ANNUAL REPORT

2015-16

NATIONAL INSTITUTE OF PATHOLOGY (ICMR)

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NATIONAL INSTITUTE OF PATHOLOGY (ICMR)

Executive Summary

It is my privilege to present Annual Report of National Institute of Pathology, providing the panoramic view of progress, achievements and other activities undertaken during the year *2015-2016.* During the year under report the scientists have continued their pursuit for cutting edge research in the thrust areas of research mainly tumor biology, infectious diseases including *leishmaniasis, tuberculosis* and *chlamydiasis*, stem cell biology and environmental toxicology. The scientists conducted 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**.

Under translational research at NIP we have developed monoclonal antibody to *C.trachomatis,* the commercial kit is under third party validation. Loop mediated Isothermal Amlification (LAMP) assay has been developed for detection of *L. donovani* in clinical samples; efforts are undergoing to develop Live attenuated Leishmania Vaccine. A cost-effective & improved technology has been established to grow cultured epithelial autografts (CEA) for burns patients which is ready for clinical trials.

Studies in Tumor biology are focused on breast, genitor-urinary and brain cancers. In Breast cancer study of micro RNA signature associated with Breast Cancer Stem Cells showed differential expression of target genes for 88 microRNAs in cancer stem cells compared to bulk cancer cells. Evaluation of contribution of SOX family genes showed possible involvement of *SOX1*, and *SOX3* genes in formation of breast cancer stem cells. Whole genome sequencing to identify sequence variations and chromosomal rearrangements of deregulated genes in early onset breast cancers showed 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. Analysis of variants showed involvement of cAMP, axon guidance, ECM receptor signaling TNF signaling pathways in early onset cancers and regulation of endocytosis,

regulation of keratins, Rap1 signaling pathways in late onset tumors. Beside more than 30 gene fusions were also identified in three chromosomes, 1, 7 and 17.

The array CGH analysis for genetic alterations in prostate cancer of varying histological grades and aggressiveness is undergoing. The preliminary study showed marked copy number variations in 75% of primary prostate cancer tissue with losses mainly on chromosomes 8 (p23.3), 10 (q11.22), 6 on both p and q arms and chromosome 15 (q11.2) and gains in chromosome 16 (p11.2) in 50% of the cases . Large chromosomal loss was found on chromosome 13 (q11-q34). Array CGH results indicated that losses of several chromosomal regions were common genetic changes in primary tumors, suggesting that inactivation of putative tumor suppressor genes in these chromosomal sites is likely to initiate development of prostate cancer.

Study of Differential Protein Profile of recurrent Urothelial Cancer had been under taken to identify predictive biomarker for recurrence. A total of 1895 deregulated proteins (15381 peptides) were identified in tumor tissue including 1137down regulated and 758 upregulated by subjecting tumor and normal tissue to liquid chromatography and mass spectrometery (MS/MS). Further data analysis showed 64 proteins present in all tumour samples, while 100 proteins were found unique to low grade and 298 proteins unique to high grade tumors. 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 oxidoreductase class.

In Glioblastoma multiforme (GBM) study has been undertaken to identify important chemokine axis associated with GBM growth and development which could be used for therapeutic interventions. The chemokine genes *CXCR4*, *CCRL2*, *CCR5*, *PF4V1*, *CXCL6*, and *CXCL8* (*IL8)* which are upregulated in GBM but not astrocytoma were sorted. CXCL8 appeared significant biomarker for GBM compared to DA ($p< 0.001$), however, no significant difference was observed between GBM and DA for its receptors CXCR1 and CXCR2. Validation of results in cell lines by targeting cxcl8 with antibodies and drugs *in- vitro is undergoing*. Study on expression of gonadotropin releasing hormone receptor (GnRH) in glioblastoma cell line-derived exosomes had been under taken to explore

their potential as circulatory marker. GnRH receptor was observed to be expressed both at the gene and protein level in GBM cell line, LN229, and at protein level in LN229 cell line-derived exosomes. Significant enrichment of GnRH receptor protein was found 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.

One of the important facets in deciphering pathogenesis of tuberculosis is to understand the role of molecular three-dimensional structure of its proteins and their functions. In silico analysis of disordered proteins PE/PPE, Mce, MmPL and secretome of M.tb along with prediction of protein binding sites and ELM search had been carried out and validated with one of the member of PE/PPE. The RipA secretory protein has been shown to possess p60 domain that is capable of hydrolyzing dipeptide, D-glutamyl-meso-diaminopimelic acid. 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. Secreted RipA protein interacts with other proteins and gets cleaved to start its peptidoglycan hydrolase activity. The properties of RipA protein such as virulence, invasion, secretion and cell-wall association makes it an ideal candidate to evaluate the potential efficacy as a possible vaccine candidate. M.tb has also 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 secretory proteins, cyclophilins, PpiA and PpiB which interacts with several host proteins such as those involved in iron regulation, immune defence 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. 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 $CoCl_{2.}$ *M.tb* Ppiases also play role in modulating host immune responses and enhance persistence of the pathogen within the host by subverting host cell generated stresses.

Analysis of clinical efficacy of two different dosage regimens of miltefosine (Regimen I- 50mg twice daily for 90 days and Regimen II- 50 mg thrice for 60 days) in treatment of post kala azar dermal leishmaniasis (PKDL) in India showed approximately 4% patients relapsed by the end of 12 months follow-up while a total of 15% 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. Increasing incidence of relapse in VL cases treated with miltefosine raised the concern for its immediate surveillance in the field to safeguard efficacy. Study of the parasitic factors involved in miltefosine unresponsiveness in natural population of *Leishmania donovani* showed significantly lower accumulation of miltefosine in LdRelapse (relapsed VL & PKDL) and LdM30 (experimental MIL resistant)parasites compared to LdPre-TX (pre treatment) parasites ($p<0.05$). Transcriptome profiling had been done to understand the parasitic factors and pathways responsible for miltefosine unresponsiveness in VL and PKDL. Several genes involved in antioxidant defense mechanism, metabolic process, transporters, cell component and cell motility were preferentially expressed in LdM30 and LdRelapse parasites than wild type *L. donovani* parasites. 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 microarray analysis to study mechanism of resistance towards paromomycin in *Leishmania donovani* showed 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. Analysis of chromosome map identified that higher number of up regulated genes located on chromosome 6, 12, 32, 35 and 36. Maximum numbers of down regulated genes were found located on chromosome 23 in PMM-R isolates. Study has also been initiated for identification of genes associated with artemisinin resistance in *Leishmania donovani.* Natural in vitro sensitivity of different

field isolates of *L. donovani* towards artesunate was determined. Artesunate resistance *Leishmania* parasites were generated in laboratory to investigate the mechanism of drug resistance. Study on characterization of amastigote specific gene A1 b had been completed. A1 gene was found localized near kinetoplast of the parasite. A1 is a *L. donovani* specific gene and $A^{-/-}$ knock out could not be generated indicating the essential nature of the gene for the parasite. *Ld*$A1^{+/-}$ also showed different phenotype, these cells were more rounded instead of regular elongated, however, infectivity of the parasite was unaltered.

In *Chlamydia trachomatis* induced Reactive Arthritis (ReA) it has been noticed that Th1 and Th2 cytokines or blunting of initial cytokine response might be important in the disease manifestation. 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.

A new cell culture process for growing cultured epidermis for application in burns had earlier been standardized. The technique involved culture of human epidermal keratinocytes in the presence of a specific sub-set of SWISS 3T3 feeders cells which were growth arrested with low concentration of Mitomycin C using an innovative dose derivation and a Prototype has been prepared. 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 have been completed. As a pilot trial, a small piece of autograft skin from a 26 year old male was processed in the lab 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. Coordinated efforts are being made to simultaneously obtain grants and cGMP construction to facilitate such trial.

The Biomedical informatics centre (BIC), NIP has developed database of genes associated

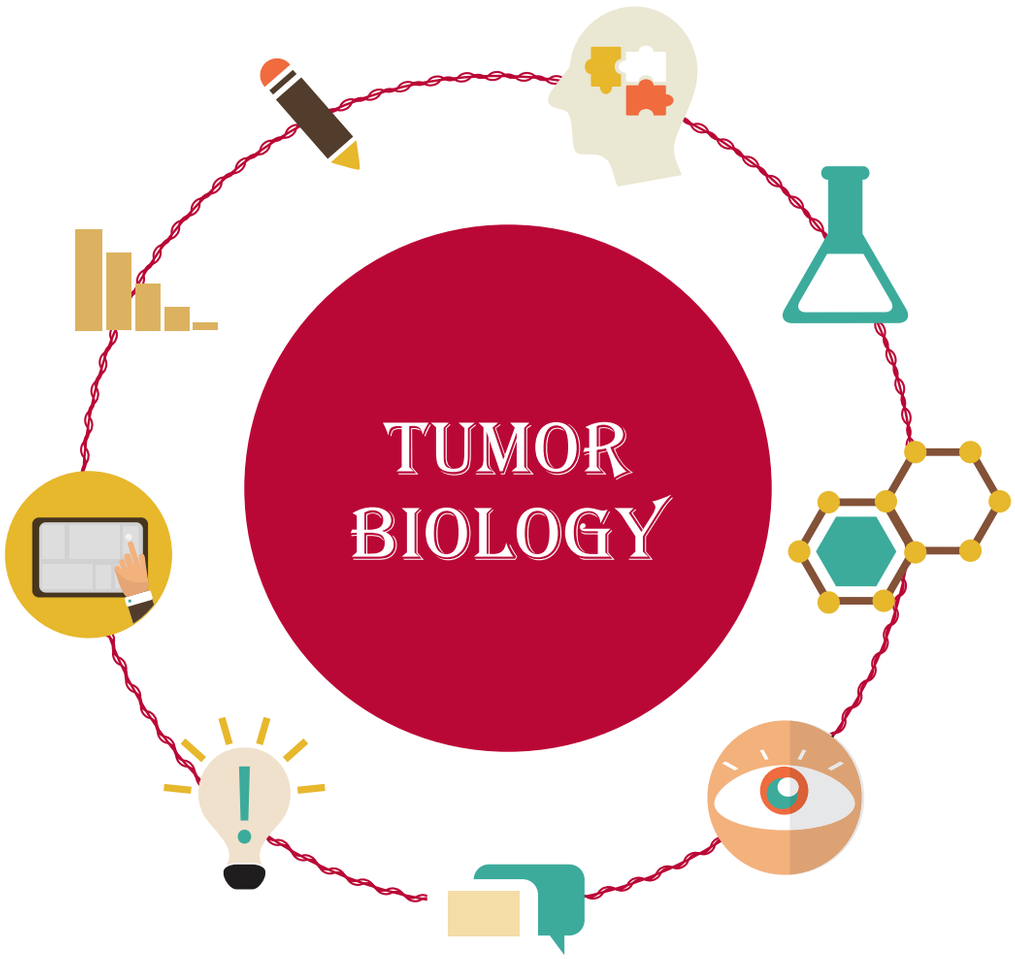
with psoriasis (dbGAP) encompassing all genetic markers associated with psoriasis (http://bmicnip.in/dbgap/). BIC has also developed TiD, a standalone software which would be useful in identifying potential drug targets from whole proteomes of bacteria (input size = ~5000 sequences) within two hours. The tool would be useful in identifying reserve pool of drug target of a bacterial pathogen, which in turn would be useful in combating the threat of antibiotic resistance. The BIC has standardized a computer aided antibody engineering techniques for *in silico* design of IL6 lead antibodies. Small molecule inhibitors targeting BMX non-receptor tyrosine kinase, JAK2, TNF have been also identified through pharmacophore modelling and structure based virtual screening. The screened small molecule and designed lead antibodies can be used as starting step in biologic development against autoimmune and inflammatory diseases.

Overall the year 2015-16 had been very productive through significant contributions in areas of basic, translational and clinical research. Two patents have been filed by Dr. L.K.Yerneni on "A method for processing of feeder cells suitable for adult stem cell proliferation" and Dr. Nasreen Ethesham on "Genetic Markers for Diagnosis of Tuberculosis caused by Mycobacterium tuberculosis". 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. Dr. AP Singh has been awarded 'Dr Bishnupriya Devi award' for the best original article published in Indian Journal of Dermatology, Venereology and Leprology for the year 2015 by IADVL. Dr. Poonam Salotra was invited as a delegate at "Kala-azar Elimination Program" partners' consultative meeting held at WHO, Geneva, Switzerland and conferred TWAS fellowship at the 26th General Meeting of The World Academy of Sciences (TWAS) at Vienna, Austria. Dr. Usha Agrawal was awarded HRD fellowship for short-term foreign fellowship in "Quality control and Quality assessment" in Biobanking at BC Cancer Agency, Victoria,BC, Canada. To transfer recent technologies to medical and biomedical students three interactive workshops, special training programs have been organized on "Next Generation Sequencing in Disease Diagnosis", "Research Methodology fot Medical and Biomedical scientists" and "Hands on training on Proteomics" during the year. The academic activities have been continued for training PhD and DNB students with vigor. Dr. Kiran Katoch gave "Smt. Pushpa Sriramachari" oration on "Improving health of

rural population by advances in technology" on 1st May, the Foundation day of institute. Dr. S. Sriramachari" Young scientist award" was given to Ms. Vanila Sharma. I take this opportunity to convey my thanks to Dr. Soumya Swaminathan, Director General, ICMR and Secretary, Department of Health Research for her support for infrastructure development and encouragement for scientific and academic programs. I acknowledge my sincere thanks to my scientific, technical and administrative staff for their unstinted support for carrying out various academic and non academic activities for welfare of institute.

DR. SUNITA SAXENA


RESEARCH
ACTIVITIES


TUMOR
BIOLOGY

TUMOR BIOLOGY

1. Study on micro-RNA signatures associated with Breast Cancer Stem like Cells (CSCs) and their role in drug response

Scientific staff : Dr. Sunita Saxena, Dr. S. A. Raju Bagadi, Ms. Renu Yadav

Duration : 4 years (2013-16)

Aims, Objectives and Background:

Stem cells are defined by their capacity for self-renewal. Either one or both of the daughter cells remain undifferentiated and can give rise to another stem cell. The paradigm of the Cancer Stem Cells is derived from studies of specific cell populations identified and characterized in human leukaemia, that form a minority fraction of cancer cells with the capacity for self renewal and tumorigenisity in immunodeficient mice at low dilutions. Recent work has demonstrated the existence of small fraction of such cancer stem cells or tumour-initiating cells in various solid tumors such as breast, brain, prostate, pancreas and colon cancers. These cancer stem cells may play an important role in cancer establishment, progression, and resistance to therapy. Traditional cancer therapies are effective in debulking the tumors but often fail to completely eradicate all cancer cells and thus, fail to stop recurrence or metastasis to distant organs, this is possibly due to their inability to eradicate, a small fraction of tumour-initiating or cancer stem cells present within the tumour. Hence it is important to understand the molecular mechanisms that regulate self-renewal and differentiation. 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.

Work done during the year:

In the previous year we have screened cell lines namely, T47D, MDA-MB-453, MDA-MB-468, and ZR-75-1 to estimate the proportion of breast cancer stem cells, using the CD44+/CD24- markers. Further cancer stem cells were enriched in these cell lines using mammosphere assay, which were further utilized for profiling of microRNAs that expressed uniquely in these breast cancer stem cells. We have identified 88 miRNAs which differentially expressed in breast cancer stem cells compared to bulk cells that are 2 fold differential using RNA seq. We identified targets genes for these 88 microRNAs which were significantly differentially expressed in cancer stem cells, several key cancer associated genes that are targets for these miRNAs, which form part of several pathways that are associated with cancer. One such pathway identified is SOX family genes. 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 in formation of breast cancer stem cells (Figure 1). The expression of SOX family genes will be further extended to more number of cancer stem cells to confirm such involvement.

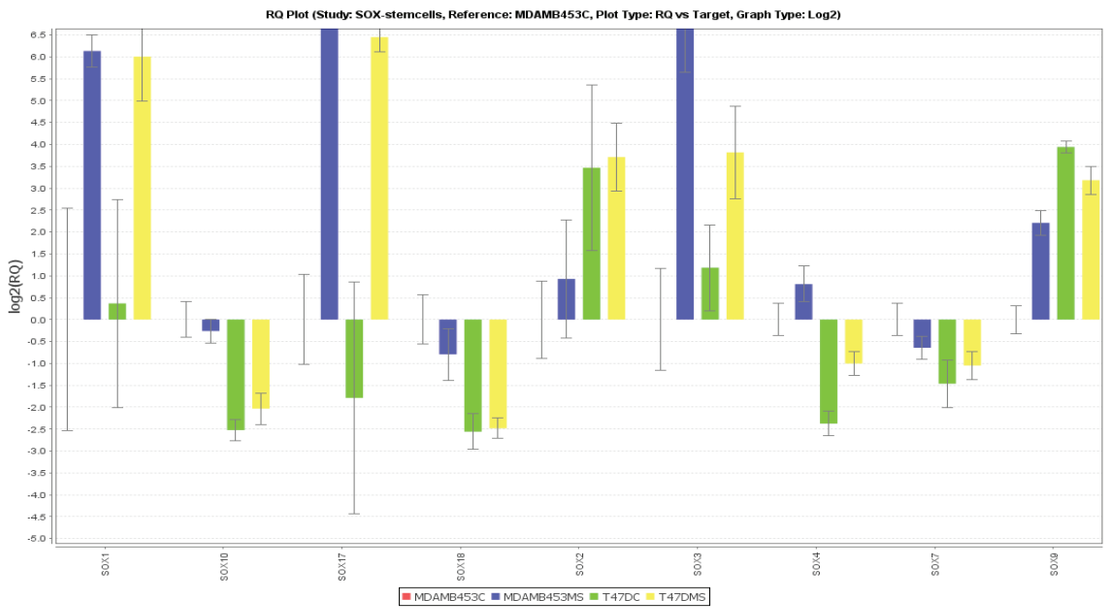


Fig. 1: showing differential expression of SOX family genes in breast cancer stem cells.

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

Scientific staff : Dr. Sunita Saxena, Dr. S. A. Raju Bagadi, Ms. Shreshtha Malvia, Dr. Chintamani, Dr. A.Bhatnagar, Dr. Mohil, Dr. Deepshikha Arora, Dr. Ramesh Sarin

Duration : 2013-16 (ICMR)

Aims, Objectives and Background:

Breast cancer diagnosed at young age is well recognized more clinically different than breast cancers diagnosed at older ages. Younger patients more frequently exhibit aggressive features such as large tumor size, high histologic grade, positive lymph nodes, absence of steroid receptors and high S-phase fraction, and young age itself has been shown to be an independent predictor of adverse prognosis. Hence understanding genomic changes associated with early onset tumors may lead to identification of novel biological markers for early detection. The project aimed to identify sequence variations and chromosomal rearrangements of deregulated genes in early breast cancer in early onset breast cancers.

Work done during the year:

Earlier we have done whole exome sequencing of 12 cases belonging to early and late onset tumors. In the present year we have analysed the data to identify genetic variations that are associated with early onset and late onset tumors, using partek flow and partek genomics suite. We found 2886 single nucleotide variations, 239 indels that are associated with early onset breast tumors, 5232 single nucleotide variations and 521 indels found to be associated with late onset breast tumors. We found 1991 single nucleotide variations and 137 indels that are common in both early and late onset tumors. We have analysed chromosome wise distribution of the variants, Highest number of variants were found in chromosome 1 followed by chromosome 2 and 6. In the current year, 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 chromosome 13, 18, and 21 (Figure 1). Further, we have carried out whole exome sequencing for 22 more breast cancer cases and 4 controls, the data of current year will be merged with the previous data and would be analysed to identify genetic variants that are unique to early and late onset breast tumors.

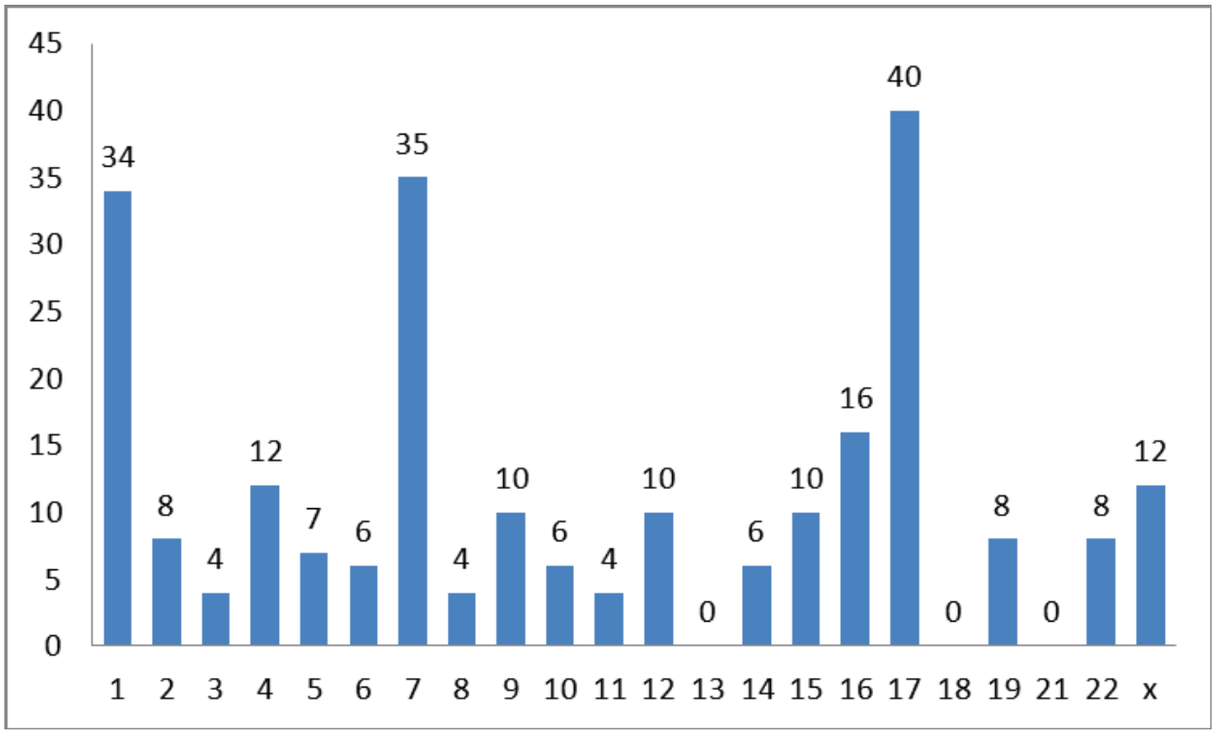


Fig. 1: Showing distribution of chromosome wise gene fusions

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

Scientific staff	:	Dr. Sunita Saxena, Dr. Anju Bansal, Ms Ananya Choudhary
Collaboration with	:	Dr. Anup Kumar, Department of Urology, Safdarjung Hospital, New Delhi
Duration	:	2015-18

Aims, Objectives and Background:

Prostate cancer is the second most common cancer and fifth leading cause of death from cancer in men. The worldwide prostate cancer burden is currently 1.1 million and growing. The incidence and mortality rates for prostate cancer in India show an increasing trend since the last few decades. The genetic changes underlying the development and progression of prostate cancer are poorly understood. Comparative genomic hybridization (CGH) for amplification and loss of DNA sites allows screening of the whole genome for sequence copy number alterations. Array CGH analysis can substantially narrow down the regions of interest relevant in clinical tumor specimens. The aim of this study is to identify those chromosome regions that contain genes important for the development of prostate cancer and to identify genetic markers of tumor progression. The present study has been undertaken to identify a link between chromosomal aberrations, genetic variations, LOH and copy number alterations in patients with prostate cancer of varying histological grades and aggressiveness.

Work done during the year:

Target study group were patients who were screened by serum PSA testing and Digital rectal examinations (DRE). Such patients were scheduled for 12 core TRUS guided prostatic biopsies at the Dept. of Urology, Safdarjung hospital. Prostatic tissue biopsy specimens from 40 patients were collected by means of TRUS guided biopsy for histo-pathological analyses and further genome studies. DNA extraction

was done using Qiagen DNAEasy Mini kit followed by array CGH based analyses. CGH+SNP based study was carried out on tissue samples of prostate cancer biopsies using Agilent Sureprint CGH+SNP 4x180K microarray chips. Data analysis was done using Agilent cytogenomics software. Genomic DNA from two cell populations was differentially labeled and hybridized to a microarray. Labeled patient DNA, reference DNA, and unlabeled Cot-1 DNA were hybridized. CGH results were analysed using Agilent Cytogenomics CGH + SNP software version 4.0. The fluorescent ratios on each array spot were calculated and normalized so that the median log2 ratio was 0. Based on hybridization results from control samples (Agilent Euro male hybridized against Male patient), chromosomal regions with copy number ratios under 0.85 were considered as lost, and above 1.2 as gained, in the chromosomal CGH analysis.

CGH microarray analysis revealed numerous recurrent copy number changes in prostate cancer tissue samples. Four samples of prostate cancer were analyzed. Major deletions, loss, gains and LOH were found. In all four samples major deletions were found on chromosome 2, 4, 8, 9, 10, 12, 15, 21. Deletion in q13.2 of chromosome 4 was found in all the four cases studied. Deletion at q34 of chromosome 2, p11.22 of chromosome 10, p13.31 of chromosome of chromosome 12, p11.1-11.2 of chromosome 15 shown in 75% cases. 50% cases showed deletions in q11.22 of chromosome 10, q22.2-22.3 of chromosome 21, p24.3-24.2 of chromosome 9. Similarly noticeable gains were seen in 2p11.2,14 q32.33, 19q13.43 in all the four cases. In 75% cases gains were seen at 20q13.32, Xq28, 1p36.13, 2p21, 7q11.2, 8p11.1. In 50% cases gains were found at 10q24.31- q24.32, 3q24, 11p15.5,16p11.2-11.1, Xp22.33. LOH was found in three chromosomes i.e. p22.3-p24.3 of chromosome 9, q22.1-q24.3 of chromosome 16, q22.2-q22.3 of chromosome 21 in 50% 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.

