitourinary reactive arthritis patients in India. ***Int Res J Medical Sci,*** 1: 16-20 (2013).

18. Gannavaram S, Connelly PS, Daniels MP, Duncan R, **Salotra P,** Nakhasi HL. Deletion of mitochondrial associated ubiquitin fold modifier protein Ufm1 in *Leishmania donovani* results in loss of β-oxidation of fatty acids and blocks cell division in the amastigote stage. ***Mol Microbiol,*** 86(1): 187-98 (2012).

19. Subba Raju BV, Gurumurthy S, Kuhls K, Bhandari V, Schnonian G, **Salotra P.** Genetic typing reveals monomorphism between antimony sensitive and resistant *Leishmania donovani* isolates from visceral leishmaniasis or post-kala-azar dermal leishmaniasis cases in India. ***Parasitol Res,*** 111(4): 1559-68 (2012).

20. Katara GK, Ansari NA, Singh A, Ramesh V, **Salotra P.** Evidence for involvement of Th-17 type responses in Post-Kala-Aazar Dermal Leishmaniasis (PKDL). ***PLoS Negl Trop Dis,*** 6(6): e1703 (2012).

21. **Salotra P,** Carter K, Sundar S, Rijal S, Dujardin JC, Cos P, Maes L. Experimental induction of paromomycin resistance in antimony-resistant strains of *Leishmania donovani*: Outcome dependent on *in vitro* selection protocol. ***PLoS Negl Trop Dis,*** 6(5): e1664 (2012).

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23. Hendrickx S, Inocêncio da Luz RA, Bhandari V, Kuypers K, Shaw CD, Lonchamp J, **Salotra P,** Carter K, Sundar S, Rijal S, Dujardin JC, Cos P, Maes L. Experimental induction of paromomycin resistance in antimony-resistant strains of *L. donovani*: Outcome dependent on in vitro selection protocol. ***PLoS Negl Trop Dis,*** 6(5): e1664 (2012).

24. Kumar D, Singh R, Bhandari V, Kulshrestha A, Negi NS, **Salotra P.** Biomarkers of antimony resistance: Need for expression analysis of multiple genes to distinguish resistance phenotype in clinical isolates of *Leishmania donovani*. ***Parasitol Res,*** 111(1): 223-30 (2012).

25. Srividya G, Kulshrestha A, Singh R, **Salotra P.** Diagnosis of visceral leishmaniasis: Developments over the last decade. ***Parasitol Res,*** 110(3): 1065-78 (2012).

26. Selvapandiyan A, Dey R, Gannavaram S, Lakhal-Naouar I, Duncan R, **Salotra P,** Nakhasi HL. Immunity to visceral leishmaniasis using genetically defined live-attenuated parasites. ***J Trop Med,*** 2012: 631460 (2012).

27. **Singh A,** Sawhney M, Das S. Granular cell tumor of skin diagnosed on fine needle aspiration cytology. ***Ind J Dermatol,*** 57: 33-331 (2012).

28. Sipayya V, Sharma I, Sharma KC, **Singh A.** Immunohistochemical expression of IDH1 in gliomas: A tissue microarray based approach. ***J Cancer Ther***, 8: 71-74 (2012).

29. Sharma DK, Manral A, Saini V, **Singh A,** Srinivasan BP, Tiwari M. Novel diallyldisulfide analogs ameliorate cardiovascular remodeling in rats with L-NAME-induced hypertension. ***Eur J Pharmacol***, 691:198-208 (2012).

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31. Bihaqi SW, **Singh AP,** Tiwari M. Supplementation of *Convolvulus pluricaulis* attenuates scopolamine-induced increase in tau and amyloid precursor protein (AβPP) expression in rat brain. ***Ind J Pharmacol,*** 44(5): 593-98 (2012).

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33. Puri P, RameshV, **Singh A,** Muralidhar S, Capoor MR. Facial eruption in a human immunodeficiency virus (HIV) seropositive patient: *Penicillium marneiffei* as a manifestation of immune restoration disease. ***Int J Dermatol,*** 51: 777-779 (2012).

34. **Singh A,** Mishra AK, Ylaya K, Hewitt SM, Sharma KC, **Saxena S.** Wilms tumor-1, claudin-1 and ezrin are useful immunohistochemical markers that help to distinguish schwannoma from fibroblastic meningioma. ***Pathol Oncol Research,*** 18: 383-389 (2012).

35. Kathuria S, Ramesh V, **Singh A.** Pentazocine induced ulceration of the buttocks. ***Ind J Dermatol Venereol Leprol,*** 78: 521-3 (2012).