4. Differential protein profile for identification of markers in recurrent urothelial cancer

Scientific Staff : Dr Usha Agrawal, Ms Nitu Kumari

In collaboration with : Dr Anup Kumar, Dr Pawan Vasudeva, Dept. of Urology, S J Hospital, New Delhi

Duration of the Project : 2014-16

Aims, Objectives and Background:

Bladder cancer is the 7th most common cancer worldwide while in India it is the fifth most common cancer in men. The non-muscle invasive cancers show recurrences in 70% of cases and 30% may progress to muscle-invasive tumours. Hence, there is a need for identifying biomarkers to monitor the recurrence of disease. The present study was planned to identify differentially expressed tumor protein for use as biomarkers of recurrence.

Work done during the year:

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 down regulated 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) (Figure 1). 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.

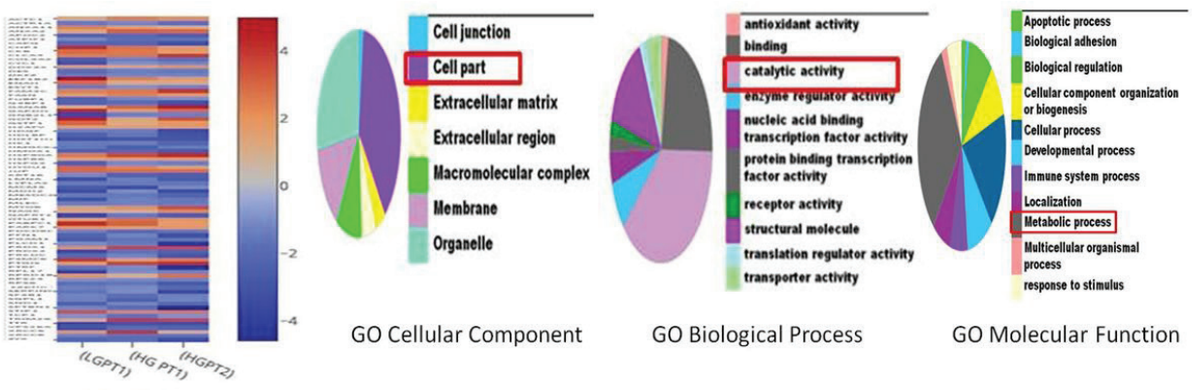


Fig. 1: Heatmap showed deregulation of protein in the three groups of bladder cancer including low grade non-invasive, high grade non-invasive and high grade invasive. Gene ontology terms showed most of the proteins involved are involved in catalytic activity and metabolic process.

5. Understanding the role of chemokines and their receptors in growth and development of glioblastoma

Scientific Staff : Dr. Avninder Singh, Dr. Sunita Saxena, Ms. Ira Sharma

In collaboration with : Dr. K.C. Sharma, Dept. of Neurosurgery, Safdarjung Hospital, New Delhi

Duration : 2014-16

Aims, Objectives and Background:

Tumors of the central nervous system (CNS) are rare neoplasms constituting 1-2% of all malignancies. GBM is the most lethal neoplasm of Central Nervous System (CNS) with less than 12 months survival in 40 % of cases. It may arise de novo (primary GBM) or may develop from progression of a pre-existing low-grade astrocytoma (secondary GBM). Due to limited success in current treatment modalities, new pathways are being explored. Chemokine signalling pathway has been implicated in various cancers. They are involved in myriad of biological processes affecting different aspects of cancer. They could directly influence the tumor growth by

activating pathways related to cell survival and cell proliferation or indirectly by promoting angiogenesis.

The aim of this study is

(a) To study differential gene expression of chemokines and their receptors in low grade and high grade glioma (GBM) using Real Time PCR array.

(b) To identify and validate differentially expressed chemokine and their receptors through tissue micro array and immunohistochemistry.

(c) To assess therapeutic potential of key targets identified in gene expression and immunohistochemistry through in vitro study.

Work done during the year:

To identify and validate differentially expressed chemokine and their receptors through tissue microarray and immunohistochemistry.

Functional classification of upregulated genes in GBM: Geneontology analysis using online tool PANTHER was performed. Chemokine genes involved in biological functions such as cell proliferation and cell cycle were selected. Those genes which wereupregulated in GBM and not Diffuse astrocytoma were sorted. Those genes are *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 $p< 0.001$. (Figure 3G).Expression of CXCL8 was primarily found in tumor astrocytes confirmed by co-expression study done with the help of immunofluorescence (Figure 3 H). However, no significant difference was observed between GBM and DA for its receptors CXCR1 and CXCR2 (Figure 1A-H).

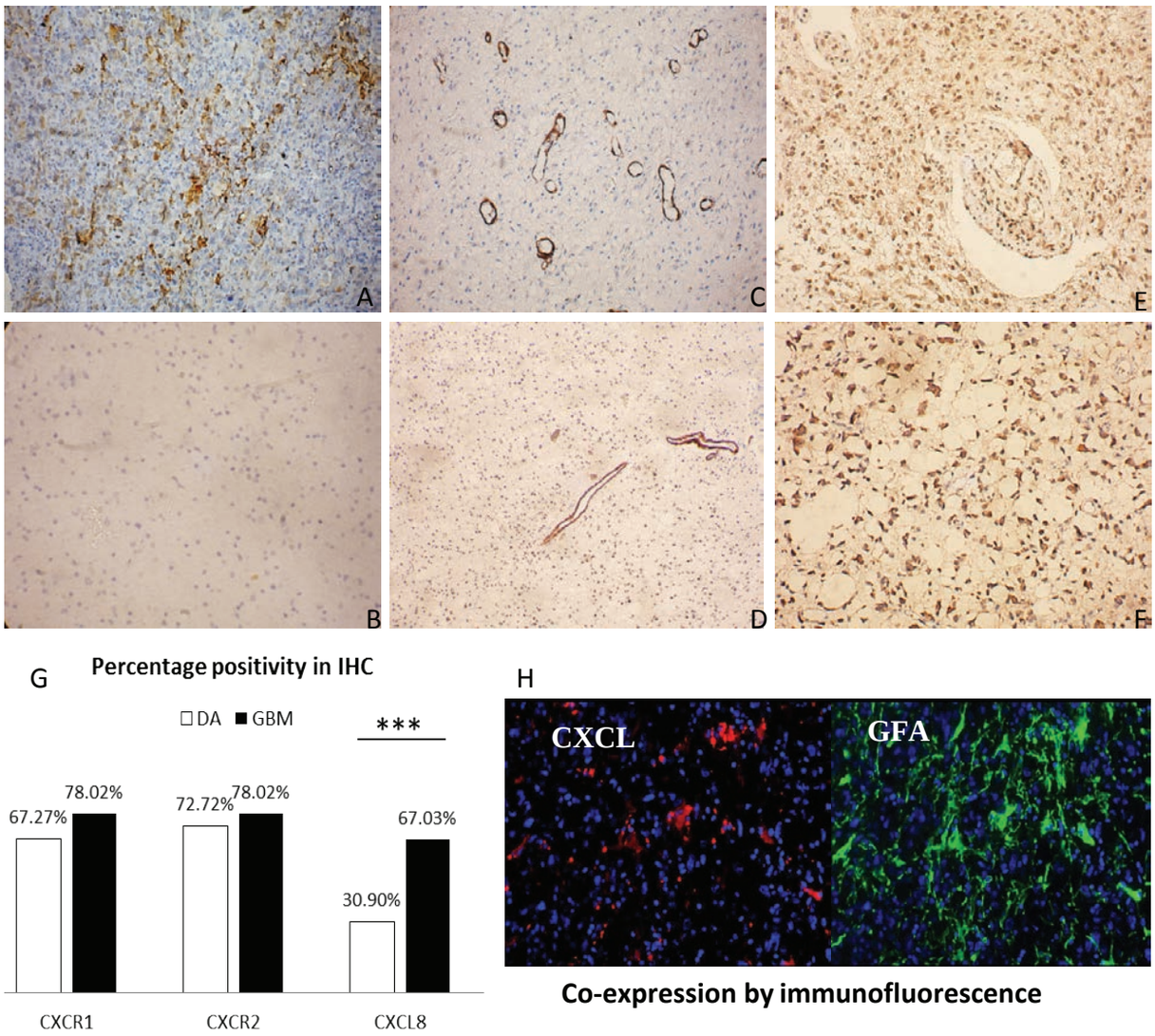


Fig. 1: Immunohistochemical staining of GBM (A) Anti-human CXCL8, (C) Anti-human CXCR1, (E) Anti-human CXCR2 and DA (B) Anti-human CXCL8, (D) Anti-human CXCR1, (F) Anti-human CXCR2. (G) Bar graph showing percentage positivity for CXCR1, CXCR2 and CXCL8 in DA and GBM. (H) Double immunofluorescence staining for CXCL8 and GFAP in GBM

6. Identification of novel therapeutic targets associated with gonadotropin-releasing hormone signaling in glioblastoma

Scientific staff	:	Dr. Poonam Gautam, Dr. Fouzia Siraj, Ms. Priyanka H. Tripathi
In collaboration with	:	Dr. Ravindra Kumar Saran, G. B. Pant Hospital, New Delhi Dr. Ravi Sirdeshmukh, Institute of Bioinformatics, Bangalore
Duration	:	2016-19

Aims, Objectives and Background:

Glioblastoma multiforme (GBM) is one of the most malignant and aggressive forms of primary brain tumors and despite major advances in high-throughput technologies and multi-dimensional profiling in the last decade, management of GBM remains a clinical challenge and new drugs are needed for treatment of this aggressive tumor. Protein kinase-mediated signaling pathways are known to be altered in GBM and other cancers and are prominent targets in cancer treatment. For this purpose, we focused on deregulated kinases in GBM from highthrougput omics studies. Bioinformatic analysis using Ingenuity pathway knowledgebase revealed these kinases to be associated with **gonadotropin releasing hormone (GnRH) signaling pathway**. Expression of GnRH and GnRH receptor has been reported in GBM and treatment with GnRH agonist showed significant reduction in cell proliferation *in vitro* and reduction in 70% tumor growth *in vivo*. However, the molecular targets associated with GnRH receptor are not clearly understood in GBM and other cancers. The present study aims to identify the therapeutic targets associated with GnRH signaling in glioblastoma.

Work done during the year:

We have analyzed the expression of GnRH receptor both at gene and protein level in GBM cell lines, LN229 and U87MG. Primers were designed for GnRH receptor and its gene expression was studied using RT-PCR analysis. We observed a PCR product with 100 bp confirming the expression of GnRH receptor in both LN229 and U87MG cell

lines. Protein expression of GnRH receptor was studied in cell lysate using Western blot analysis. Western blot analysis showed expression of GnRH receptor in both the cell lines at ~52 kD.

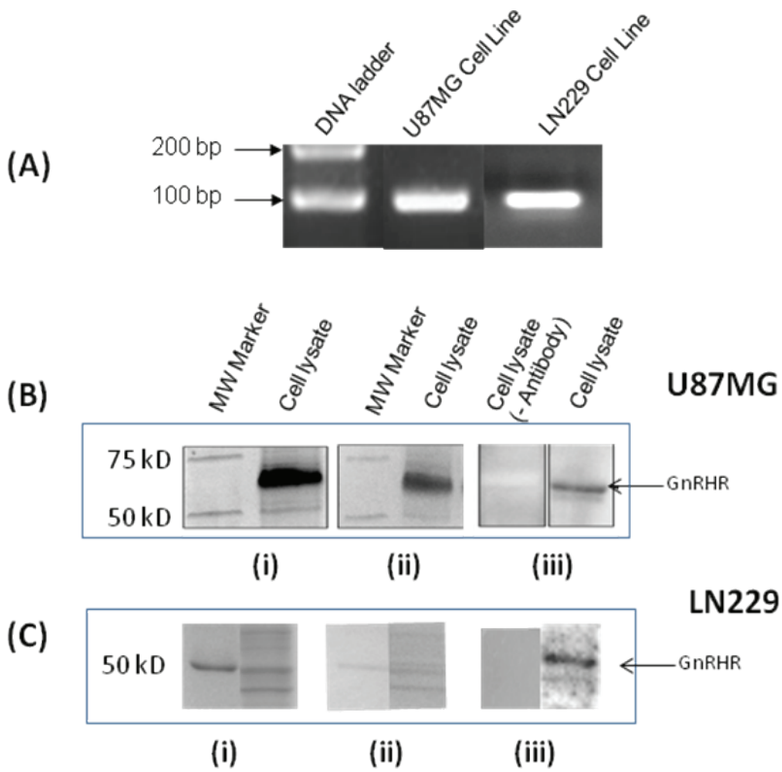


Figure **Gene and protein expression of GnRH receptor in U87 MG and LN229 GBM cell lines using RT-PCR and Western blot analysis.** RNA isolation followed by RT-PCR analysis showed expression of GNRH receptor at gene level in both the cell lines (A). Western blot analysis showed expression of GnRH receptor at protein level in both the cell lines. For Western blot analysis, a total of 30 µg and 20 µg protein was resolved by SDS-PAGE for U87MG (B) and LN229 cell line (C) respectively and were electrotransferred onto PVDF membrane and immunoblotting was performed. The blot was developed using Clarity Western Enhanced chemiluminescence (ECL) Substrate (Bio Rad). (i) SDS-PAGE (ii) Ponseau S stained image after the blotting (iii) immunoblot showing expression of GnRH receptor in cell lysate. Antibody used: Santacruz for LN229 (sc-8682, polyclonal) and Thermo for U87MG (MA5-11538, monoclonal)

Future plan of action:

GBM cell lines, LN229 and U87MG, will be treated with GnRH agonist and high throughput iTRAQ-based quantitative proteomic analysis will be performed to identify proteins and signalling pathways associated with GnRH receptor in GBM. Some of the novel targets may be further explored for therapeutic applications in GnRH receptor positive GBM cases.

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

Scientific staff : Dr. Sunita Saxena, Dr. Fouzia Siraj, Ms. Asheema Khanna

Duration : 2015-18

Aims, Objectives and Background:

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. Current ALL treatment protocols use combinations of multiple cytotoxic chemotherapeutics drugs with overlapping toxicity and the potential for long term sequelae, especially in the most intensively treated patients. Therefore, treatment of leukemia remains a challenge for clinicians. New agents with activity against ALL are needed, and targeted biologic agents have the potential to add efficacy without additional toxicity in patients with ALL. 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).

There is not much data available on mTOR expression in relation to leukemia etiology. The objective of the study was to study 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.

Work done during the year:

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. RNA was isolated from ALL cells using TRIZOL reagent and complementary DNA (cDNA) was generated using high capacity cDNA archive kit (Applied Biosystems). Quality control of cDNA was done through relative quantitation of

expression level of 18s rRNA endogenous control gene using Taqman assay by real time PCR step one plus. Expression of mTOR gene in response to induction chemotherapy. (50 ALL samples and 20 Healthy control)

mTOR expression was found to be higher in acute lymphoblastic leukemia patients in comparison healthy controls. (Fig.1)

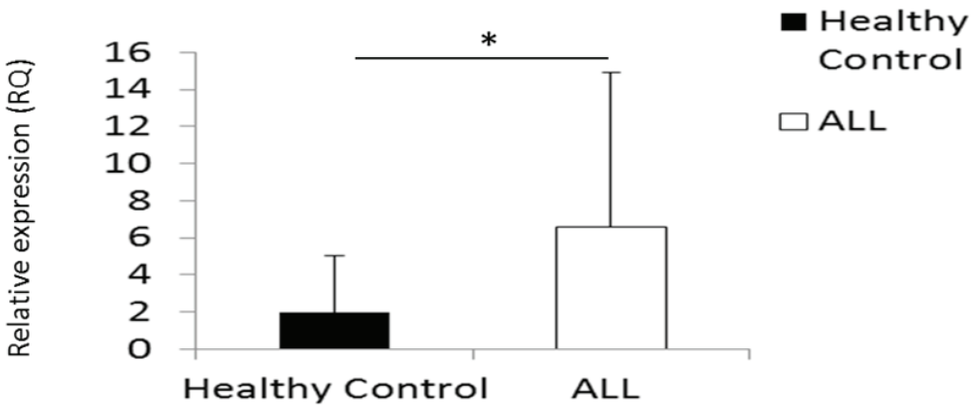


Fig. 1: Relative expression of mTOR gene in ALL samples compared to normal PBMC cells

Correlation with Induction chemotherapy:

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 (Fig 2).

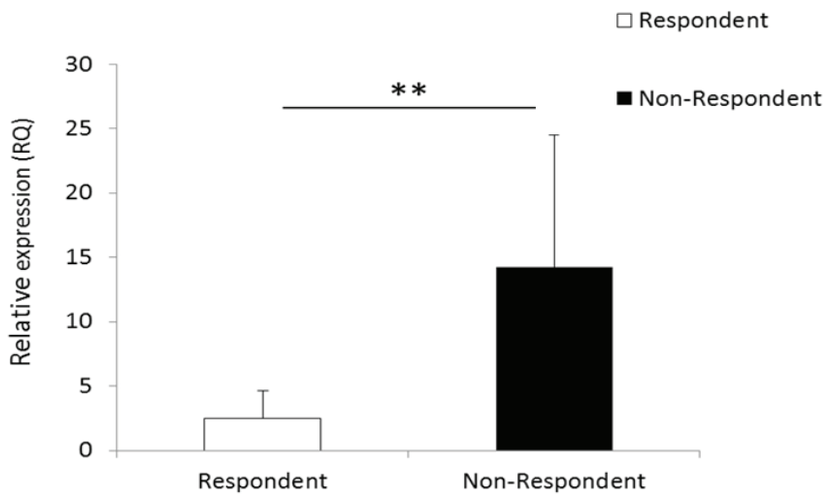


Fig. 2: Expression of mTOR gene in ALL samples responded and non-responded to induction chemotherapy


INFECTIOUS
DISEASES

LEISHMANIASIS

1. Mechanism of resistance to Miltefosine (MIL) in *Leishmania donovani*

Scientific staff	:	Dr. Poonam Salotra, Dr. Ruchi Singh, Mr. Deepak Kumar Deep, Ms. Aditya Verma
In collaboration with	:	Dr. N S Negi, Dr. V Ramesh
Duratio	:	2012-16

Aims, Objectives and Background:

Miltefosine (MIL) is an oral antileishmanial drug, introduced in the year 2002 for visceral leishmaniasis (VL) treatment in the Indian subcontinent. Recent reports indicate a significant decline in its efficacy and high relapse rate in visceral leishmaniasis (VL) as well as post kala-azar dermal leishmaniasis (PKDL). In this situation, understanding the mechanism of development of resistance towards miltefosine in *Leishmania donovani* has become the top priority to rescue the efficacy of this drug.

Work done during the year:

In the year under report we assessed the differential accumulation of MIL, thiol content and reactive oxygen species (ROS) tolerance in *L. donovani* parasites comprising pretreatment group (LdPreTx, n=6), relapse patients of both VL and PKDL after MIL treatment (LdRelapse, n=5) as well as in experimental resistant group (LdM30, n=2). Further we assessed MIL uptake and ROS tolerance in transfected parasites ($LdLip^{++}$) overexpressing lipase precursor like protein.

MIL uptake and intracellular thiol content in *L. donovani* parasites:

MIL uptake was assessed using liquid chromatography coupled mass spectrometry (LCMS). LdRelapse isolates and LdM30 isolates showed 2 fold and 2.5 fold less accumulation of MIL respectively when compared with LdPreTx (Fig1A).

Thiol content: Intracellularthiol content was measured in *L. donovani* promastigotes from LdPreTx, LdRelapse and in LdM30 groups. The mean thiol concentration in nM of LdPre-Tx was 84±9.7, in LdRelapse 120±1.1 and in LdM30 isolates 140±5.5 (Fig1B).

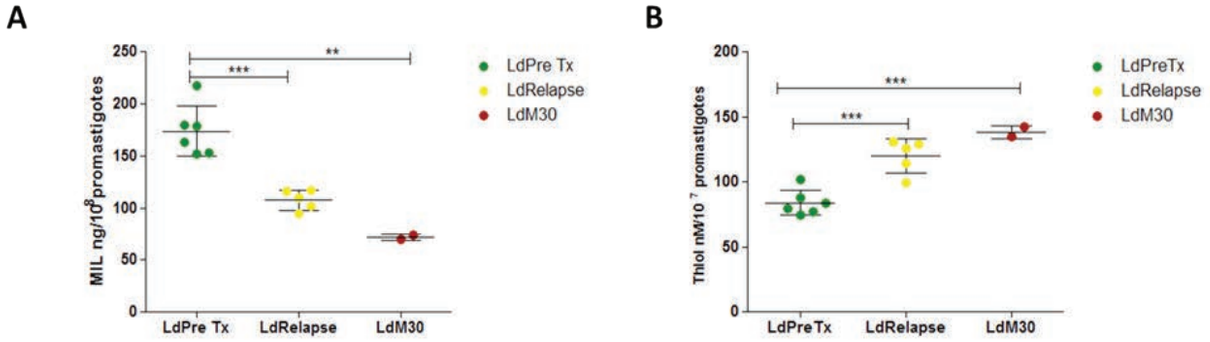


Fig. 1: A. LdRelpase and LdM30 have significantly lower accumulation of MIL compared to LdPreTx group parasites. **B.** LdRelapse and LdM30 showed significantly higher intracellular thiol content when compared to LdPreTx.

ROS tolerance at Promastigote level:

We measured the ROS accumulation in *L. donovani* promastigotes using cell permeable fluorescent probe 2',7'–dichlorodihydrofluorescin diacetate (H_2DCFDA). LdPreTx isolates showed dose dependent increase in ROS level at promastigote stages and was more evident at higher MIL concentration (20µM). LdM30 parasites did not show any significant change in ROS level with increasing MIL concentration. LdRelapse parasites showed increased level of ROS with increasing MIL pressure; however it was significantly lower to pretreatment group (Fig2A).

At amastigote level:

We evaluated the ROS tolerance at amastigote level by following macrophage-amastigote model. The level of ROS were comparable before and after exposure of MIL (20µM) in macrophages infected with LdRelapse and LdM30 parasites indicating better tolerance towards MIL induced oxidative stress. However the level of ROS in macrophages infected with LdPreTx were significantly higher after MIL exposure (Fig2B).

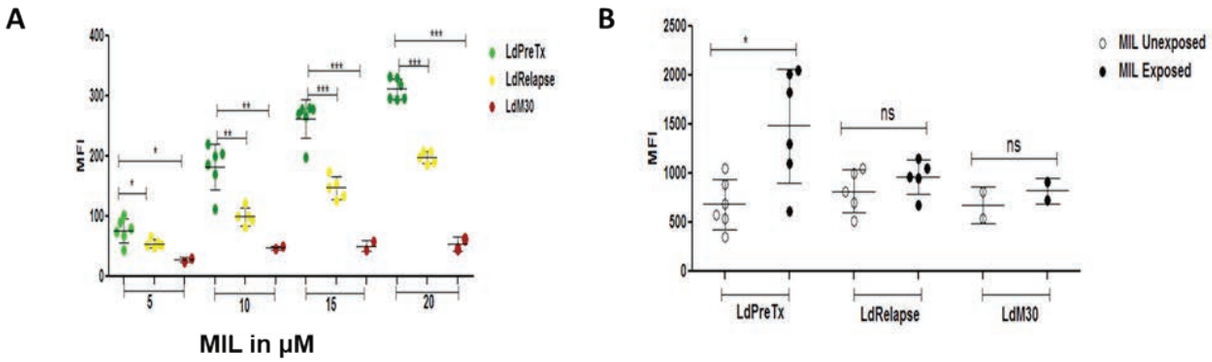


Fig.2: A. Dose dependent accumulation of ROS is significantly low in LdRelapse and in LdM30. **B.** Macrophages infected with LdRelapse and LdM30 showed comparable level of ROS before and after MIL exposure.

Characterization of LdLip^{++} parasites

We have generated Lipase overexpressing parasite line by transfecting MIL sensitive *L. donovani* (LdLip^{++}) and shown that LdLip^{++} exhibit 3 fold increase in MIL IC_{50} value. Here, we assessed lipase activity, targeted gene expression analysis, MIL uptake and ROS tolerance in LdLip^{++}. Parasites transfected with empty vector (LdNeo) served as control.

Lipase activity assay in culture supernatant and cell lysate of LdLip++

Lipase activity was measured in culture supernatant and cell lysate of LdLip^{++} parasites by using lipase activity fluorometric assay kit III from BioVision. The activity of lipase was found to be 2 fold higher in culture supernatant of LdLip^{++} when compared with LdNeo and LdWT at 37°C. The activity in cell lysate of LdLip^{++}was also 2 fold higher than in cell lysate of LdNeo and LdWT under similar condition. (Fig 3)

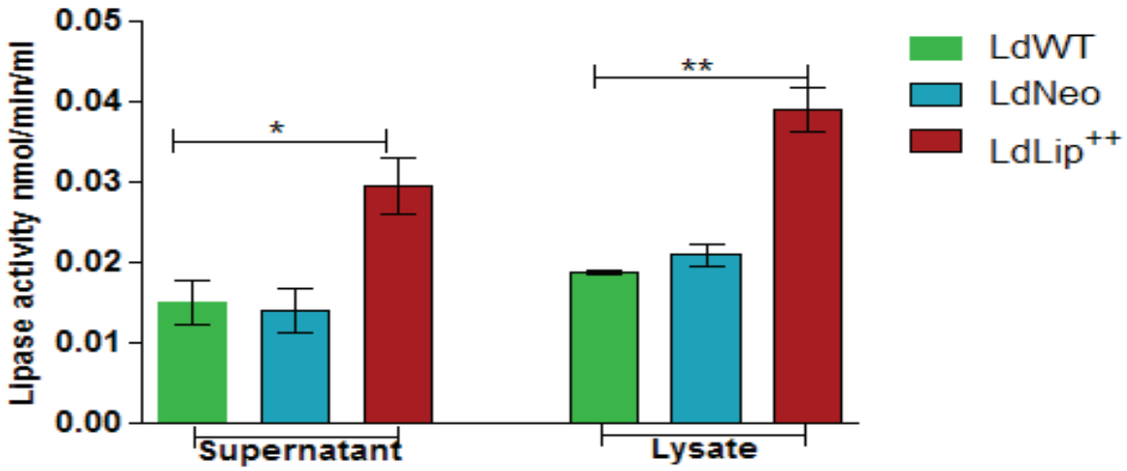


Fig. 3: Lipase activity in culture supernatant and lysate of LdLip++ parasite, LdNeo and LdWT. Values are mean ± SD of two separate assays.

MIL uptake and ROS tolerance in LdLip++ parasites

The uptake of MIL in LdLip^{++} was comparable to wild type (LdWT) and LdNeo parasites. Therefore MIL uptake machinery remained unaltered with increasing transient expression of lipase precursor protein in *L. donovani* (Fig4A).

The tolerance against MIL induced oxidative stress was increased in LdLip^{++}. The macrophages infected with LdLip^{++} showed comparable ROS level before and after MIL exposure while there was significant increase in ROS level in macrophages infected with LdNeo and LdWT parasites after MIL exposure (Fig4B).

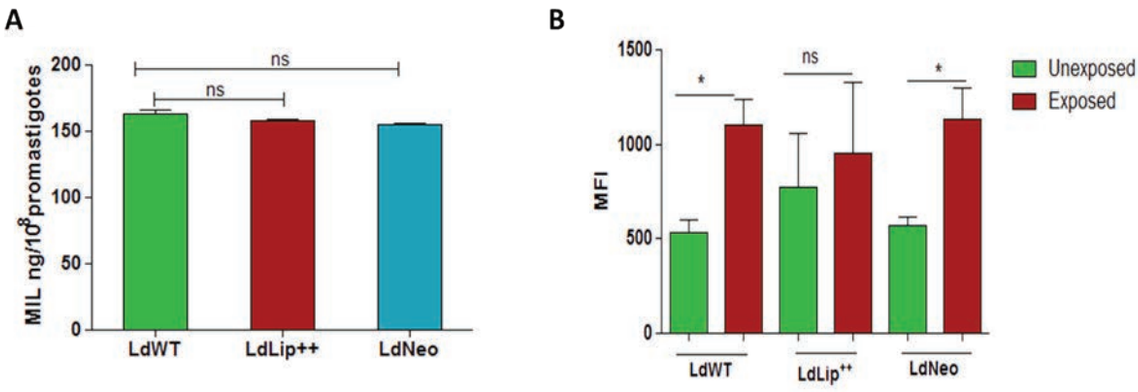


Fig. 4: A. MIL uptake was comparable in LdLip^{++}, LdNeo and LdWT parasites. **B.** LdLip^{++} infected macrophages showed comparable level of ROS before and after MIL exposure.

m RNA expression of selected genes in LdLip++ parasites

We did expression analysis of selected genes by Q-PCR in LdLip++ parasites. The level of m RNA expression in LdLip++ parasites was consistent with expression in LdWT for SMP2, PGMPUT, TSH, MDRP, ABCTrans, ABCF2, TCP, AAT and APH. The expression of TRYP and CytB5Red was upregulated in LdLip++ parasites when compared with LdWT.

2. Investigations on Paromomycin resistance in *Leishmania donovani* using molecular and biochemical tools.

Scientific Staff : Dr. Poonam Salotra, Dr. Ruchi Singh, Ms. Aditya, Mr. Deepak Kumar Deep

Duration : 2015-18

Aims, Objectives and Background:

Based on the preliminary studies in the past on mechanism of resistance towards paromomycin an extramural project funded by ICMR titled above was initiated in August 2015.

Paromomycin (PMM) is a new treatment option for VL control in India as a monotherapy and in combination therapy. It is vital to understand the mechanisms of PMM resistance to increase the life span of this drug. In order to understand the mechanisms involved in resistance towards PMM, during the period under study, we successfully explored microarray technique to analyze the genes showing modulated expression in PMM resistant parasites.

Work done during the year:

Transcriptome profiling of PMM-S and PMM-R parasites:

To investigate the global mRNA expression profiles of PMM resistant and PMM sensitive *L.donovani*, one colour microarray based gene expression profiling was carried out using *L.infantum* whole genome 60mer oligonucleotide microarray slide [8X15K format]. Gene expression studies were performed between lab generated PMM resistant *L.donovani* (K133 PMM-R, adapted to 100 µM of Paromomycin) and its corresponding wild type parasite (K133 WT). RNA was prepared from K133 PMM-R and K133 WT parasites using trizol method. Concentration and purity of the RNA were evaluated using the Nanodrop Spectrophotometer (Thermo Scientific; 1000). The integrity of the extracted RNA were analysed on the Bioanalyzer (Agilent; 2100). Complementary RNA (cRNA) was generated from 1µg of total RNA using Quick-Amp Labeling kit (Agilent technologies) that directly incorporates Cy-3 labeled CTP into the cRNA. cRNA obtained was cleaned up using Qiagen RNeasy columns. Concentration and amount of dye incorporated were determined using Nanodrop. Samples that pass the QC for specific activity were taken for hybridization. Hybridization was carried out using the Gene Expression Hybridization kit (Agilent Technologies) in Sure hybridization Chambers (Agilent) at 65° C for 16 hours. Three biological replicates of all hybridizations were performed to account for sample heterogeneity, and variations due to hybridization. Hybridized slides were washed using Agilent Gene Expression wash buffers (Agilent Technologies). The hybridized, washed microarray slides were then scanned on a G2505C scanner (Agilent Technologies). Images thus obtained were quantified using Feature Extraction Software (Version-10.7, Agilent). Feature extracted raw data was analyzed using GeneSpring GX12.6.1 microarray data and pathway analysis tool. Statistically significant differentially expressed genes were determined by t test (unpaired) for two groups; Confidence Level (p-value cut-off) < 0.05 and fold change analysis. Genes with expression ratio greater than 2.0 between paromomycin-sensitive and paromomycin-resistant parasites were considered as differentially regulated. 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. 5**. Most of the genes were similarly regulated. We identified a number of 267 genes (approx. 2.9%) were differentially modulated in

PMM-R parasites. Few genes showed ≥ 4 fold up- or down-regulation in drug resistant mutants (**Fig. 5**).

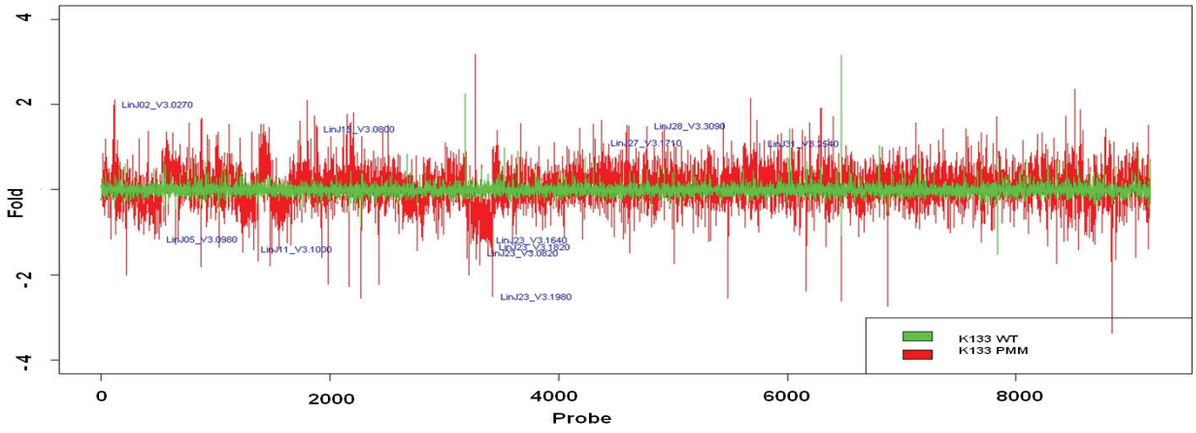


Fig 5: Overlap of log2 transformed PMM-R and PMM-S expression ratio plotted as a function of chromosomal location of probes represented the full genome microarray. The plot represents the average values of three independent hybridizations for each isolates.

There were 174 genes up-regulated and 93 genes down-regulated in PMM-R isolates. The pattern of overall up-regulated and down-regulated geneexpression is shown in **Table 1.** The largest number of genes showed 2-3 fold modulation in expression while very few showed >4 fold modulation.

Table 1: Pattern of up-regulated and down-regulated gene expression in paromomycin resistant *L. donovani* parasite

Fold Changes	PMM-R, Up-regulated	PMM-R, Down regulated
>2.0-3.0	153	78
>3.0 – 4.0	17	9
>4.0	4	6
Total genes	174	93
Percent of modulated genes*	1.89%	1.01%

*The percent modulated genes calculated from the total 9,170 genes obtained in QC after filtering.

All the modulated genes identified were then filtered by Tritrypdb and Uniport databases which resulted in a total of 231 annotated genes. The annotated genes comprised of 144 up-regulated genes and 87 down-regulated genes.

Future Work

Both up-regulated and down-regulated genes in paromomycin resistant parasites will be analysed by using BLAST2GO, AmiGO databases for functional classification of genes showing modulated expression. KEGG pathway analysis tool will be utilized to identify the pathways in which these genes were involved.

3. Identification of genes associated with Artemisinin resistance in *Leishmania donovani*

Scientific Staff : Dr. Ruchi Singh, Dr. Poonam Salotra, Ms. Aditya

Duration : 2015-18

Aims, Objectives and Background:

In the absence of an effective vaccine, chemotherapy remains the sole weapon in the arsenal against leishmaniasis. However, current treatment modalities are limited, have the potential to develop resistance and possess unacceptable toxicity. Artemisinin, a sesquiterpene endoperoxide isolated from *Artemisia annua,* is potent antimalarial compounds that have demonstrated effectiveness in experimental models of leishmaniasis. Studies to unravel the possible mechanisms of action and resistance towards this drug are necessary.

Work done during the year:

During the period under study natural in vitro sensitivity of different field isolates of *L. donovani*towards artesunate was determined. Artesunate tolerance was induced in a field isolate of *Leishmania donovani* isolated from SAG sensitive patient. The growth kinetics of the drug induced resistant parasites (AS-R) was compared with the wild

type (WT) in presence and absence of drug pressure. Drug susceptibility assays were carried out at both life stages of the *Leishmania* parasite.

Inherent sensitivity of *L.donovani* isolates towards ART:

Clinical isolates(n=8) of *Leishmania donovani* obtained from the kala-azar patients (antimonials sensitive/resistant or miltefosine pre-treatment/relapse cases) already cryopreserved in lab were propagated in Medium M199 containing 10% heat inactivated FCS at 26˚C. ART susceptibility of all the isolates was evaluated at promastigote stage. A wide range of variation ranging from 8.82±0.85 µM to 140.33±11.43 in IC_{50} values was observed. Similarly IC_{90} values were also varied from 29.29±3.25µ to 935.80±50.33 µM.

Generation of Artemisinin resistant Parasite:

L. donovani parasite (K133WT) was exposed stepwise to increasing artesunate pressure up to 50µM and stable resistance could be achieved in 48 weeks. The parasite was named as AS-R. Both AS-R *L. donovani* and wild type parasites exhibited similar morphology. The resistance in parasites was stable without drug pressure up to 8 passages.

Growth kinetics of AS-R parasites:

The resistant parasites exhibited growth comparable to the wild type parasite (Fig-6). We studied the growth of WT and AS-R parasites in absence and presence of artesunate 50 µM.

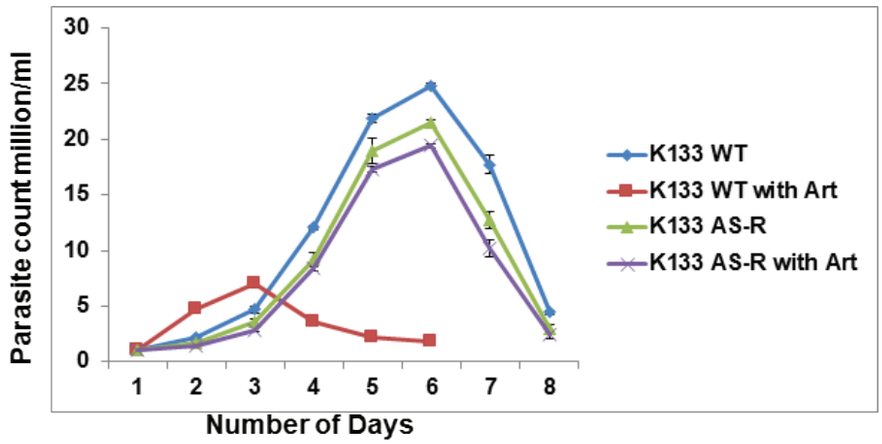


Fig. 6: Growth profile of cultured AS-R and wild type promastigotes in presence or absence of ART for 8 days. Wild type parasite (WT) and parasite resistant up to 50µM artesunate (AS-R) were counted using hemocytometer for eight days. Error bars indicate standard deviation of the mean (SD) based on 2 repeated experiments.

Artemisinin susceptibility of AS-R parasites at promastigote and amastigote stage:

The AS-R parasites showed 3.73 fold increase in IC_{50} as compared to WT and 1.80 fold increase in IC_{90} value in comparison with the WT isolates (Fig 7) for Artesunate at promastigote level. Susceptibility towards artesunate was determined at amastigote stage also. An increase of >3 fold in IC_{50} and approx. 2 fold increase in IC_{90} value was observed at intracellular amastigote level as compared to WT parasites (Fig 7).

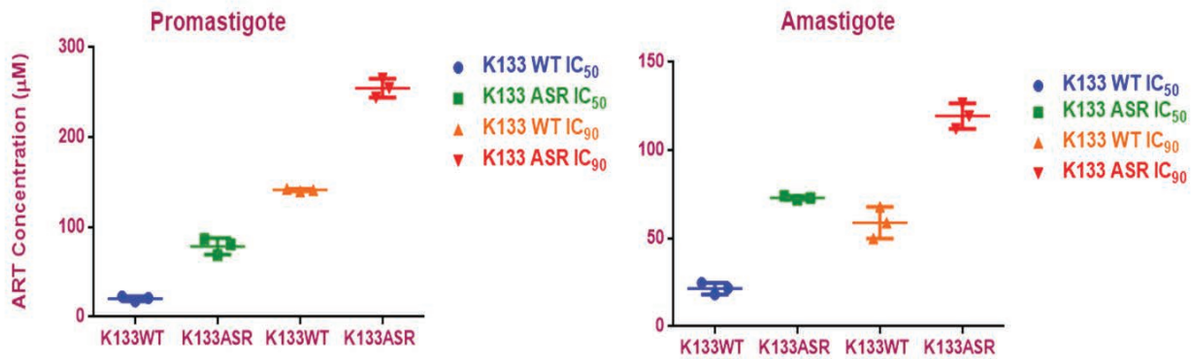


Fig. 7: Sensitivity profile of artesunate resistant and their respective wild type parasites towards artesunate at promastigote and amastigote stage. IC_{50} and IC_{90} values represented are the mean of two independent experiments performed in quadruplicate (promastigote) and triplicate (amastigote).

Future Work:

Determination of comparative tolerance of AS-R and WT parasites towards oxidative and nitrosative stress. Comparative global gene expression analysis of WT and AS-R parasites for identification of resistance associated genes.

4. Characterization of amastigote specific gene A1 by gene knock-out and over-expression

Scientific staff : Dr. Poonam Salotra, Kumar Avishek

Collaborators : Dr. Angamuthu Selvapandiyan, Dr. Hira Nakhasi

Duration : 2015-16

Aims, Objectives and Background:

The work is an extension of the study "Development of new live attenuated vaccine candidates for kala-azar". Amastigote-specific genes are likely to play central roles in survival of *Leishmania* parasite in the mammalian host and hence they are considered as virulence factors. The present study aims to characterize A1 gene, highly expressed at the intracellular amastigote stage.

Work done during the year:

Transfection for the generation of A1 gene double allele knock out mutant parasite (*Ld*A1$^{-/-}$)

In the previous year report, we had shown that the knock-out construct with 5' and 3' flanking regions (5FLK and 3FLK) of A1 and hygromycin (HYG) antibiotic resistance gene was generated (5FLK/HYG/3FLK). Now this construct was used to delete the 2nd allele of A1 gene by transfecting the 1st allele deleted *Leishmania* parasite, generated during the previous year. Transfected parasites were selected under HYG pressure. We observed that parasites were not able to survive under the antibiotic pressure. Transfection was repeated thrice with appropriate controls to rule out the possibility

of unsuccessful transfection. Inhibition in survival of parasite after transfection, indicates the possibility of an essential role of A1 gene in parasite survival. Hence, A1 double knock out mutants could not be prepared and characterization studies were performed with single A1 allele deleted parasite.

Effect of A1 single allele deletion on growth and phenotype of parasites at promastigote stage

Growth of the A1 single allele deleted *Leishmania* parasite was monitored at the promastigote stage over a period of 8 days. It was found that the growth of *Ld*A1$^{+/-}$ was reduced as compared to that of the wild type, indicating that deletion of A1 had negative effect on the parasite growth at promastigote stage **(Fig 8)**.

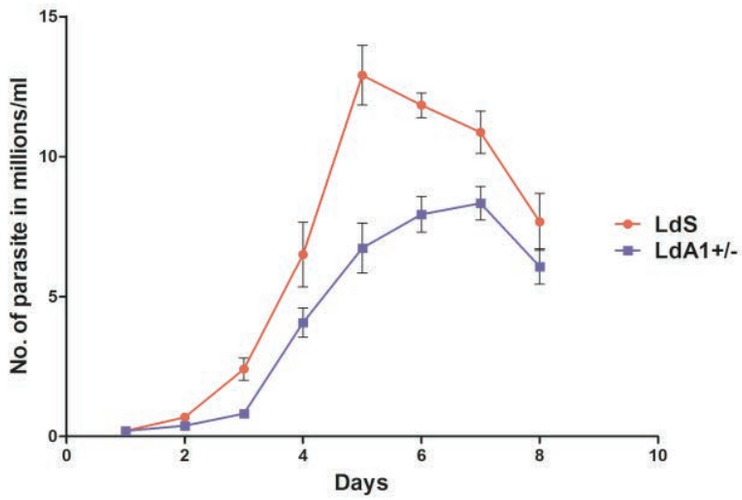


Fig 8. Growth curve of A1 single allele deleted *Leishmania* parasite (LdA1$^{+/-}$) in comparison to the wild type. X-axis of the graph represents number in days while Y-axis represents number of parasite in millions.

Further *Ld*A1$^{+/-}$ also showed different phenotype, these cells were more rounded instead of regular elongated like wild type (**Figure 9A**). Phenotypic changes and reduction in growth of *Ld*A1$^{+/-}$ was found at amastigote stage as well (**Figure 9B**).

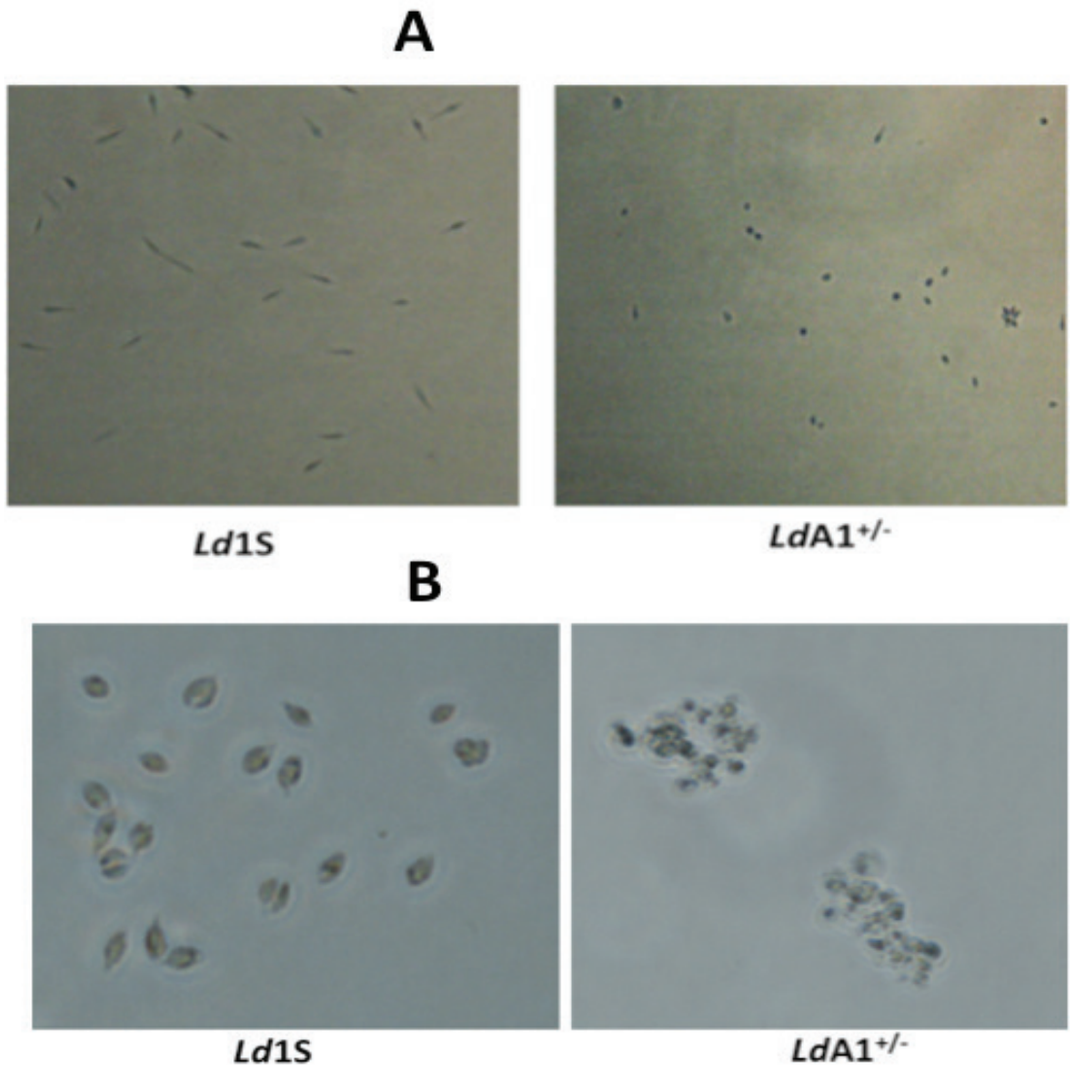


Fig. 9: Phenotypic difference between wild type (*Ld*1S) and *Ld*A1$^{+/-}$. **(A)** Promastigote stage, **(B)** Amastigote stage.

5. Clinico-epidemiological analysis of Post kala-azar dermal leishmaniasis (PKDL) cases in India over last two decades: a hospital based retrospective study

Scientific staff : Dr. Poonam Salotra, Dr. Ruchi Singh, Mr. Himanshu Kaushal

In collaboration with : Dr. V Ramesh

Duration : 2014-15

Aims, Objectives and Background:

The present work is retrospective study to explore trends in clinical and epidemiological features of PKDL cases over last two decades (1995-2014) in a tertiary hospital setting (Safdarjung Hospital, New Delhi), constituting the largest study undertaken so far in Indian PKDL, that may have important implications for control of VL.

Work done during the year:

PKDL predominant in areas of high endemicity for VL

A total of 282 PKDL cases (Male, n=225, Female, n=57) were registered over last two decades since the year 1995 **(Fig. 10)**. Majority (94.3%, n=266) of them originated from Bihar, and the rest (5.7%, n=16) were from the adjoining states namely Eastern Uttar Pradesh (3.5%, n=10), West Bengal (1.4%, n=4) and Jharkhand (0.7%, n=2). VL endemic areas in the state of Bihar have been categorised into high-, meso-, and low endemic areas based on the presence of degree of antimony resistance. Adjoining states (Eastern Uttar Pradesh, West Bengal and Jharkhand) were categorised as low endemic region. Based on this classification, 63.5% (n=179) of PKDL cases originated from high endemic area as against 30.1% (n=85) and 6.4% (n=18) from meso- and low-endemic areas respectively **(Fig. 10)**. We observed an upward trend in reporting of PKDL cases to Safdarjung Hospital, New Delhi, India as 51 PKDL cases were reported duing the 5-year period between 2000-04 as against 86 during 2010-14.