36. Mallya V, **Singh A,** Sharma KC. Clear cell meningioma of the cauda equina in an adult. ***Ind J Pathol Microbiol,*** 55: 262-4 (2012).

37. **Gautam P,** Nair SC, Gupta MK, Sharma R, Polisetty RV, Uppin MS, Sundaram C, Puligopu AK, Ankathi P, Purohit AK, Chandak GR, Harsha HC, Sirdeshmukh R. Proteins with altered levels in plasma from glioblastoma patients as revealed by iTRAQ-based quantitative proteomic analysis. ***PLoS One,*** 7(9): e46153 (2012).

ACCEPTED

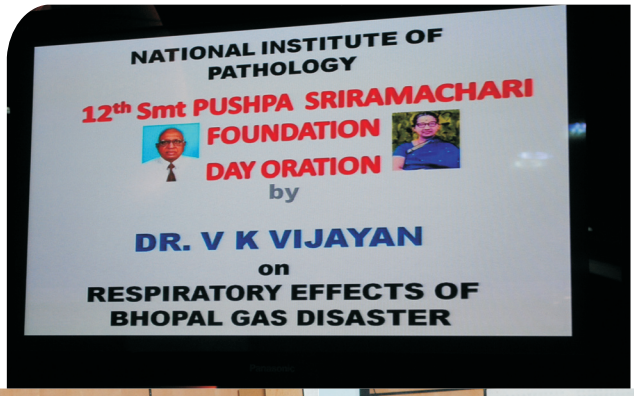
1. Ihsan R, Chauhan PS, Mishra AK, **Singh LC,** Sharma JD, Zomawia E, Verma Y, **Kapur S, Saxena S.** Investigation on copy number polymorphism of GSTM1 and GSTT11 in susceptibility to lung cancer in a high-risk population from north-east India. ***Ind J Med Res*** (2013).

WORKSHOPS/ SEMINARS ORGANIZED AT NATIONAL INSTITUTE OF PATHOLOGY

1. Organized **Interactive Radio Counseling** session on "*How to prepare for the DNB Examinations*" on 12th April 2012 broadcast on Radio Gyanvani at 5:00 PM.

2. **Quarterly IAPM Delhi Chapter Meet** was organized by NIP-SJ hospital on 27th April 2012.

3. **12th Smt. Pushpa Sriramachari Foundation Day Oration** titled, "*Respiratory Effects of Bhopal Gas Disaster"* was delivered by **Dr. VK Vijayan**, Advisor to DG, ICMR for BMHRC



and NIREH, Bhopal and Former Director, VPCI, University of Delhi at NIP on 15th May 2012. Chief Guest: Dr. VM Katoch, Secretary, Department of Health Research & Director-General (ICMR).

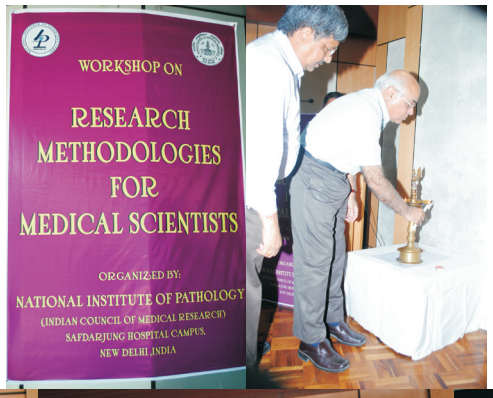
4. On the eve of **15th August 2012** and **26th January 2013**, flag hoisting was done at NIP (ICMR).

5. Organized "**Workshop on Scholarly Publishing for Scientists and Medical Professionals**" on 21st September 2012 in collaboration with ICMR.

6. **Guest lecture** entitled "*Fighting cancer– A non-toxic approach*" was delivered by **Dr. Robert W Gorter,** M.D. Ph.D., Founder Director of Medical Centre, Germany, at NIP on 24th September 2012.

7. **ICMR and NIP Training Programme on "Hybridoma" & technology given to Accurex, Mumbai** at Tissue Culture Laboratory, NIP from 24th September-5th October 2012.

8. Workshop on **"Research Methodologies for Medical Scientists"** was conducted from 9th-12th October 2012 at NIP.



9. Dr. Sunita Saxena (NIP) and Prof. Raisuddin (Jamia Hamdard) signed a **Memorandum of Understanding between NIP and Jamia Hamdard University, New Delhi** for initiating Ph.D. on 19th October 2012.

10. Organized **Scientific Advisory Committee meeting** at NIP on 16th November 2012.

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