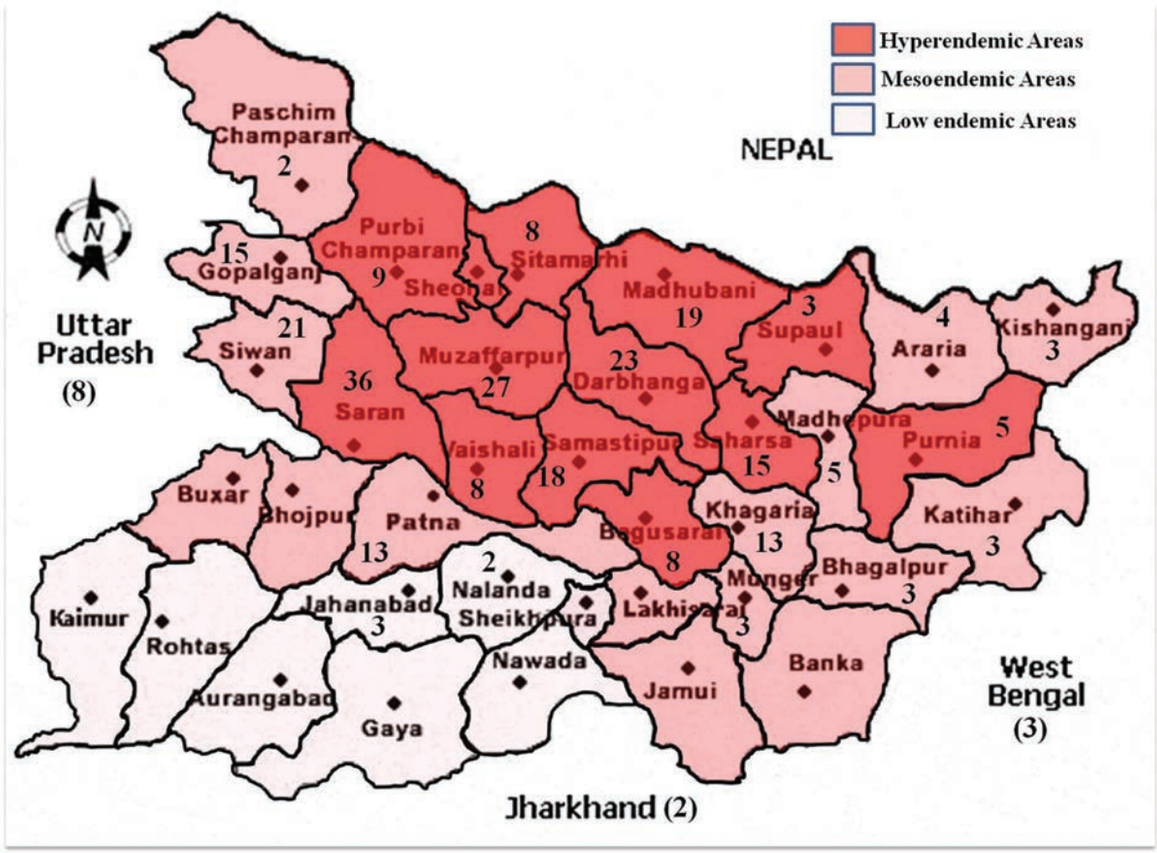


Fig. 10: Distribution of PKDL cases in Bihar and the adjoining states. Map showing the distribution of PKDL cases in the state of Bihar and adjoining states, based on the area of high, moderate and low endemicity for VL, designated as per Sundar et al. Trop Med Int Health (2002). Number shown in the figure is the number of PKDL cases from the district.

Clinico-epidemiological characteristics

The clinical characteristics of PKDL patients are summarized in table 1. Our study recorded 79.8% (n=225) male and 20.2% (n=57) female with overall median age of 22 years (range 5-65 years). The median age of females (20 years, range = 8-60) was comparable to that of males (22 years, range = 5-65, p = 0.789). Majority of cases belonged to the age group 19-44 years (64.5%, n=182), followed by paediatric cases aged ≤ 18 years (30.1%, n=85) and the group aged ≥ 45 years (5.3%, n =15).

Mixed/polymorphic form of lesions were predominant in 53.5% cases (n=151) followed by macular lesions (23.1%, n=65) and papulonodular lesions (21.6%, n=61). Unusual clinical lesions such as erythrodermic, fibroid type, plaque, ulcerated skin lesions were observed in 1.8% (n=5) cases. Polymorphic/mixed forms were

predominantly present in both genders (53.3% males, n=120; 54.4% females, n=31) followed by papulonodular form in males (24.9%, n=56) and macular in females (36.8%, n=21). Papulonodular lesions were the least frequent in female (8.8%, n=5) cases whereas the macular lesions were the least frequent in males (19.5%, n=44). The unusual clinical lesions were exclusively observed in male cases (2.2%, n=5). There was a significant association between type of lesions and age ($\chi 2$ = 40.775 (6), p = 0.001), polymorphic/mixed lesions were predominant in adults aged between 19-44 years. Furthermore, type of clinical presentation were not associated with PKDL cases by the area of origin (χ^2 = 3.960(6), p = 0.682). Besides, we observed mucosal lesions in 34 PKDL cases, all males. Majority of them (n=33) received SAG treatment and one received amphotericin B treatment for VL. Mucosal involvement showed no association with lesion types (χ^2 = 4.916(3), p = 0.178).

In the present study, 79.8% (n= 225) of PKDL patients reported history of VL. Among PKDL patients with history of VL, 52.9% (n=119) had mixed/polymorphic lesions, 24.9% (n=56) had only macular lesions and 20.9% (n=47) had either papular and/or nodular. The remaining 1.3% (n=3) cases had unusual clinical presentations like erythrodermic, fibroid or plaque. There was no association between type of clinical presentation and history of VL (χ^2 = 2.289 (3) p = 0.515). In addition, there was an evidence of association ($\chi 2$ = 9.681(2), p=0.008) between cases with history of VL and the place of origin, indicating that the majority of the PKDL cases with history of VL originated from high endemic zones. Overall, the median time of manifestation of PKDL after VL treatment was 36 months (range = 1 - 384 months). PKDL lesions developed within 1 year in 13.3% (n=30), within 2-5 years in 56% (n=126) and after 5 years or more in 30.7% (n=69) cases, after apparent cure from VL **(Table 2)**. Majority of cases with history of VL (93.3%, n=210) had been treated for VL with SAG while the remaining were treated with amphotericin B (5.3%, n=12) or miltefosine (1.3%, n=3). The median time lapse after VL treatment with SAG was 36 months (range, 1-384 months) which was less compared with amphotericin B (48 months, 3.6 – 84 months) but more compared to miltefosine treatment (21 months, range 12-36 months) **(Table 3).**

Table 2: Clinical presentation with respect to time lapse after VL

Time lapse after VL (yrs)	No. of macular lesions	No. of papulon-odular lesions	No. of mixed lesions	No. of unusual clinical presenta-tions	Total No. (%)	p
< 1	9	3	18	0	30(13.34)	$\chi2 = 13.324$ (6), $p = 0.038$
1 - 5	33	30	63	0	126(56)	
> 5	13	15	38	3	69(30.67)	
Total No. (%)	55(24.44)	48(21.33)	119(52.88)	3(1.34)	225	

Table 3: Time lapse between VL and PKDL with respect to the drug used for VL

Anti-leishmanial drug	No. (%) patients	Median time lapse after VL treatment (months)	Range (months)
SAG	210 (93.33)	36	1-384
Amphotericin B	12 (5.33)	48	3.6-84
Miltefosine	3 (1.33)	21	12-36

Besides, our data indicated that there was no association between type of drug used for VL treatment and the time lapse between VL treatment and PKDL incidence ($\chi2$=1.994 (2), p = 0.369). We observed significant evidence of association ($\chi2$ =20.407(4), p<0.001) between time lapse after VL treatment and age groups. The data indicated that majority of cases belonging to age group 19-44 yrs had onset of PKDL within 1-5 yrs post VL treatment. However, endemicity ($\chi2$ = 0.332(4), p = 0.988) was independent of time lapse after VL treatment and onset of PKDL.

PKDL diagnosis

In the present study, the median time between appearance of lesions and diagnosis was 30 months (range 1-240 months). Approx. 20.6% (n=57) of cases reported within 12 months and 54.9% (n=155) within 60 months after the appearance of lesions. The *Leishmania* amastigotes were evident in 31.5% (89/282) tissue biopsy samples and in 36.2% (107/282) slit aspirate samples. Diagnosis in cases negative by microscopy

was confirmed by PCR/qPCR. Approximately, 26.95% (n=76) PKDL cases were initially misdiagnosed at primary health centres before they reported to our centre, of which, 77.63% (n=59) cases had been misdiagnosed as cases of leprosy and received either complete or partial treatment for the same. The other misdiagnoses made were sarcoidosis in 11.84% (n=9) cases, secondary syphilis in 3.94% (n=3), rosacea in 3.94% (n=3) and pityriasis versicolor in 2.63% (n=2) cases.

PKDL treatment

During the period from 1995 to 2008, a total of 59.57% (n=168) cases were diagnosed for PKDL and treated with SAG singly or in combination. Approximately 31.6% (n=89) PKDL cases were exclusively treated with SAG, of which only 14.6% (n=13) patients completed full treatment **(Table 4)**. Combination of antimonials with weak antileishmanial drugs like allopurinol, rifampicin, allopurinol and rifampicin, Mw (Immunvac) were given in 79 cases. In the group receiving SAG with allopurinol (n=56), only 17.8% cases completed treatment and achieved cure; the corresponding figure in the group treated with SAG plus rifampicin group (n=9) was 22.2%. In SAG + rifampicin + allopurinol group (n=6), none could continue injections beyond 1½ months. In the group where Mw vaccine was combined with SAG (n=8), only 25% adhered to complete treatment and attained cure.

From 2009 onwards, a total of 113 patients were treated either with miltefosine alone (n=107) or in combination with amphotericin B (n=6). Of cases treated exclusively with miltefosine, 85% (n=91) completed treatment and achieved cure, while 14.9% (n=16) remained absconded. In the 18 months follow up period, we observed 13.2% (n=12) relapses **(Table 4).** Combination of miltefosine along with amphotericin B was given to 6 PKDL cases. One patient absconded and the remaining 5 completed treatment and none relapsed. One PKDL patient who was treated exclusively with amphotericin B remained cured.

Table 4: Outcome of treatment – compliance and relapse

Treatment	No. (%) patients	No. (%) of patients who completed treatment	No. (%) of relapse
SAG	89 (31.56)	13 (14.61)	-
SAG + Allopurinol	56 (19.86)	10 (17.85)	-
SAG + Rifampicin	9 (3.19)	2 (22.23)	Not followed
SAG + Allopurinol + Rifampicin	6 (2.12)	0 (0)	-
SAG + Mw Vaccine	8 (2.83)	2 (25)	-
Miltefosine	107 (37.94)	91 (85.05)	12 (13.18)
Amphotericin B	1 (0.35)	1(100)	-
Miltefosine + Amphotericin B	6 (2.12)	5(83.33)	-
Total	**282**	**124 (43.97)**	**12 (9.67)**

This study is published in BMC Public Health Oct, 2015.

6. Evaluation of cellular immunological responses in mono- and polymorphic clinical forms of Post Kala-Azar Dermal Leishmaniasis in India

Scientific staff : Dr. Poonam Salotra, Mr. Himanshu Kaushal, Mr. Kumar Avishek, Mr. Deepak Kumar Deep

In collaboration with : Dr. V. Ramesh

Duration : 2015-16

Aims, Objectives and Background:

The current study aimed to dissect *Leishmania*-specific cell mediated immune response in polymorphic and monomorphic forms of PKDL, measuring lymphoproliferation, cytokines and granzyme B levels, T lymphocyte activation following *in vitro*stimulation of PBMCs with total soluble *Leishmania* antigen (TSLA). Taken together, the study provides precise understanding of immunopathological differences between the two distinct clinical forms prevalent in Indian PKDL.

Work done during the year:

The data in study reported regarding evaluation of PSA-2 as a vaccine candidate was taken up for analysis to compare the TSLA induced immune responses in two groups of PKDL.

Lymphoproliferative response to *L. donovani* TSLA

Cellular response was analyzed in PKDL (n=16) and naive group (n=19) in terms of lymphoproliferative response *in vitro* to TSLA, using PHA-M as a positive control. All cases showed high proliferation with PHA-M (Naive PI mean ± SE, 9.873±0.775; PKDL, 9.949±0.98). In response to TSLA stimulation, the group mean of monomorphic PKDL was found comparable to naïve group **(Fig. 11)**. However, the response in polymorphic PKDL group was found significantly high compared to naïve.

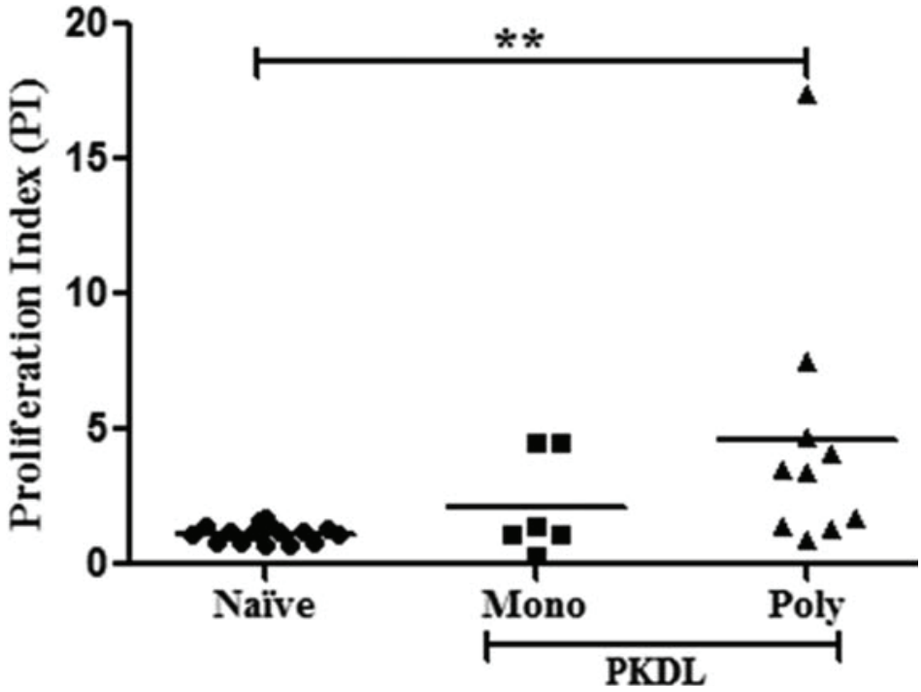


Fig. 11: Lymphoproliferative response to total soluble *Leishmania* antigen (TSLA) in post-kala-azar dermal leishmaniasis (PKDL). Data were analysed between groups by the non-parametric Kruskal–Wallis test followed by the post-hoc Dunn's multiple comparison test. The horizontal lines indicate mean value. *P < 0.05 is considered statistically significant.

Cytokine profile upon TSLA stimulation

PBMCs from all the study groups were examined for cytokine profile in response to TSLA **(Fig. 12 A-C)**. The Cell Mediated Immune (CMI) response (IFN-γ and TNF-α) in polymorphic groups was found significantly higher compared to the naïve group. The polymorphic group showed significantly high (p< 0.001) IFN-γ level compared to naive, while mean of monomorphic PKDL group was found comparable to naïve. Similarly, significantly high TNF-α production was observed in response to TSLA stimulation in polymorphic group compared to naive, while group mean of monomorphic PKDL was found comparable to naïve. For IL-10 cytokine, the measured values for polymorphic were low, and comparable to naive or monomorphic PKDL.

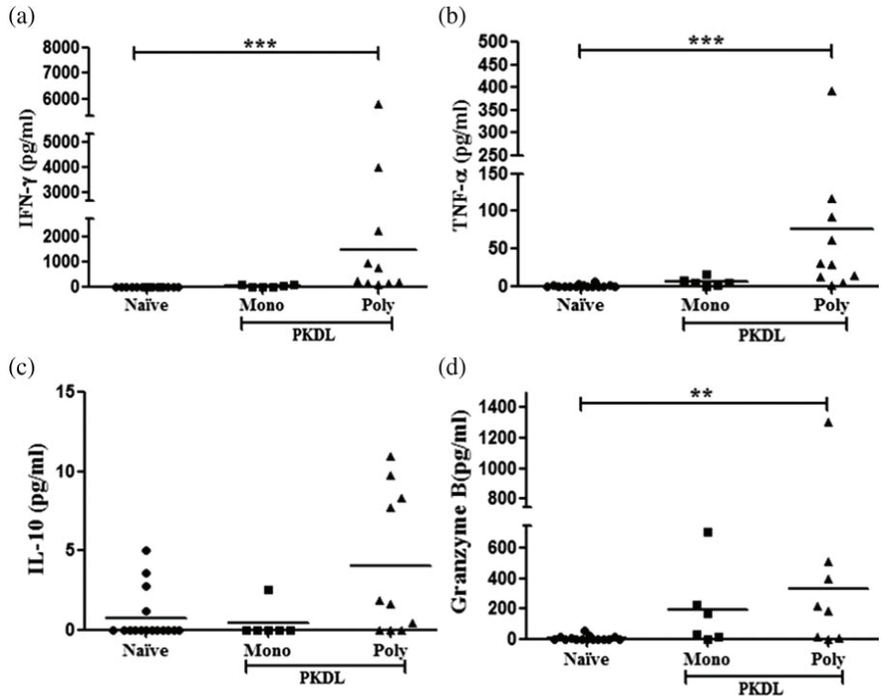


Fig. 12*: In vitro Leishmania*-specific cellular immune response in PKDL. PBMCs were cultured in presence of TSLA for 120 hrs. (A-C) Cytokines (IFN-γ, TNF-α and IL-10) were measured in the culture supernatant of PKDL and naive groups and (D) Granzyme B were analyzed in the culture supernatant of PKDL and naive groups using CBA. Data were analyzed between groups by the nonparametric Kruskal-Wallis test followed by the post hoc Dunn multiple comparison test. Horizontal lines indicate mean values. * $p< 0.05$, ** $p< 0.01$, *** $p< 0.001$.

Granzyme B production in response to TSLA stimulation

Granzyme B, a serine proteinase expressed by the cytotoxic lymphocytes such as $CD8^+$, $CD4^+$ T cells and Treg cells, induces apoptosis of target cells. Here, granzyme B level upon *in vitro* stimulation of PBMCs with TSLA was estimated in culture supernatant by CBA assay. The granzyme B level of polymorphic group was found significantly high compared to naive while group mean of monomorphic PKDL was found comparable to naïve group **(Fig. 12D).**

Determination of activated T cell population

Using CD69 as a marker of activation, we investigated the percentage of activated $CD4^+$ and $CD8^+$ T cell populations upon *in vitro* TSLA stimulation in polymorphic PKDL and naive groups. Both $CD4^+$ and $CD8^+$ T cells showed a pronounced activation, with significantly higher percentage of $CD8^+CD69^+$ T cell population in polymorphic

PKDL group compared to naive group. There was also a significantly higher percentage of $CD4^+CD69^+$ T cell compared to naive group **(Fig. 13).** The values of unstimulated cells were substracted from TSLA stimulated cells. Cell viability test using 7AAD staining of samples confirmed that the gated lymphocytes were >99% viable for both PKDL and naive groups.

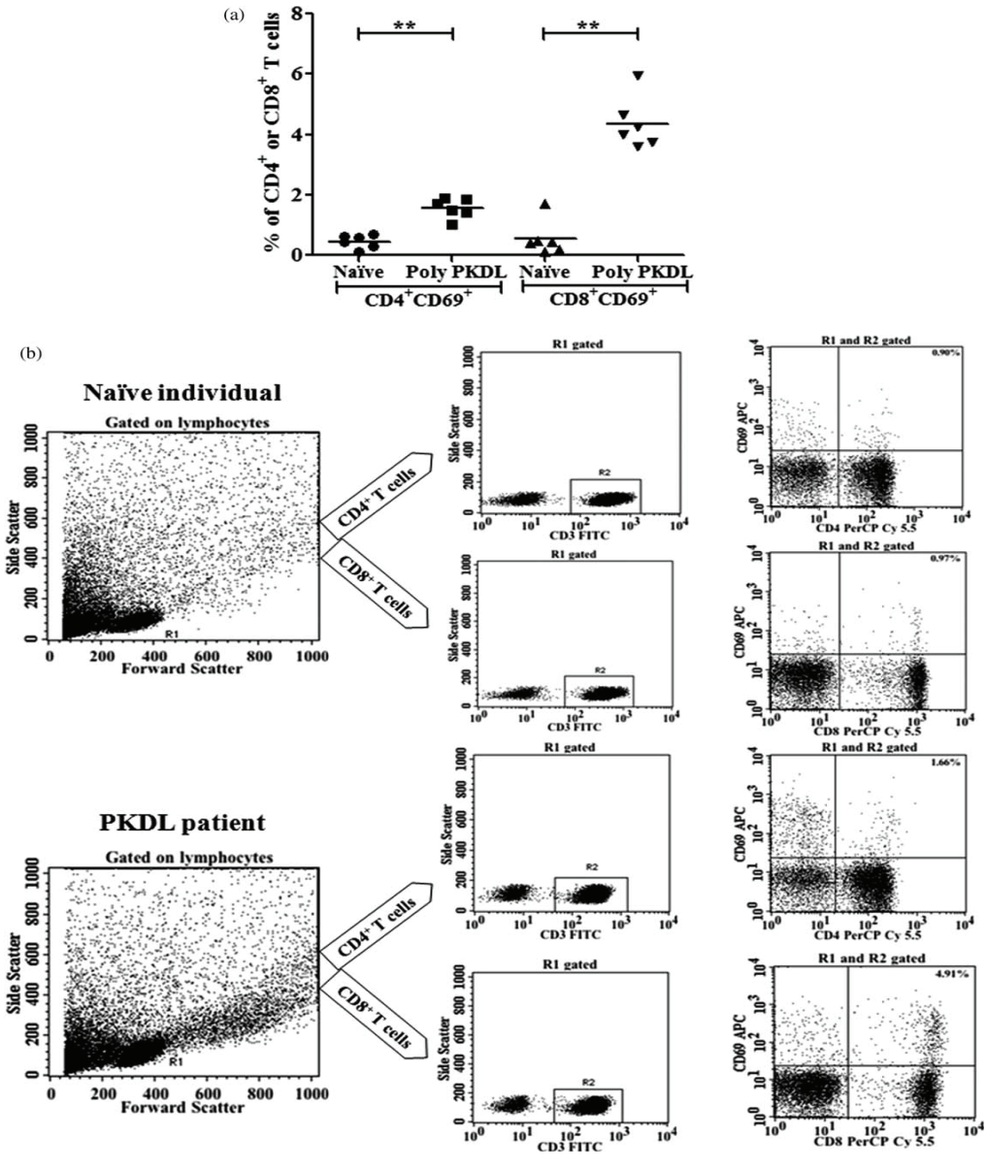


Fig. 13: Determination of activated T cell population in PKDL. (A) Percentage of TSLA-activated $CD4^+$ and $CD8^+$ T cells in polymorphic PKDL and naive groups. Analysis gates were set for lymphocytes using forward and side scatter properties and the frequencies of activated $CD4^+$ and $CD8^+$ cells were acquired on $CD3^+$ T cells. (B) The data show a representative cytofluorimetric analysis in one of the PKDL individuals. Data were analysed using nonparametric Mann-Whitney U test between groups. Horizontal lines indicate mean values.** $p< 0.01$.

This study is accepted for publication in Clin Exp Immunol.

CHLAMYDIASIS

1. Immunopathogenesis of Reactive Arthritis induced by *Chlamydia trachomatis*

Scientific Staff : Dr. Sangita Rastogi, Mr. Praveen Kumar

In collaboration with : Brig. (Dr.) DS Bhakuni, Army (R & R) Hospital, New Delhi
Dr. S Batra, Dr. G Khanna, Dr. VK Sharma, CIO, VMMC & SJ Hospital, New Delhi

Duration : 2010-15 (4.6 years) (ICMR-SRF project)

Aims, Objectives and Background:

The immunopathogenic mechanism involved in *Chlamydia trachomatis* infection induced-Reactive Arthritis (ReA) is still unknown. It was hypothesized that certain cytokines could be involved in the pathogenesis of ReA and critical in determining susceptibility to disease. A better understanding of the role of cytokines in the pathogenesis of ReA could contribute to better management of such patients and subsequent treatment. The aim of the study was to ascertain the expression of few Th1/ Th2/ Th17 cytokines, *viz.:* IFN-gamma, IL-4, IL-17 and IL-6 in the immunopathogenesis of *C. trachomatis*-induced ReA/ undifferentiated spondyloarthropathy (uSpA) patients.

Work done during the year:

Clinical details

In *C. trachomatis*-infected ReA/ uSpA patients, the median age was 26 years (IQR 21 -33); median number of SF leucocytes was 14200/ mm^3 (range: 8200 - 24600) while in *Chlamydia*-negative patients, the median age was 26.5 years (Inter-Quartile Range (IQR): 21.5 - 41.5); median number of SF leucocytes was 11200/ mm^3 (8400 - 24800). The median age of RA patients was 38 years (IQR 31 - 42); median number of SF leucocytes was 8300/ mm^3 (IQR 6300 - 12900), while in OA patients, the median age was 48 years (IQR 45 - 60); while median number of SF leucocytes was 400/ mm^3 (IQR 165 - 465).

Estimation of cytokines

Commercial ELISA kits were used to estimate IFN-gamma (*eBiosciences, USA*), IL-4 (*eBiosciences, USA*), IL-6 (*BD Biosciences, USA*) and IL-17 (*Krishgen BioSystems, CA*) in arthritic patients as per the manufacturer's instructions. Detection limit of these kits were as follows: IFN-gamma: 4 - 500 pg/ ml, IL-4: 2 - 200 pg/ ml, IL-6: 2.2 - 300 pg/ ml, IL-17: 15.6 - 1000 pg/ ml.

In synovial fluid

Level of IFN-gamma in SF of *C. trachomatis*-positive ReA/ uSpA patients (median-45.15 pg/ ml; IQR: 39.7 - 62.8) was significantly higher ('p' = 0.0003) than the negative patients (median - 23.5 pg/ ml; IQR: 12.8 - 33.3) as well as inflammatory control RA (median-22 pg/ ml; IQR: 21 - 27; 'p' = 0.0001) and non-inflammatory control OA (median 1.32 pg/ ml; IQR: 0.3 - 2.1; 'p' = 0.0001) (Fig. 1a). IL-4 was significantly higher ('p' = 0.01) in *C. trachomatis*-infected ReA/ uSpA patients (median- 19.3 pg/ ml; IQR: 14.1 - 23.4) in comparison to non-infected ReA/ uSpA patients (median- 13.7 pg/ ml; IQR: 10.5 - 19.2). The level of IL-4 in *C. trachomatis* infected ReA/ uSpA patients was also significantly upregulated in comparison to RA (median- 10 pg/ ml; IQR: 8.4 - 12.2; 'p' = 0.0001) and OA (median- 1 pg/ ml; IQR: 0.24 - 2.1; 'p' = 0.0001) (Fig. 1c).

There was no significant difference observed in the level of the pleiotropic cytokine IL-6 in *C. trachomatis*-infected ReA/ uSpA patients (median- 265.8 pg/ ml; IQR: 225.6 - 275.2; 'p' = 0.6) in comparison to non-infected patients (median- 261.1 pg/ ml; IQR: 216 - 295.8), however, it was significantly higher than both control groups (RA; median- 156.3 pg/ ml; IQR: 139.8 - 201.3; 'p' = 0.0001 and OA; median- 1.9 pg/ ml; IQR: 0.0 - 4.8; 'p' = 0.0001) (Fig. 2c).

IL-17 was comparable ('p' = 0.9) between the *C. trachomatis*-infected ReA/ uSpA patients (median- 77.6 pg/ ml; IQR: 48.8 - 91.3; 'p' = 0.0001) and non-infected patients (median- 77.3 pg/ ml; IQR: 48.8 - 91.3) and RA patients (median- 77.3 pg/ ml; IQR: 39.9 - 102.4; 'p' = 0.5). However, it was significantly high in comparison to OA patients (median- 1.8 pg/ ml; IQR: 1.1 - 2.5; 'p' = 0.0001) (Fig. 2a).

In serum

IFN-gamma level in serum of *C. trachomatis*-infected ReA/ uSpA patients was higher ('p' = 0.06 (non-significant), median- 29 pg/ ml; IQR: 21.8 - 40.9) than the *C. trachomatis*-non-infected patients (median- 21.1 pg/ ml; IQR: 12.3 - 27.7). Level of serum IFN-gamma in *C. trachomatis*-infected ReA/ uSpA patients was significantly upregulated in comparison to RA ('p' = 0.009; median- 19.6 pg/ ml; IQR: 11.8 - 23.1) and OA ('p' = 0.0001; median- 1.32 pg/ ml; IQR: 0.3 - 2.1) (Fig. 1b). Serum levels of IL-4 in *C. trachomatis*-infected ReA/ uSpA patients were significantly elevated ('p' = 0.006; median- 14.1 pg/ ml; IQR: 10.4 - 22.2) than the non-infected ReA/ uSpA patients (median- 10.5 pg/ ml, IQR: 8.3 - 11.7) Furthermore, the level of IL-4 was significantly high in the *C. trachomatis*-positive ReA/ uSpA patients in comparison to RA ('p' = 0.001; median- 9.2 pg/ ml; IQR: 7.4 -11.6) and OA ('p' = 0.0001; median- 1 pg/ ml; IQR: 0.24 - 2.1) (Fig. 1d). In contrast to the synovial findings, the pleiotropic cytokine IL-6 was significantly enhanced in *C. trachomatis*-infected ReA/ uSpA patients ('p' = 0.04; median- 15.2 pg/ ml; IQR: 8.6 - 28.1) in comparison to patients without *C. trachomatis* infection (median- 6.96 pg/ ml; IQR: 3.5 - 21.5). Also, *C. trachomatis*-infected ReA/ uSpA patients had higher levels of IL-6 in comparison to RA ('p' = 0.02; median- 6.98 pg/ ml; IQR: 4.4 - 21.6) and OA ('p' = 0.0001; median- 2.3 pg/ ml; IQR: 1.8 - 4.8) (Fig. 2d). IL-17 levels were differential in contrast to SF findings but there was no significant difference between *C. trachomatis*-infected ('p' = 0.6; median- 100 pg/ ml; IQR: 41.9 - 149.6) and non-infected ReA/ uSpA (median- 58 pg/ ml; IQR: 39.2 - 152.3) patients. It was found that the level of IL-17 in infected ReA/ uSpA was non-significant in comparison to RA ('p' = 0.07; median- 77.3 pg/ ml; IQR: 39.9 - 102.4) while it was significantly elevated as compared to OA ('p' = 0.0001; median- 1.8 pg/ ml; IQR: 1.1 - 2.5) (Fig. 2b).

Correlation between synovial fluid and serum cytokines

Cytokine findings in *C. trachomatis*-infected ReA/ uSpA patients (n- 12) were compared in both SF and serum by Kruskal Wallis test ('p' = 0.0001) and correlated to each other using Spearman's rank correlation test. In SF, IL-6 was found to be positively correlated (r = 0.72; 95% CI: 0.23 - 0.91; 'p' = 0.007) with IFN-gamma (Fig.3). Also, SF IFN-gamma was found to be correlated with serum IFN-gamma ($p < 0.05$).

Cytokine levels in SF and serum of *C. trachomatis*-positive ReA/ uSpA patients were further analyzed using non-parametric Mann-Whitney test. Upon analysis, both IFN-gamma and IL-6 were increased ('p' = 0.4 and 'p' = 0.0001, respectively) in SF in comparison to serum; while IL-17 was higher ('p' = 0.4) in serum than in SF. However, IL-4 was comparable ('p' = 0.7) in both SF and serum.

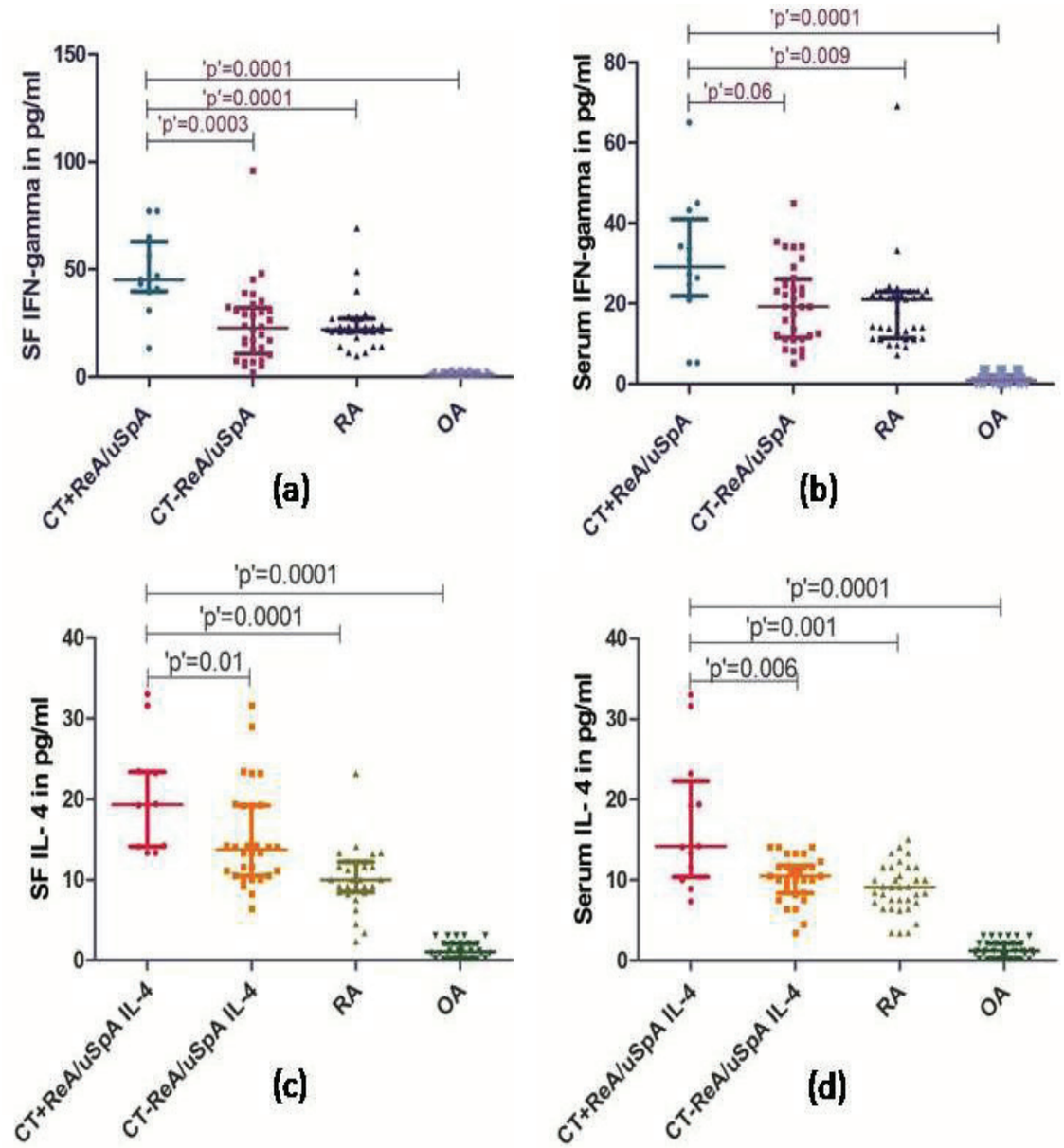


Fig. 1: IFN-gamma level in (a) synovial fluid and (b) serum of arthritic patients & IL-4 levels in (c) synovial fluid and (d) serum of arthritic patients

Abbreviations: CT- *Chlamydia trachomatis*; ReA- Reactive Arthritis; uSpA- Undifferentiated Spondyloarthropathy; RA- Rheumatoid arthritis; OA- Osteoarthritis; CT+ *C. trachomatis*-positive; CT- *C. trachomatis* non-infected

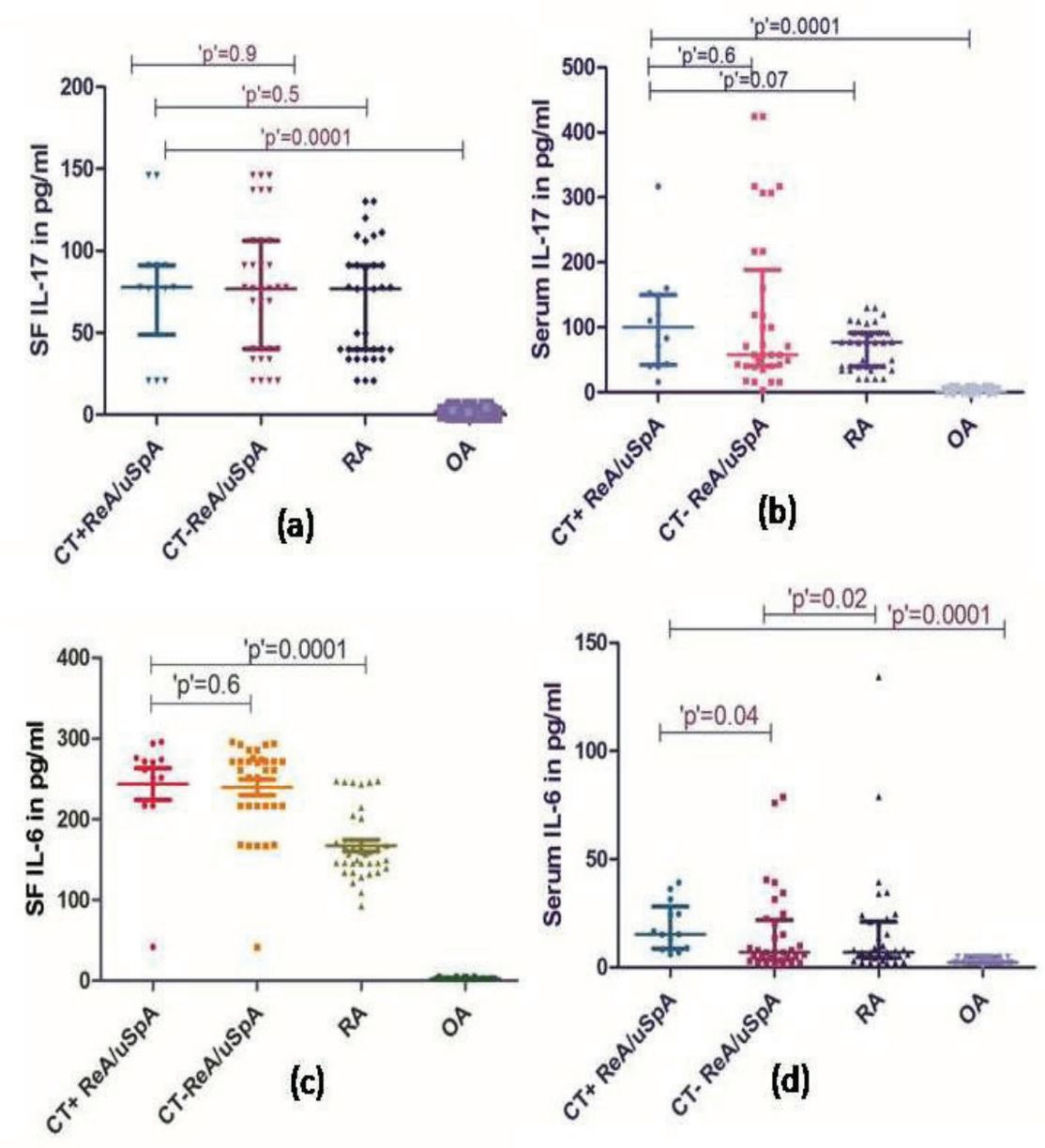


Fig. 2: IL-17 levels in (a) synovial fluid and (b) serum of arthritic patients & IL-6 level in (c) synovial fluid and (d) serum of arthritic patients.

Abbreviations: CT- *Chlamydia trachomatis*; ReA- Reactive Arthritis; uSpA- Undifferentiated Spondyloarthropathy; RA- Rheumatoid arthritis; OA- Osteoarthritis; CT+ *C. trachomatis*-positive; CT- *C. trachomatis* non-infected

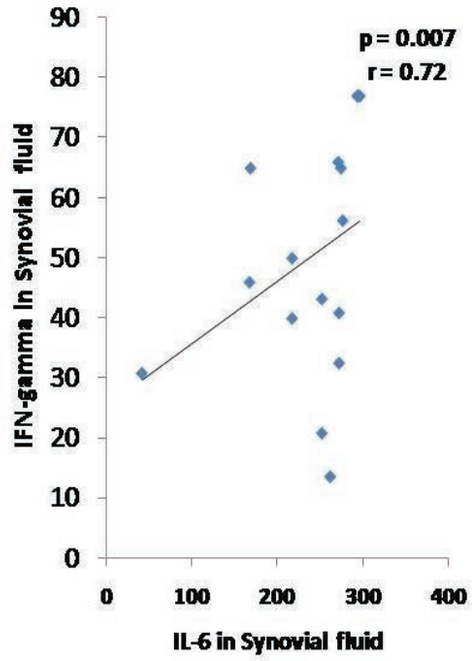


Fig. 3: Correlation between IFN-gamma and IL-6 cytokines in synovial fluid in *Chlamydia trachomatis*-positive reactive arthritis/undifferentiated spondyloarthropathy patients by Spearman's Rank Correlation test.

TUBERCULOSIS

1. To study the immunomodulatory role and antigenicity of Co-operonic *PE32/PPE65* genes belonging to the *Region of difference-8 (RD-8)* of the *Mycobacterium tuberculosis* genome.

Scientific Staff	:	Dr. Nasreen Z. Ehtesham, Dr. Mohd Khubaib, Dr. Javaid Sheikh
In collaboration with	:	Prof. Seyed E. Hasnain, IIT, Delhi
Duration	:	2015-17

Aims, Objectives and Background:

The PE/PPE family of proteins constitute about 10% of total genome of *Mycobacterium tuberculosis*. The role of these highly expanded and enigmatic protein families are not fully understood. Many members of these gene families have been described to have multiple functions (moonlighting) by gene cooption as a consequence of genomic reduction. Several *PE/PPE* genes are co-operonic and are present in clusters along with ESX and/or other proteins. PE/PPE genes, present in cluster with ESAT-6 like genes, are suspected to have a role in antigenic variation and virulence of *Mycobacterium tuberculosis.* Their roles in immune evasion and immune modulation of host are also well documented.

This study demonstrates the operonic organization of PE32/PPE65 genepairpresentin RD-8 region of *Mtb* genome and their role in antigenicity & immune modulation of host.

Work done during the year:

PE32 and PPE65 are organized in an operon and transcribed as a single mRNA

In-silico analysis predicted several PE/PPE genes, including the PE32/PPE65 pair to be organized as operons in the genome.This was confirmed by RT-PCR using specific

reverse and forward primers of PPE65 and PE32.For amplification of PE32 (P1, P2), PPE65 (P3, P4) and PE32+PPE65 (P1, P4) were used. Agarose gel electrophoresis of the amplified product clearly shows amplification fragment of 300bp corresponding to PE32, 1.2kb of PPE65 and 1.5kb of PE32+PPE65 (Figure 1A). These results validated the *in-silico* observation on the co-operonic natureof PE32/PPE65 gene pair.

Co-translation of PE32 and PPE65

The complete cassette of PE32+PPE65 gene with the native intergenic region was cloned in pETDuet1. This was cloned under the transcriptional control of T7 promoter with RBS and His tag fused to the PE32 gene (Figure1, B). Assuming that a single mRNA will be transcribed for both the genes, RBS present in vector will carry out the translation of PE32 while native RBS present before the PPE65 gene will initiate translation of PPE65. This indeed could be seen when the protein expression product present in the supernatant of culture lysate was loaded on 10% tricine SDS gel. Over expression of PE32 (10kDa) and PPE65 (40.7kDa) can be observed (Figure1, C) as compared to un-induced culture. These data confirms the co-operonic nature of PE32/ PPE65 gene pair.

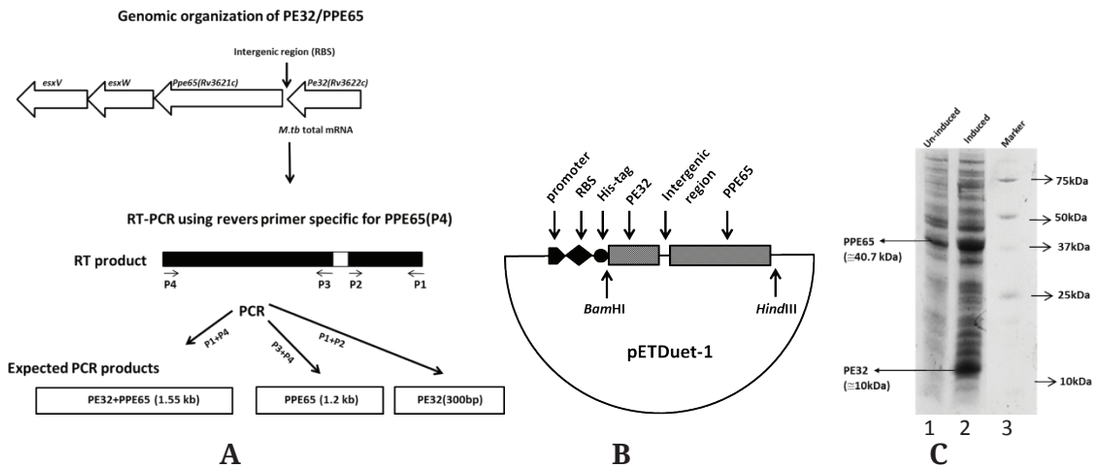


Fig. 1: Genomic organization and expression analysis reveals PE32 and PPE65 as functionally linked co-operonic gene pair. PE32/PPE65 (Rv3622c/Rv3621c) gene pair is organized in an operon. (A) Schematic representation of co-operonic genes. Primer pair specific to PE32 produced amplicon of the size 300bp, specific primer pair for PPE65 yielded amplification of 1.2kb, and forward primer for PE32 and reverse primer for PPE65 revealed an amplification product of 1.5 kb. (B) PE32/PPE65 genes are expressed together at protein level. Expression of PE32/PPE65 gene was checked on 10% tricine SDS gel. Lane 1 is un-induced culture, Lane 2 is induced culture showing the expression of PE32 (10 kDa) and PPE65 (40.7 kDa), Lane 3 is protein ladder.

PE32/PPE65 proteins reduce pro-inflammatory response and concomitantly enhance anti-inflammatory response.

Varying concentration of purified recombinant PE32 and PPE65 with N-terminal Histidine tag,were usedto stimulate RAW264.7 cells. PPE65 was shown to increase the level of anti-inflammatory cytokine IL-10 in a dose dependent manner (Figure2, A). PE32 also increased IL-10 production, though not significantly. Combinations of the two proteins also lead to significant elevation in IL-10 secretion. Simultaneously we evaluated pro-inflammatory cytokines. IL-6 and TNF-α secretion by RAW 264.7 cells decreases (Figure 2, B and C) as a direct function of concentration of recombinant proteins. These results demonstrate that both PE32 and PPE65 proteins are immunomodulatory in nature and play role in either establishment or maintenance of infection to favor survival of pathogen by altering the cytokine milieu.

B-cell response against PPE32/PPE65 in immunized mice

Among several operonic PE/PPE proteins, PE had been seen to be less or non-immunogenic in mice as well as in humans. We used DNASTAR prediction tool and expectedly found that PPE65 has numerous antigenic patches as compared to PE32 which had a few antigenic stretches indicating that PE32 could be less immunogenic. *In-vivo* antigenicity assay was carried out by immunizing mice with PE32, PPE65 and PE32+PPE65 followed by booster at 15th day of primary immunization. Serum was collected after 4 weeks of primary immunization. ELISA was performed for IgG1, IgG2a and IgG2b response against PE32, PPE65 and PE32+PPE65. IgG1 was the predominant subtype in immunized animal as compared to other subtypes (Figure3, A).Higher IgG2a and IgG2b response could also be observed in PPE65 and PE32+PPE65 immunized mice as compared to that of PE32 immunized mice which displayed low levels of all the isotypes (Figure3, B&C). Further, IgG1 and IgG2a ratio revealed that PPE65 and PE32+PPE65 induce Th2 response (Figure3, D). IgG isotype ratio clearly suggested predominance of Th2 response over Th1 that impairs anti-mycobacterial immunity.

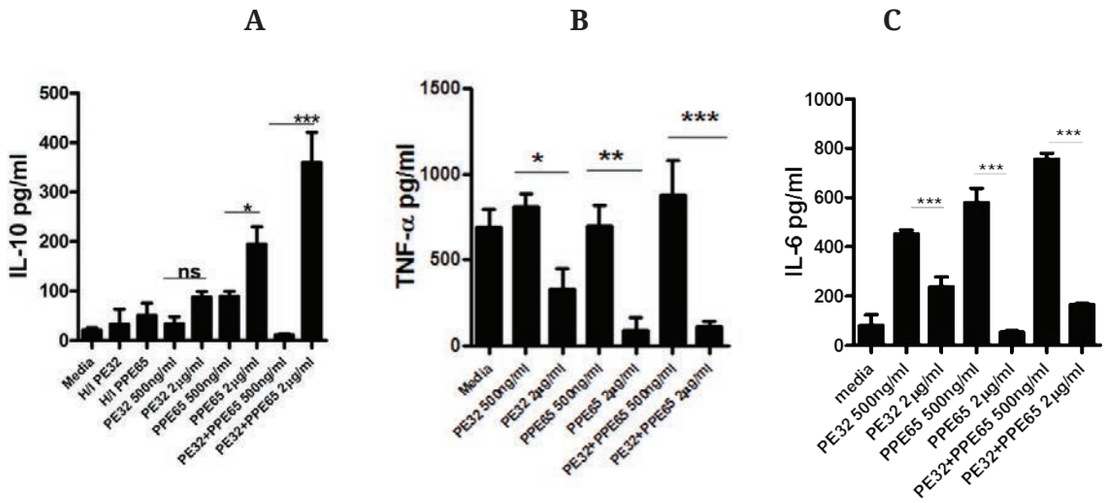


Fig. 2: PE32 and PPE65 modulate the host immune pathway by suppressing pro inflammatory cytokine production. RAW 264.7 cells were treated with purified recombinant PE/PPE proteins in different concentration for 24 hours, culture supernatants were collected for measuring various cytokine levels through sandwich ELISA. **(A)** PE32, PPE65 induce IL-10 level in RAW 264.7 cells as a direct function of protein concentration whether used singly or together. **(B&C)** Note the decrease in the level of Th1 cytokine (IL-6 and TNF-α) in RAW 264.7 cells with increase in protein concentration Experiments were performed at least twice; SEM is represented by error bar for biological triplicates. *p<0.05, ***p<0.001.

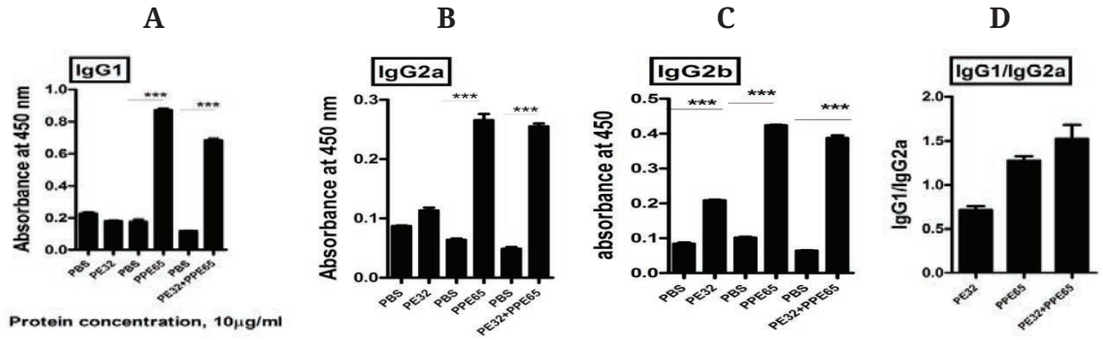


Fig. 3: Antibody isotype analysis reveals the skewing of immune phenotype towards Th2 type of response. Sera from immunized mice were used to perform ELISA to measure IgG1, IgG2a and IgG2b. (A) Mice immunized with PE32 show low IgG1 while those immunized with PPE65 and PE32+PPE65 proteins showed higher levels of IgG1 against respective proteins. (B&C) Similar observation was made for IgG2a and IgG2b. IgG1 vs IgG2a ratio is important to determine whether the response is Th1 or Th2. (D) The IgG1/IgG2a> 1 shows Th2 response for PPE65 and PE32+PPE65. Error bar represents SEM of average of 3 animals in biological triplicates. ***p<0.001

PE32/PPE65 proteins suppress Th1 cytokines production in splenocytes.

Treatment of macrophage cell lines with PE32/PPE65 proteins demonstrated decrease in pro-inflammatory cytokines along with increase in anti-inflammatory cytokine. Result of IgG subtype responses also corroborated a shift towards Th2 type of immune response. Expectedly, animals immunized with these proteins either singly or in combination also depicted a decrease in IFN-γ level in splenocytes culture supernatant with increasing concentration of PE32, PPE65 and PE32+PPE65 (Figure4).These finding directly point to the role of these proteins in dampening the anti-mycobacterial Th1 response. Having observed decrease in IFN-γ levels in culture supernatant of splenocytes treated with recombinant proteins, we assayed for T-cell activity by measuring $CD4^+/CD8^+$ cells positive for IFN-γand IL-2 by flow cytometry. A decrease in $CD4^+$ and $CD8^+$ cells positive for IFN-γ and IL-2 could be seen after treatment with these PE/PPE proteins, with maximum decrease observed in splenocytes primed and treated with PE32+PPE65 (Figure5, A&B).

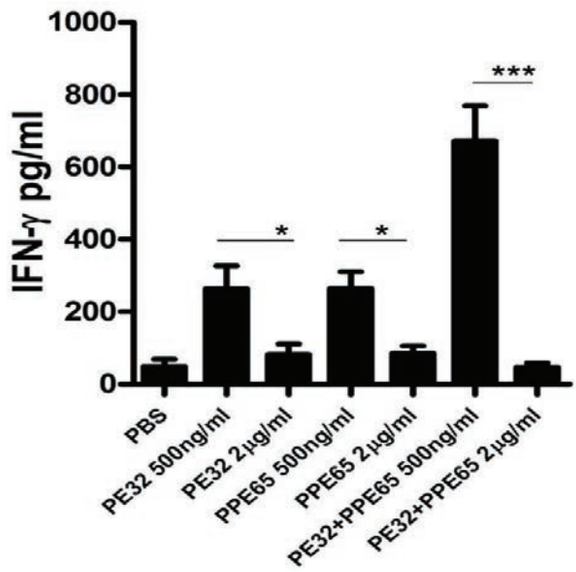


Fig. 4: PE32 and PPE65 proteins reduce the secretion of IFN-γ in splenocytes of mice immunized with respective proteins.Splenocytes isolated from mice which were immunized with PE32, PPE65 and PE32+PPE65 proteins were again challenged with corresponding proteins in culture for 72 hours, supernatants were harvested and sandwich ELISA for IFNγ was performed. Note the decrease in IFNγ level with increase in protein concentration. Experiments were performed at least twice; SEM is represented by error bar for biological triplicates. *p<0.05, ***p<0.001.

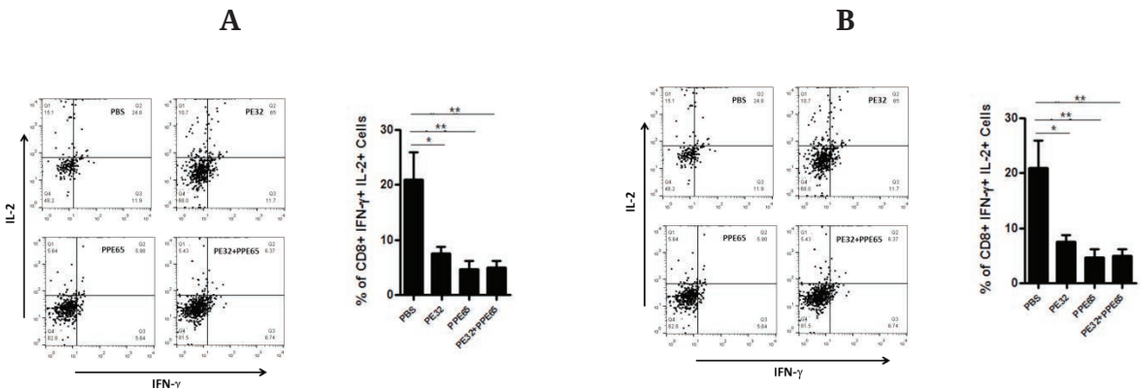


Fig. 5: PE32 and PPE65 proteins reduce polyfunctional $CD4^+$ and $CD8^+$ cells in immunized mice. Splenocytes isolated from mice which were immunized with PE32, PPE65 and PE32+PPE65 proteins were again challenged with same proteins in culture for 8 hours and stained for intracellular IFN-γ and IL-2. **(A)** PE32 and PPE65 proteins decrease intracellular IFN-γ and IL-2 level as depicted by graphical representation of change in number of $CD4^+$ cells positive for IFNγ and IL-2. **(B)** These proteins also decreased frequency of $CD8^+$ cells expressing IFNγ and IL-2 as evident from graphical representation of change in frequency of $CD8^+$ cells positive for IFNγ and IL-2. Dot plots are representative plots from at least 4 animals for each group. *$p<0.05$.

2. Understanding the structural flexibility and functional diversity of PE/PPE protein family of *Mycobacterium tuberculosis*: Identification and role of unstructured/ disordered regions in this family using *in-silico* tools

Scientific Staff	:	Dr. Nasreen Z. Ehtesham, Javeed Ahmad
In Collaboration with	:	Dr. A. Srinivasan, AIIMS and Prof. Seyed E. Hasnain, IIT Delhi
Duration	:	2012-18

Aims, Objectives and Background:

Mycobacterium tuberculosis (*M.tb*) is one of the most successful obligate human pathogens that exhibits a surprising capacity for adaptation to the host immune system. PE and PPE family proteins, present exclusively in the *Mycobacterium*, constitute about 10% of the *M.tb* coding capacity. These genes are so named due to the presence of Pro-Glu (PE) and Pro-Pro-Glu (PPE) signature motifs near the N-terminus of their gene products. Our previous study on comparison of these proteins between virulent and non virulent strains highlights their importance in disease process. Work from our group as well as others studies support their role in antigenic variation, immune evasion, persistence and interaction with the host macrophages.

Work done during the year:

We performed the disordered protein analysis in the PE/PPE, Mce, MmpL and secretome of H37Rv, prediction of protein binding sites and ELM search was also carried out. Functional characterization of one of the member of PE PPE family, PPE37 coded by Rv2123 was done to validate our *in-silico* analysis.

PE_PGRS family of Proteins Harbors Highest Disorder:

Intrinsically unstructured/disordered proteins (IUPs/IDPs) function via binding specifically to other macromolecules that involves a disorder-to-order transition. The structural flexibility of IUPs/IDPs helps in multiple interaction to the protein with

least specificity. Our hypothesis was that disordered proteins might help in host-pathogen interactions, host modulation through molecular mimicry and provide functional diversity in *Mycobacterium tuberculosis* proteome, where reductive evolution is seen during the course of pathogenesis. Interestingly, we found PE_PGRS subfamily of PE PPE family as highly disordered among the selected families and with rest of the proteome taken as control group Fig.1. This family is known to help the pathogen in host-pathogen interactions and antigenic variation. Significant amount of disorder as expected was also observed in secretome proteins as well.

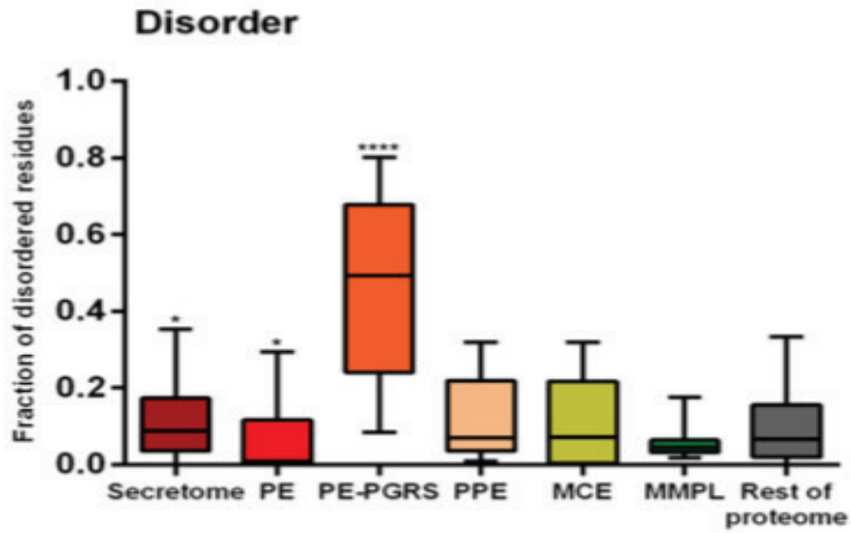


Fig. 1: Boxplots shows Protein disorder content of secretome, PE, PE_PGRS3, PPE, Mce and MmpL families with rest of the proteome. Disorder content of each group is plotted on Y-axis and families on X-axis. From the box plots we can see PE_PGRS family has highest disorder in comparison with rest of the proteome (which includes H37Rv proteome without these selected families)

Disordered proteins regions contain important functional elements, which can be analyzed through different computational tools. We used ANCHOR (http://anchor.enzim.hu/) tool for prediction of protein binding regions in disordered regions. We analyzed the protein binding regions in all selected families and found that PE_PGRS family with the higher number of binding sites in concurrence with its disorder content, results were calculated as binding sites per 100 residues of each member (Fig. 2)

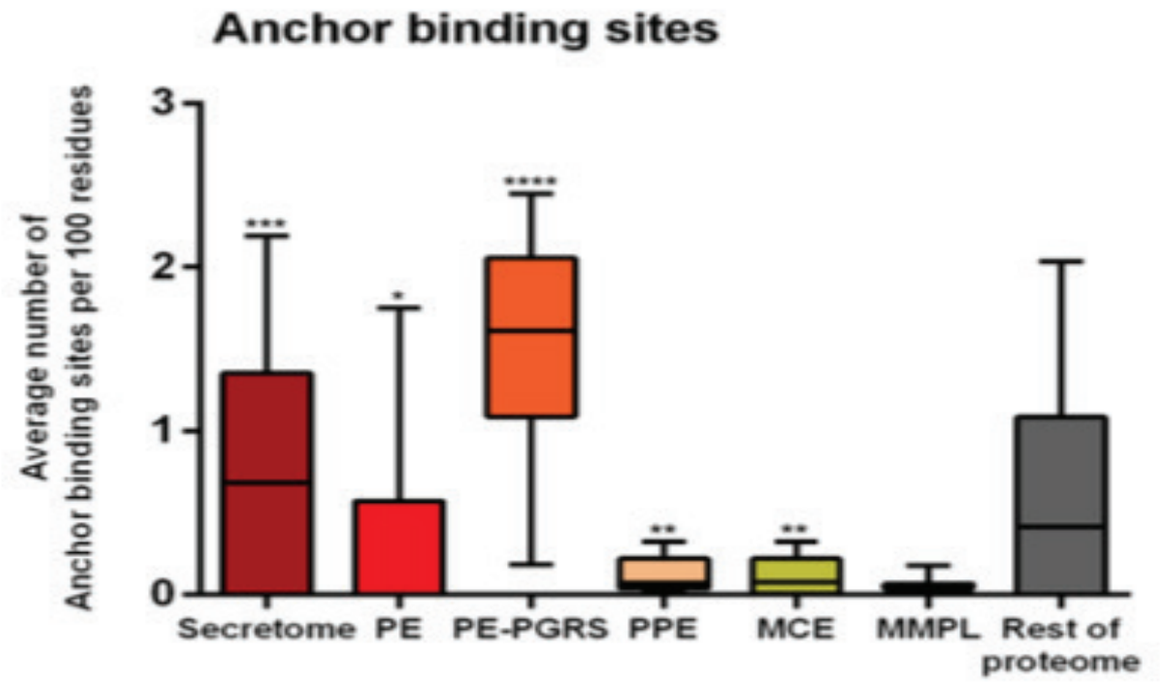


Fig. 2: Boxplots showing average number of ANCHOR binding sites per 100 residues in each group and from figure PE_PGRS family is highly enriched with protein binding sites in disordered regions. PE subfamily and MmpL protein family are having least disorder content and ANCHOR binding sites on comparison.

ELM patterns are more prevalent in PE_PGRS subfamily and Secretome:

Prediction of binding sites in disordered regions by ANCHOR does not give any information about the interacting partner. A complementary approach is to search for ELMs in disordered binding regions. It is believed that protein-protein interactions are mediated through specific linear motifs that capture key residues responsible for binding. The presence of linear motifs reduces the complex task of finding putative protein binding sites to a simple pattern-matching problem. We next scanned for ELMs with the previously identified ELMs in all selected families. The average number of ELMs was searched in the region, which is disordered and has anchor-binding sites as well per 100 residue of the protein. This search will decrease the number of false positive prediction sites. We found that ELMs which fall in disordered regions and ANCHOR binding sites are more prevalent in PE_PGRS family (Fig.3) followed by secretome while as rest members don't not show such significant number of matching ELMs in the protein sequence. False positive rate prediction in

the ELM search is very high and therefore, to validate our results we did the low probability ELMs search in all families and rest of the proteome as control group. We found that PE_PGRS family shows significantly lower number of ELMs with low probability on comparison with rest of the proteome and other groups selected for analysis. The higher probability occurrence of ELMs in PE_PGRS family proteins increases their role in molecular mimicry of the host and modulation of host machinery in favor of pathogen.

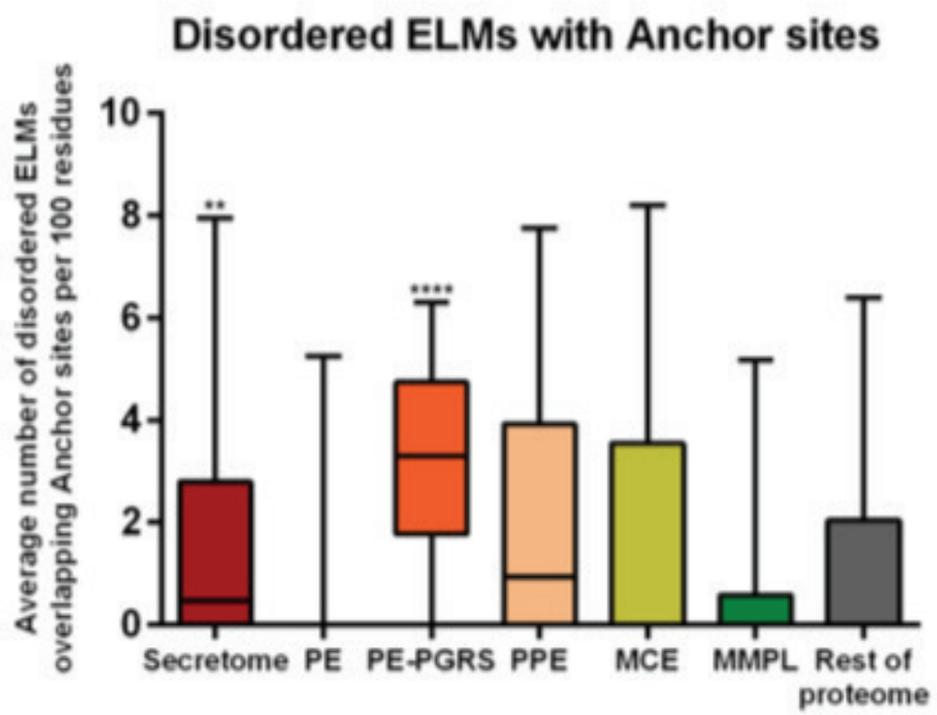


Fig. 3: Boxplot analysis of Average number of ELMs overlapping ANCHOR binding sites and disordered regions. PE_PGRS and secretome are highly enriched with ELMs overlapping in disorder regions and ANCHOR binding sites while as MmPL and PE families least ELM content

PPE37, a partially unstructured protein has an iron binding motif in its N-terminal segment and a nuclear localization signal (NLS) in the C-terminal segment

Computational analyses of PPE37 sequence revealed that the protein consists of a N-terminal PPE domain and a C-terminal intrinsically unstructured separated by two central trans-membrane segments of approximately 20 amino acids with a

small helical hinge region in between. Stretches of 225 amino acids (aa), constituting N-terminal segment (P37N), and 191 amino acids constituting C-terminal segment (P37C) flank the trans-membrane anchor (Fig. 4). An iron binding motif (DFLE) at the N-terminal is present close to the trans-membrane anchor and an NLS is predicted within the unstructured C-terminal segment.

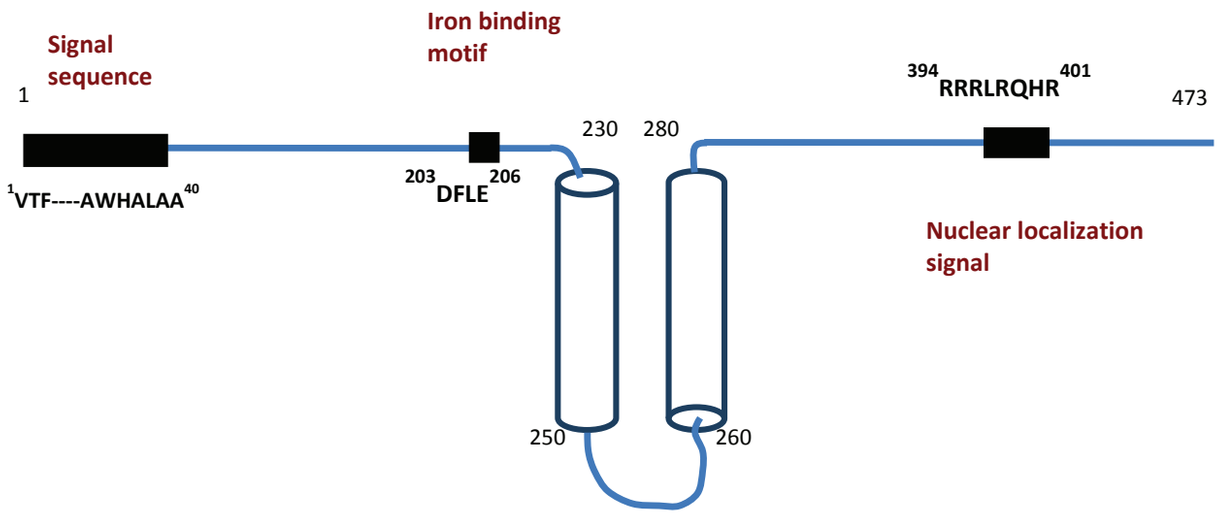


Fig. 4: Structural analysis of PPE37 revealed presence Iron binding motif in N-terminal segment whereas Nuclear Localization Signal and number of important Eukaryotic Like Motifs in C-terminal region.

PPE37 localizes to the cell membrane of *M.tb*:-

M.tb fractions collected from cultures grown for 36 hours under low iron conditions shows induction as a function of time, as PPE37 gene is under IdeR regulator and is induced by low iron conditions (Fig 5A). Immunoblot analysis of various cell fractions such as culture filtrate (CF), cytosol (C), cell wall (CW) and cell membrane (CM) using anti-P37FL (anti-PPE37 full length) antibody indicated the presence of 48 kDa protein band corresponding to PPE37 mainly in the cell membrane fraction (Fig. 5B; lane 4) but not in the culture filtrate (lane 1), cytosol (lane 2) or cell wall (lane 3) fraction.

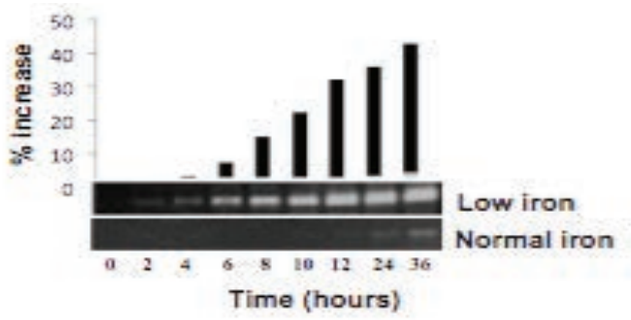


Figure 5A

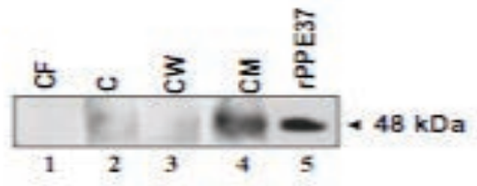


Figure 5B

Fig. 5: (A) PPE37 Induced under low iron conditions and RT-PCR analysis at different time points, 0, 2, 4, 6, 8, 10, 12, 24 and 36hrs show increased transcription of PPE37 in H37Rv cultures. (B) Immunoblot analysis of various cell fractions of *M.tb* H37Rv using anti-P37FL (anti-PPE37 full length) antibody shows presence of protein corresponding to PPE37 mainly in the cell membrane fraction.

N-terminal domain (P37N) leads to cell proliferation and differentiation whereas C-terminal domain (P37C) is localized in nucleus and induces apoptosis:-

To evaluate the functional consequence of the differential localization of N and C terminal segments of PPE37 within the host cell, morphological analysis of THP-1 cells was carried out after transfection with pC-P37FL, pC-P37N and pC-P37C. The expression and cellular localization of the resultant proteins after transfection was first ascertained by immunoblotting of the nuclear and cytoplasmic lysates. The presence of N-terminal segment in the cytoplasmic fraction (Fig. 6A, upper panel, lane 2) and the C-terminal segment in the nuclear fraction (Fig. 6A, lower panel, lane 3) is evident. Phase contrast microscopy was then carried out to assess the effect of N- and C-terminal segment on the overall morphology of monocytic THP-1 cell line. After 24 hours of transfection with pC-P37N (Fig. 6B,e) or incubation with rP37N (Fig. 6B, g), the THP-1 cells were seen to undergo proliferation and differentiation (Fig. 6B, e2 and g2, e2i and g2i) into adherent stellate cells with dendritic cell-like morphology. These cells also divided at a higher than normal rate compared to the untransfected THP-1 cells (Fig. 6B compare, e3 and g2i with 2B, a2) or cells transfected with the vector control (Fig. 6B compare, e3 and g2i with 2B, b2) and a visible increase in the number of cells (Figure 6B, e3). In contrast, cells transfected with pC-P37C (Fig. 6B, f2), earlier shown to localize to the THP-1 nucleus, were seen to undergo apoptotic cell death, evident from blebbing and spilling of the cellular components (Fig. 6B, f2,

f2i). The expression of full-length construct, pC-P37FL, however does not lead to any significant morphological sign of cellular differentiation or apoptosis after 24 hours of transfection (Fig.6B, d2). The untransfected THP-1 cells and vector-transfected cells (Fig 6B, 'a' and 'c') grew normally. Transfection of RAW264.7 or J774 cells (instead of THP-1 cells) with pC-P37C as well as pC-P37FL also corroborated the same results.

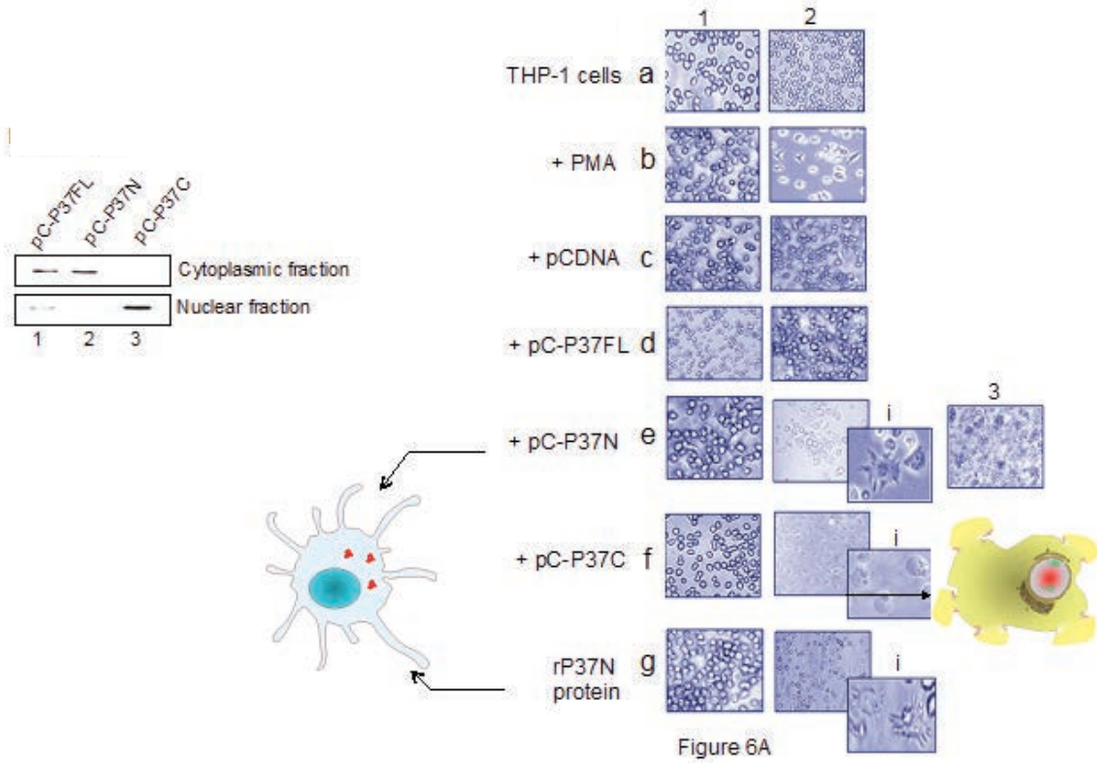


Fig. 6: N-terminal is localized to cytoplasm and promotes cell differentiation whereas C-terninal part is responsible for apoptosis. (A) Immunoblotting of cytoplasmic and nuclear lysates prepared from THP-1 cells transfected with (1) pC-P37FL, (2) pC-P37N or (3) pC-P37C was carried out. (B) Morphological analysis of (a) THP-1 cell, THP-1 cells transfected with (b) pCDNA 3.1 or (c) pC-P37FL or (d) pC-P37N or (e) pC-P37C or (f) incubated with purified rP37N (3 μg) protein. In all the cases, panel 1 shows cell morphology at the time of initiation of experiment and panel 2 is after 24 hours of incubation.

Caspase-3 activation and minimal DNA damage is the hallmark of cell death induced by the C-terminal segment:-

Cells transfected with pC-P37C undergo apoptotic cell death as could be seen from morphological (Fig. 6B) analyses. We further sought to analyze the cellular effectors that modulate this outcome. Immunoblotting of the THP-1 cell lysates at 12, 24 and

36 hours of transfection with pC-P37C demonstrated the activation of caspase-3 as evident from the presence of low molecular weight cleavage products (Fig 7).

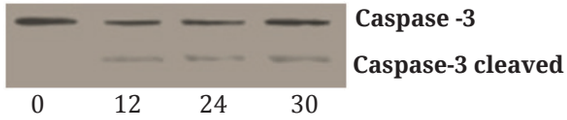


Fig. 7: Immunoblotting of whole cell lysates of THP-1 cells transfected with pC-P37C indicates the activation of caspase-3 leading to a cleaved product at 12, 24 and 30 hours of transfection.

3. *Mycobacterium tuberculosis* Unique Signature Protein Binds DNA Non-Specifically and Protects against Reactive Oxygen Species

Scientific Staff	:	Dr. Nasreen Z. Ehtesham, Javeed Ahmad. Manjunath P, Dr. Javaid Sheikh, Simran Kaur Arora
In Collaboration with	:	Prof. Seyed E. Hasnain, IIT, Delhi
Duration	:	2012-17

Aims, Objectives and Background:

In nature, the free-living species require larger genomes than parasitic species. This trend is also clearly evident from analyses of mycobacterial genomes where a distinct pattern of decreasing genomic content is seen as one moves from non-pathogenic pathogens (NP) to opportunistic pathogens (OP) to true pathogens (TP). We therefore performed genome size analysis with 44 *Mycobacterium* strains that represented NP, OP, and TP, and our analysis revealed that NP strains on average are bigger than those of OP and TP strains. Analyses of comparative metabolic pathways between different mycobacterium species pointed to the presence of novel alternative pathways in *M .tuberculosis* with implications for pathogenesis and survival in the human host and identification of new drug targets and vaccine candidates.

We used *in-silico* approach to identifying the unique proteins of *M.tb* followed by their characterization using different wet lab experiments.

Work done during the year:

In-silico analysis of Signature Protein:

Previously, we have reported two unique proteins (SP1 and SP2) that are signature to *M.tb*. One of the signature protein (SP2) displays iron binding activity and protection of DNA from hydroxyl radical damage. Now using bioinformatic analysis methyltransferase and DNA binding leucine zipper motif was identified. Secondary structure prediction showed the protein has highly ordered structure with very less disorder content around.

Signature Protein Shows S-adenosyl dependent DNA- Methyltransferase Activity:

Methyltransferase activity of Signature protein 2 was validated using different concentrations of purified recombinant SP2 for colorimetric assay of methylation to the universal substrate coated to the wells.BSA was used as test negative control.1

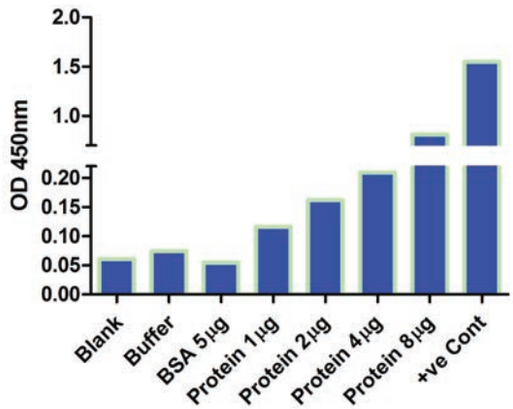


Fig. 1: Different concentration of recombinant signature protein, BSA and positive control were incubated with assay buffer supplemented with SAM. Figure shows the absorabance maxima of different controls and varying concentrations of test protein. Positive control used according to manafactures guidelines . BSA used at a concentration of 5µg and protein storage buffer were used as test negative control.

Signature Protein Shows Non-specific DNA Binding:

DNA binding ability of Signature protein was analysed using Fluorescence

Spectroscopy in both concentration and time dependent manner. The spectra measured in presence of increasing concentrations of DNA get decreased relative to the spectrum collected in absence of DNA as shown in Figure 2A. The spectra obtained at different time points 5, 10 and 15 minutes post DNA addition at single concentration showed greater shift or decrease in fluorescence compared to spectrum in absence of DNA or were increasing concentrations of DNA were used. Figure 2B.

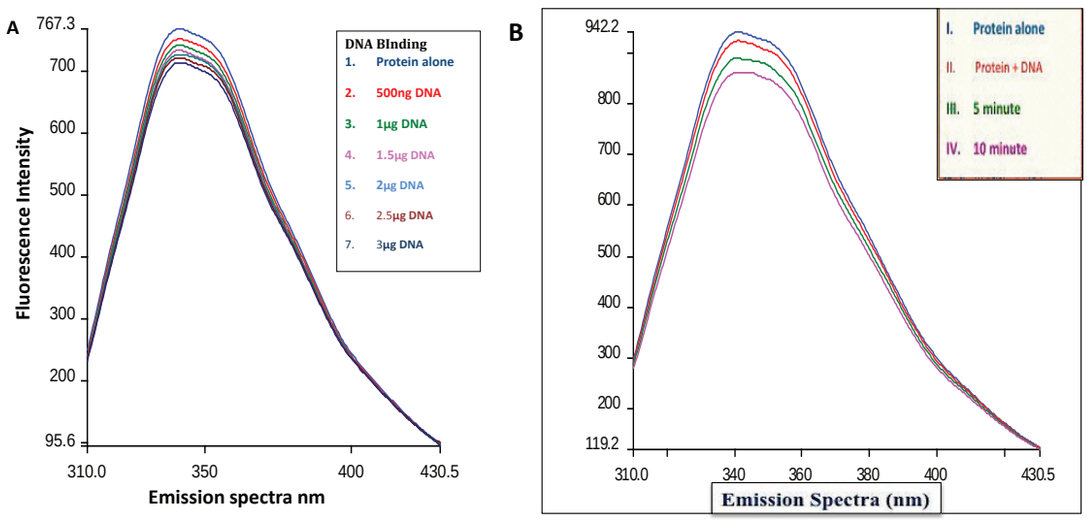


Fig. 2: Concentration and time dependent DNA Binding of Signature Protein

Signature Protein Protects *E.coli* from Oxidatve Stress:

In order to gain insight into anti-oxidant property of signature protein.we investigated whether SP2 can rescue *E.coli* cells from oxidative stress induced by H_2O_2 treatment. *E.coli* Bl21(DE3*)* cells were transformed with recombinant construct expressing SP2 or vector alone. After one hour post induction with 1mM IPTG the cultures were exposed to different concentrations of H_2O_2 for 90 minutes. Culture from each sample was plated on Kan+ plates for CFU counts. It is evident from the graphs that *E.coli* cells expressing SP2 survived better in oxidative stress in all three different concentrations (50mM, 100mM and 150mM H_2O_2) Figure 3.

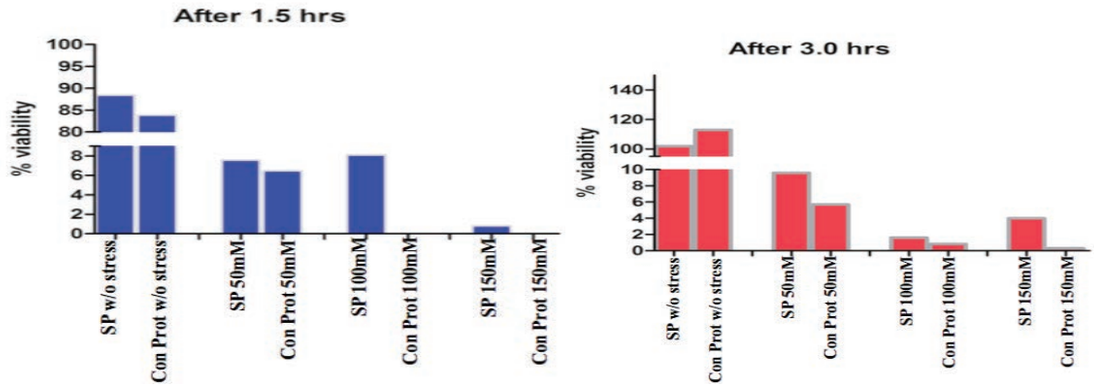


Fig. 3: Surival of *E.coli* BI21(DE3) exposed to oxidative cells expressing SP at different time points.

Recombinant Signature Protein antigens Displayed B-cell response tuberculosis Patients:.

Signature protein being present only in pathogenic Mycobacterium tuberculosis. we also tried to evaluate its antigenic potential. IEDB for epitope analysis shows signature protein 2 is highly antigenic as shown in figure 4A.

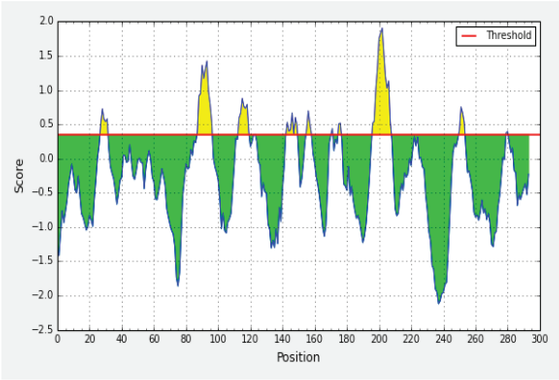


Fig. 4A: IDEB analysis for Epitope prediction

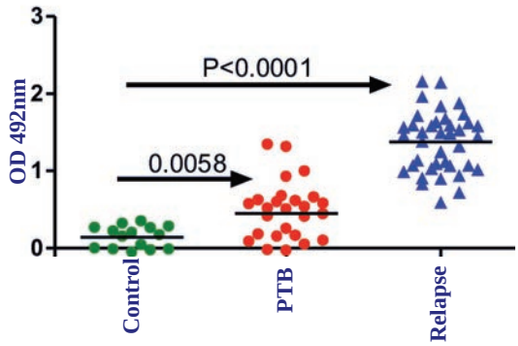


Fig. 4B: IgG response against SP

The humoral immune responses directed against the recombinant protein by patients with pulmonary tuberculosis (n=27), pulmonary relapse(n=38) cases and healthy controls (n=15) were compared. The data Fig.4B reveal that the sera of all the infected patients mounted significantly higher antibody responses against signature Protein antigen than those of the healthy controls ($P < 0.0058$ for PTB and $P<0.0001$ for Relapse PTB cases). This value is significant enough to claim these signature antigens as markers for tuberculosis diagnosis.

4. Role of RipA and its interaction with chaperonic protein of *M. tuberculosis* H37Rv strain

Scientific Staff : Dr. Nasreen Z. Ehtesham, Dr. Manish Bhuwan

Duration : 2014-17

Aims, Objectives and Background:

Functionally, **R**eactivation promoting factor **I**nteracting **P**rotein **A** (RipA), of *M. tuberculosis* H37Rv is a secretory protein that also has endopeptidase activity. The MoxR1 protein belongs to ATPases and is associated with various cellular activities. AAA+ proteins have been reported as novel molecular chaperone in bacteria and are involved in maturation or refolding of specific protein complexes. We had previously demonstrated the protein protein interactions of RipA with MoxR1 and thermal aggregation prevention assay for MoxR1 protein. During this year we had further extended our study to prove the chaperonic property of MoxR1 protein and its role in secretion of RipA protein.

Work done during the year:

MoxR1 protein protects enzyme activity of NdeI from thermal denaturation

Experiments were designed to prove that MoxR1 has the ability to act as a chaperone and can protect the functional activity of a protein from thermal denaturation. The enzymatic activity of restriction enzyme *Nde*I was assessed after heat denaturation at 60°C for 20 min in the presence or absence of MoxR1. Linearized, pcDNA 3.1(+) vector was used to investigate the enzyme activity of *Nde*I. Heat-denatured *Nde*I could not cleave pcDNA 3.1(+); however, when *Nde*I was heat denatured in presence of MoxR1, the enzyme activity was protected, as seen from the appearance of a cleavage product of 1.6 kb. Control protein bovine serum albumin (BSA), could not protect *Nde*I activity (Figure. 1). This ability of MoxR1 to protect the functional activity of restriction enzyme *Nde*I from thermal inactivation clearly demonstrates that it can act as a chaperone.

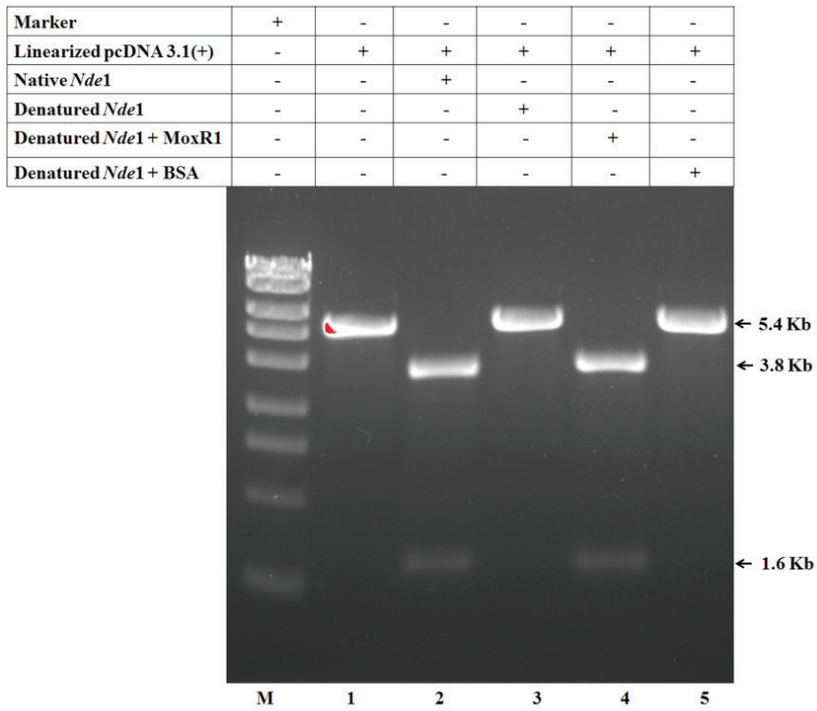


Fig. 1: Restriction enzyme activity of *Nde*I enzyme is protected from thermal denaturation in the presence of MoxR1 protein. Linearized pcDNA3.1 (+) plasmid is present at 5.4 kb (lane 1). Digestion with *Nde*I restriction enzyme cleaves pcDNA 3.1(+) to generate two bands of 3.8 kb and 1.6 kb (lane 2). These bands are seen in the case of native *Nde*I (lane 2) and denatured *Nde*I plus MoxR1 proteins (lane 4).

Coexpression of MoxR1 with RipA enhances the proper folding of recombinant RipA.

Physical interaction of RipA and MoxR1 and the experimentally demonstrated chaperone like activity of MoxR1, prompted us to investigate the functional consequence of coexpressing these two proteins. Overexpression of RipA protein alone showed the presence of RipA only in the pellet as inclusion bodies (Fig. 8A, lanes 1 to 4). However, when MoxR1 was coexpressed with RipA, solubility of RipA was enhanced (Fig. 8A, lane 6). Densitometric analysis clearly showed that in the presence of MoxR1 protein, about 20% of the RipA protein was properly folded and present in the soluble fraction (Fig.8B). These results indicate the functional significance of interaction between RipA and MoxR1 in terms of proper folding and secretion of the former with the help of the chaperone function of the latter.

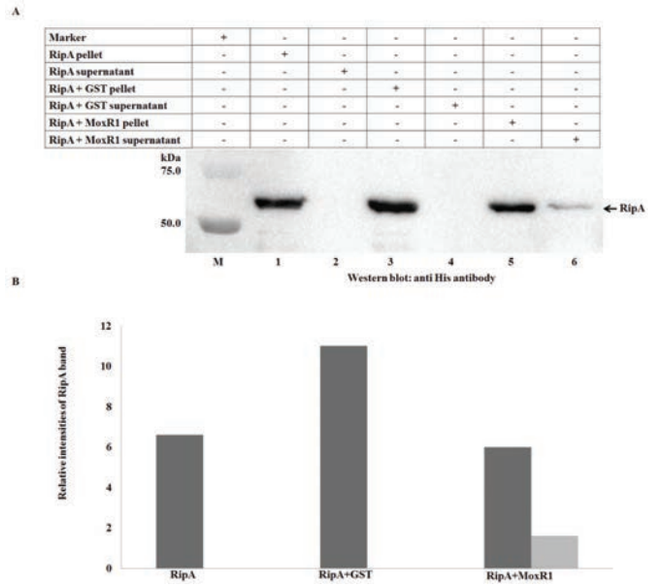


Fig. 2: (A) *In vivo* folding of RipA protein in the presence of MoxR1 chaperone activity. The different lanes are as follows: Lane M, marker; lanes 1 and 2, RipA pellet (lane 1) and folded RipA (supernatant) (lane 2) in the absence of any chaperone. RipA proteins in the pellet (lane 3) and supernatant fraction (lane 4) in the presence of GST are also shown. Insoluble RipA and folded RipA (supernatant) in the presence of MoxR1 can be seen in lanes 5 and 6, respectively. (B) Change in the levels of folded RipA in the presence of GST and MoxR1 at 37°C *In vivo*. The dark gray bars represent the RipA concentration in the pellet, and light gray represents the RipA concentration in the supernatant fraction.

MoxR1 is essential for Secretion of rRipA protein

E.coli BL21 (DE3) strains co-transformed with MoxR1 and RipA plasmids were grown in 100 ml of LB broth separately. The culture filtrate protein of the co-transformants was resolved on 12% SDS-PAGE, transferred to PVDF membrane and western blotted using the mouse monoclonal anti-His antibody as the primary. The signal was developed using Clarity ™ western ECL substrate.

RipA protein contains a potential TAT signal peptide was clearly evident from the S-score value. C and Y-scores indicate the positioning of a potential cleavage site (Fig. 3A). Moreover, the N-terminal region of RipA protein consists of signal peptide sequences, which were experimentally identified from the N-terminal mature protein. The N-terminal signal sequence of RipA consists of conserved twin-arginine sequence of Tat signal peptide and the signal cleavage site as depicted in Figure. 3B. Experiments were designed to identify the RipA secretion system and evaluate the role of MoxR1. Recombinant constructs encoding the His_6-RipA and GST-MoxR1

proteins were co expressed in *E. coli* BL21 (DE3). These cells possess the TAT secretion system and are also deficient in proteases that protect cleavage of the recombinant proteins. *E. coli* BL21 (DE3) cells expressing c-terminal His_6-RipA and GST alone were used for expression of unfolded RipA. The presence of precursor His_6-RipA expressed intracellularly in the *E. coli* BL21(DE3) cells at a predicted size of ~53 kDa (along with the His_6 tag) was detected using anti-His monoclonal antibody, and mature His_6-RipA at the predicted size of ~47 kDa (Fig. 3C, lane 9) was found only in the culture filtrate when expressed with GST-MoxR1. These results point to the role of MoxR1 as a chaperone in folding RipA in the cytoplasm before its secretion.

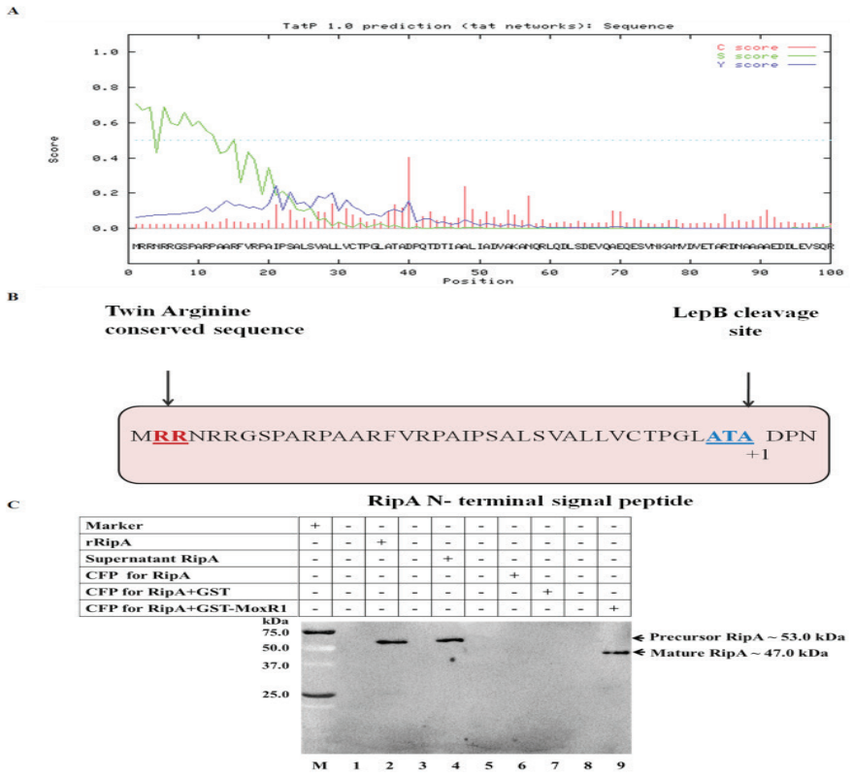


Fig. 3: (A) The N-terminal region of RipA protein consists of TAT signal peptide. (B) Schematic of RipA N-terminal signal peptide consensus amino acid. The conserved twin arginine is underlined, and the conserved 3-amino-acid LepB cleavage site starts at the 37th amino acid. The mature protein starting at the amino acid aspartate (D) is shown as +1. (C) MoxR1 protein aids in secretion of mature RipA through the TAT secretion pathway. The different lanes show the protein marker (lane 1), recombinant C-terminal His6-RipA used as a positive control (lane 2), *E. coli* BL21 (DE3) cells expressing RipA protein in the soluble fraction used as an intracellular control (lane 5), and culture filtrate protein from transformed *E. coli* cells expressing RipA alone (lane 6), as well as both RipA and GST alone (lane 7), probed for mature RipA protein. Similarly, the culture filtrate proteins from cotransformed *E. coli* cells expressing both His6-RipA and GSTMoxR1 were probed for mature RipA peptide and can be seen as a ~47.0-kDa band (lane 9).

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.

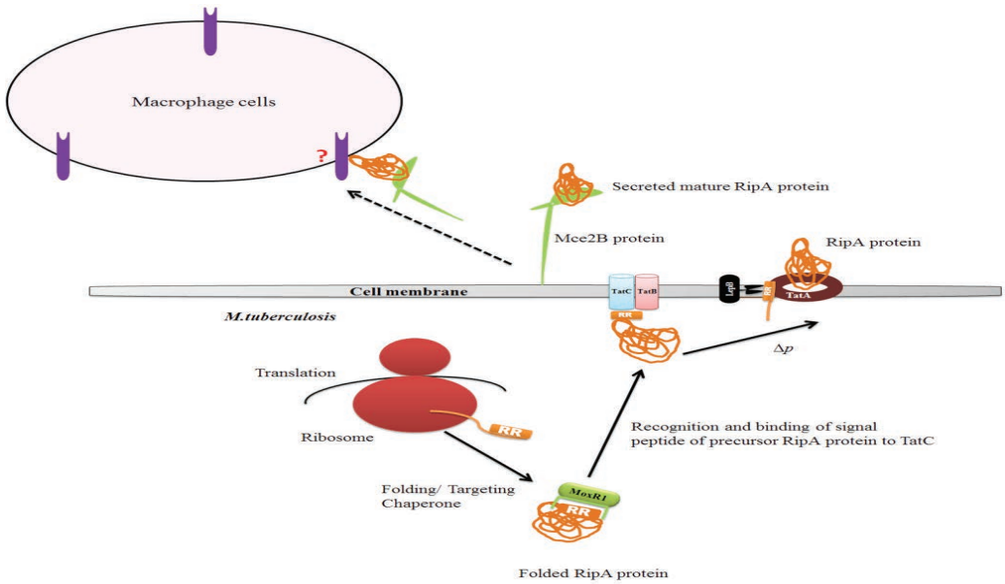


Fig. 4: Proposed model for RipA secretion through TAT secretion system in *M.tuberculosis*.

5. Host signal transduction modulation by *M. tuberculosis*: Moonlighting functions of M.tb PPIases?

Scientific Staff : Dr. Nasreen Z. Ehtesham, Saurabh Pandey, M. Khubaib Dr. Javaid Sheikh, Simran Kaur Arora

In Collaboration with : Prof. Seyed E. Hasnain, IIT Delhi

Duration : 2011-16

Aims, Objective and Background:

Peptidyl-prolyl *cis/trans* isomerases of *Mycobacterium tuberculosis* (*M.tb*), though primarily belong to foldases class of enzyme, isomerize the peptide bond preceding the proline bond. *M.tb* has two isoforms of prolyl isomerases, named PpiA and PpiB. These are ubiquitously expressing proteins, in absence of which serious folding bottlenecks are created. PpiA of *Mtb* is known to be structurally and phylogenetically related to eukaryotic cyclophilins (fig 1). This enzyme being a component of the secretome of the pathogen, is speculated to play a vital role in survival of *Mtb* in the harsh environment of macrophage.

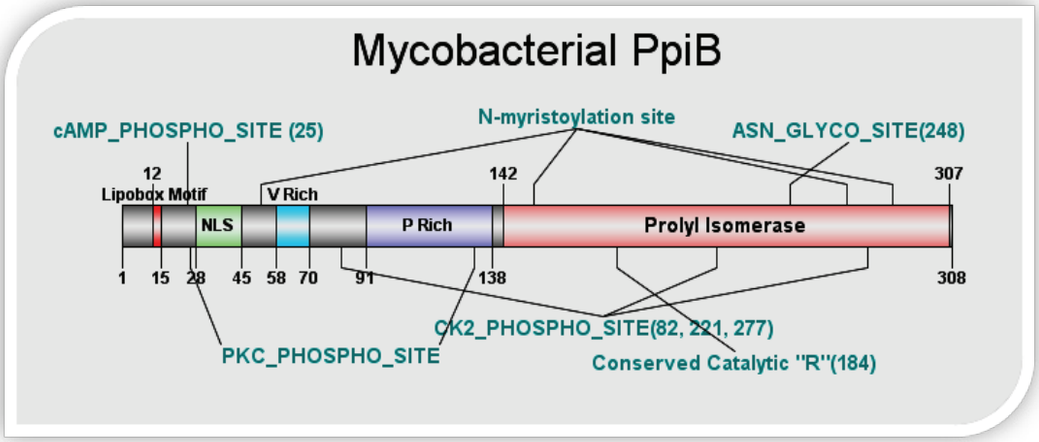


Fig. 1: Domain architecture of PpiB of *M.tb.* Signal sequence of PpiB comprises lipobox motif, nuclear localization sequence, valine rich and proline rich motifs and minor sites along with enzymatic moity.

Work done during the year:

Role of peptidyl Prolyl isomerases (PpiB and PpiA) as chaperone:

In an attempt to functionally characterize recombinant PpiA andPpiB for its potential to act as chaperone, its ability to sustain heat induced aggregation was assessed. Having shown its structure stability its ability to prevent the temperature induced aggregation of heat labile protein, Maltodextringlucosidase (MalZ) was measured by fluorometer (Fig 2A & 3A). Binding of ANS dye to PpiA and PpiB shifts the peak to the Blue region with very significant with increase in fluorescent intensity (Fig 2B &3B), measure of surface hydrophobicity, which is known to be important for proteins with chaperone like function.

Further, to investigate the chaperone like function of rPpiB andrPpiA under physiological conditions, we investigated if *E. coli* expressing PpiB andPpiA of *M.tb* exhibit resistance to thermal shock as compared to the control strains (vector control). Our results showed that*E. coli* cells transformed with *PpiB* and *PpiA* gene of *M.tb* showed approximately ten folds more survival in comparison to the *E.coli* transformed with vector alone. These results provide conclusive evidence that rPpiB and rPpiA show chaperone like function both in in-vitro conditions and under physiological conditions (Fig 2C & 3C).

PpiB: Chaperone Activity Assay

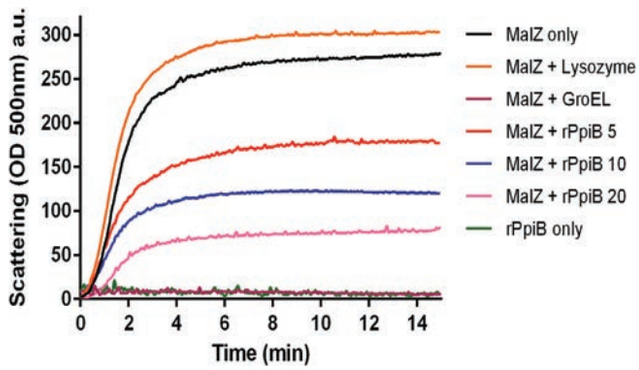


Fig. 2A: Suppression of MalZ aggregation by rPpiB. Light scattering at O.D. 500 nm with excitation and emission slit width 5 and 2.5, respectively was recorded to monitor the aggregation pattern. GroEL was used as a positive control. Lysozyme was used as a negative control.

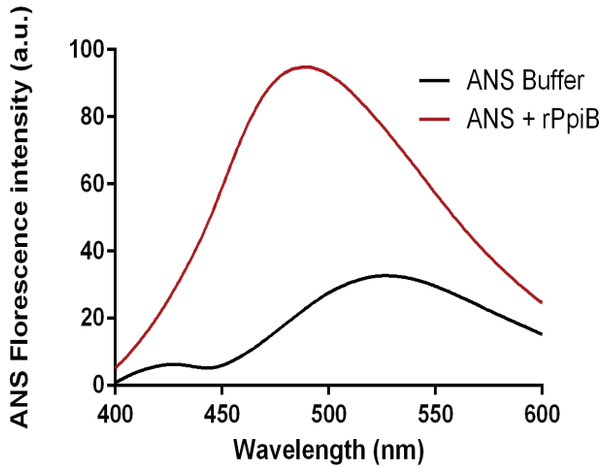


Fig. 2B: ANS Fluorescence spectra reveal surface hydrophobicity in *M.tb*rPpiB: Concentration of ANS and rPpiB used were 20µM and 0.1 mg/ml respectively. Blue shift in the position of peak and increase in the intensity of peak was observed on addition of rPpiB. The ANS emission was scanned in the range of 400 to 600 nm.

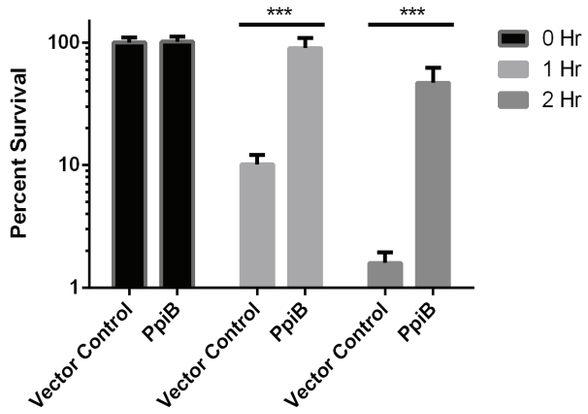


Fig. 2C: rPpiB can rescue *E. coli* cells from thermal shock: *E. coli* BL21 cells were transformed with pGEX6p1 only and pGEXppiB. After heat treatment, at one hour interval at 50ºC, ***E. coli*** transformed with *ppiB*exhibited approximately 10 fold more survival compared with vector control.

PpiA Chaperone activity Assay:

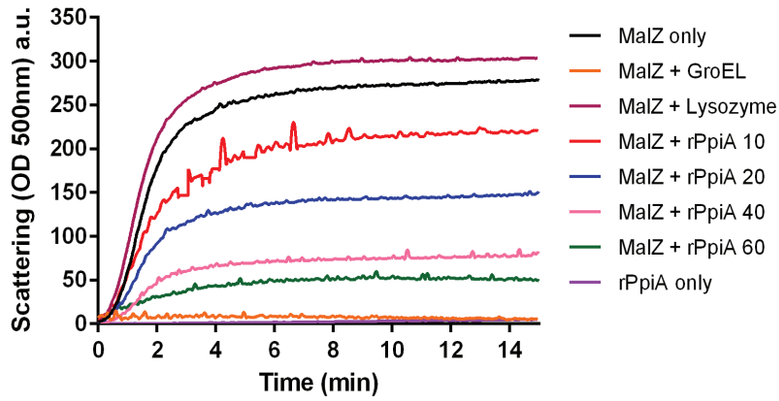


Fig. 3A: *M.tb*rPpiA suppresses aggregation of MalZ protein: The aggregation pattern was monitored by light scattering at O.D. 500 mM with excitation and emission slit width 5 and 2.5, respectively was recorded to monitor the aggregation pattern. GroEL was used as a positive control. Lysozyme was used as a negative control.

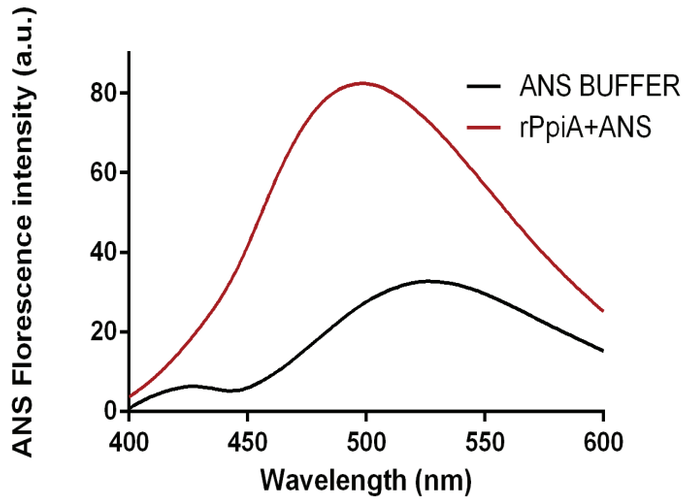


Fig. 3B: ANS Fluorescence spectra reveals surface hydrophobicity in ***M.tbr*** PpiA: Concentration of ANS and rPpiA used were 20µM and 0.1 mg/ml, respectively. Blue shift in the position of peak and increase in the intensity of peak is observed on addition of rPpiA. The ANS emission was scanned in the range of 400 to 600 nm

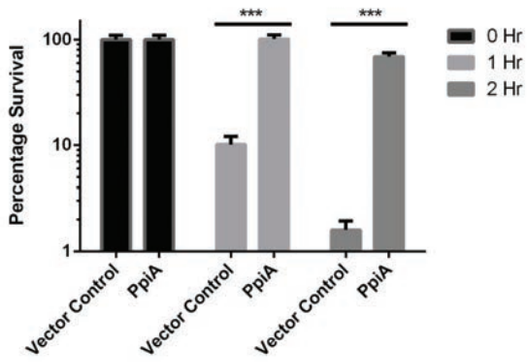


Fig. 3C: Cells overexpressing PpiA shows more tolerance to heat stress than vector only control: Time points were taken at the interval of 1hour when temperature switched from 37ºC to 50ºC. Protein induction is done at 37ºC with 1mM IPTG. Figure is representative of three individual experiments; percentage error is shown in bar graph.

Expression of PpiA in HEK cell line imparts resistance against hypoxia and oxidative stress

HEK cells were transfected with vector alone or containing gene for PpiA. HEK cells expressing PpiAwere exposed to oxidative and hypoxic stress,cell viability assay using MTT reveals significant increase in cell viability in the cells expressing PpiA as compared to vector control.

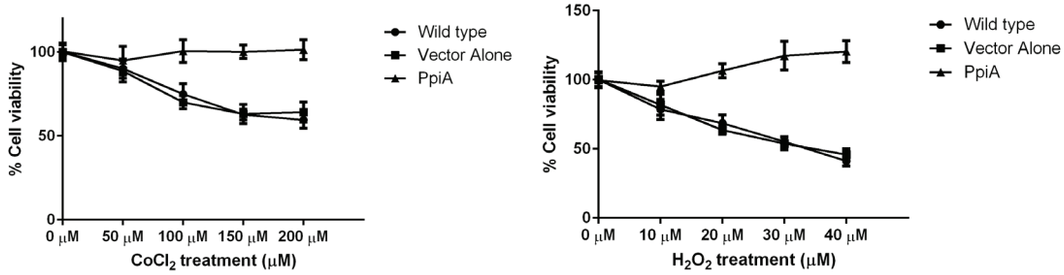
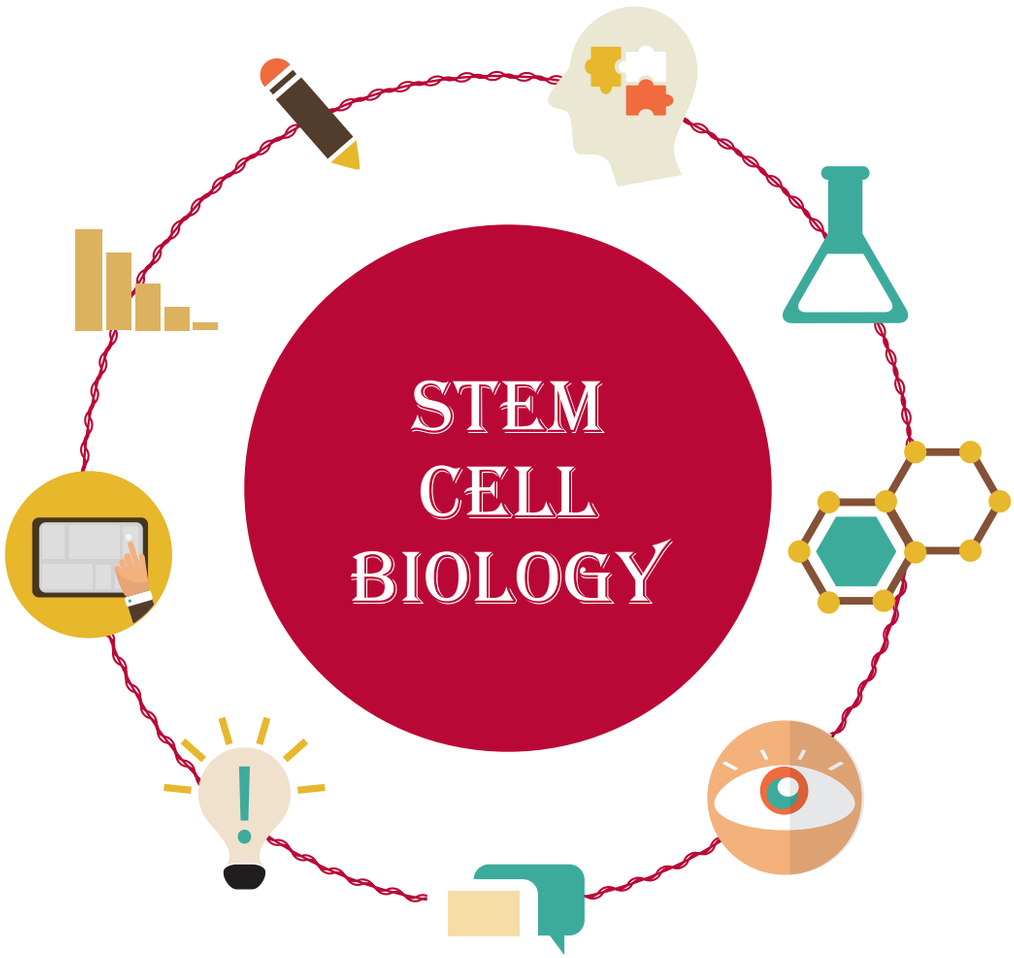


Fig. 4: PpiA imparts resistance to the HEK293t cells against Hypoxic and Oxidative stress. A, MTT assay was performed to score the % cell viability of HEK293t cells transiently expressing PpiA under the oxidative stress (H_2O_2 treatment dosage was given in 0, 10, 20, 30 and 40μM concentration). B, Under same conditions, hypoxic stress was given and % cell viability was scored ($CoCl_2$ dosage given for hypoxia was 0, 50, 100, 150 and 200μM concentration). The data is representative of three independent experiments. Error bar represents mean±S.D

These observations clearly validate a critical role of PpiA, in aiding the intracellular survival of the pathogen amid the hostile environment of infected cells.


STEM
CELL
BIOLOGY

STEM CELL BIOLOGY

1. Pre-clinical trial groundwork towards Cultured Epithelial Autograft application studies in burns patients

Scientific Staff : Dr. L.K. Yerneni, Rishi Man Chugh, Bijendra Kumar

Duration : 2014-16

Aims, Objectives and Background:

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 a low concentration of Mitomycin C using an innovative dose derivation (Chugh et al 2016). A Prototype has been prepared (Yerneni and Chugh 2014). It was suggested by the SAC to undertake a pre-clinical testing of the cultured keratinocytes for tumorigenesis in nude mice and karyotyping of late passage keratinocytes. Earlier, in order to address the Quality Control and Quality assurance issues, residues of mitomycin C in the final product and chromosomal stability in third and fourth passage cultured keratinocyte were completed.

Therefore, the objectives of the study included assessment for the absence of tumorigenesis by the cultured keratinocytes and testing for chromosomal stability in the fifth passage keratinocytes by G-Banding using the cultures established from human skin Biopsy employing the in-house culture technique.

Work done during the year:

We have completed testing of the cultured keratinocytes for tumorigenesis and the chromosomal stability in 5th passage cultured keratinocytes by G-banding.

I. Epidermal Keratinocyte cultures from biopsy:

Methods:

After obtaining the ethics committee approval, an extra unused piece of skin from the autograft from the thigh of a 26 years old male patient was brought from the burns OT of Safdarjung Hospital, New Delhi to the lab in transport medium. The biopsy was rinsed in several changes of antibiotic solutions, trypsinized, epidermal cells isolated, counted and plated along with the Swiss 3T3 feeder cells of 4-150 group prepared by our in-house technique. The cultures were incubated in Keratinocyte medium at 37^{o}C in a humidified CO_2 incubator.

Significant results:

The cultures were serially passaged and several aliquots were frozen at every passage in liquid nitrogen. 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. Cells at each passage were tested for in vitro agar-methyl cellulose transformation assay, P4 cells were used for in vivo tumorigenesis and P5 cells were used for Karyotyping.

II. In vivo Tumorigenesis testing for cultured keratinocytes

Methods:

A total of 14 nude mice of (*Mus musculus* with *nu/nu* genotype) were acquired at the animal facility of Dabur Research Foundation, Ghaziabad after obtaining the animal ethics committee approval and acclimatized for a week. For preparing the injectable stocks of epidermal keratinocytes for 8 mice, the frozen stocks of P2 keratinocytes were thawed and fresh cultures were set-up in T75 flasks in the presence of validated feeders of 4-150 group. The cells were passaged once and the sub-confluent P4 cultures were subjected to a two-step cell detachment protocol to first remove feeders by EDTA followed by keratinocyte isolation by trypsin. In order to avoid delay in cell delivery to mice as the keratinocyte recovery was time consuming; two or three flasks were handled at a time and injected subcutaneously into the posterio-lateral aspect of thorax. This resulted in differences in the recipient injected cell

number which were in the range of 5 – 7 million per mouse (Table 1). A human embryonic kidney cell line, HEK293, obtained from NCCS, Pune at 87th passage, was sub-cultured thrice to P90 to sufficiently expand enough for 6 mice which served as positive control. The cells were detached in one batch from all the flasks because of a straight forward trypsinization protocol and injected in equally divided fractions. Both the cell types at the prescribed number were suspended in 0.1 ml of medium and diluted with equal volume of Matrigel (Corning cat No.354248 having a concentration of 18-20 mg/ml).

Table 1. Details of cell injections into Nude mice.

Group	Mouse ID	Cell volume (ml)	*Matrigel Volume (ml)	Total volume (ml)	Cells injected
Positive Control cells- HEK293 cells-P90	G1A1-G1A6	0.1	0.1	0.2	5,925,925
Test cells - cultured Keratinocyte cells-P4	G2A1- G2A2	0.1	0.1	0.2	5,166,666
	G2A3-G2A5	0.1	0.1	0.2	5,814,815
	G2A6-G2A8	0.1	0.1	0.2	7,185,185

* Matrigel standard, High Concentration of 18-20 mg/ml (Corning, catalogue No. 354248)

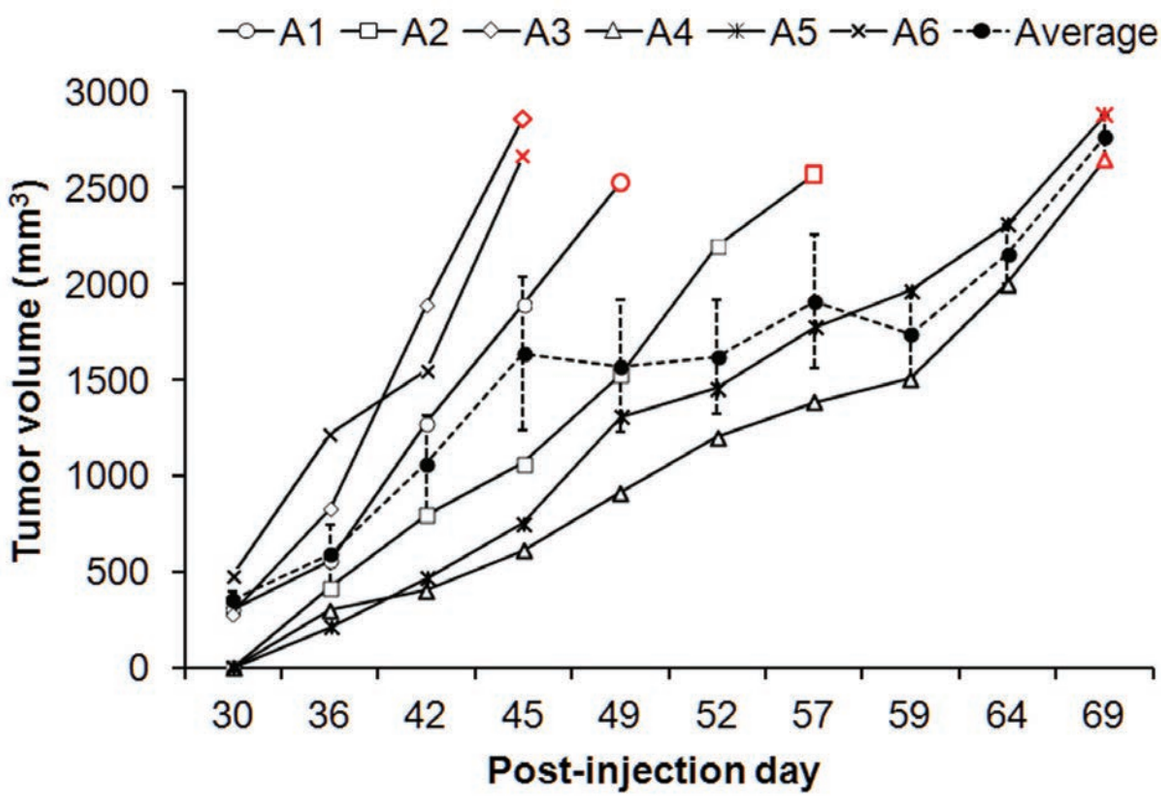


Fig. 1: Progress in tumour volume in control nude mice injected with HEK293 cells in Matrigel. The red marker indicates the day of necropsy.

The study period was concluded when the induced tumour reached a size of 2000 mm^3 or 3 months, whichever is earliest. The progress of the injected site was recorded periodically by digital photography. At the end of the study period, the animal(s) was/were sacrificed and the tumour/nodule, if present, liver, kidney, lung and the regional lymph nodes were retrieved for paraffin embedding and histology by H&E. The histological evaluation for tumour cells was independently performed by a dedicated pathologist.

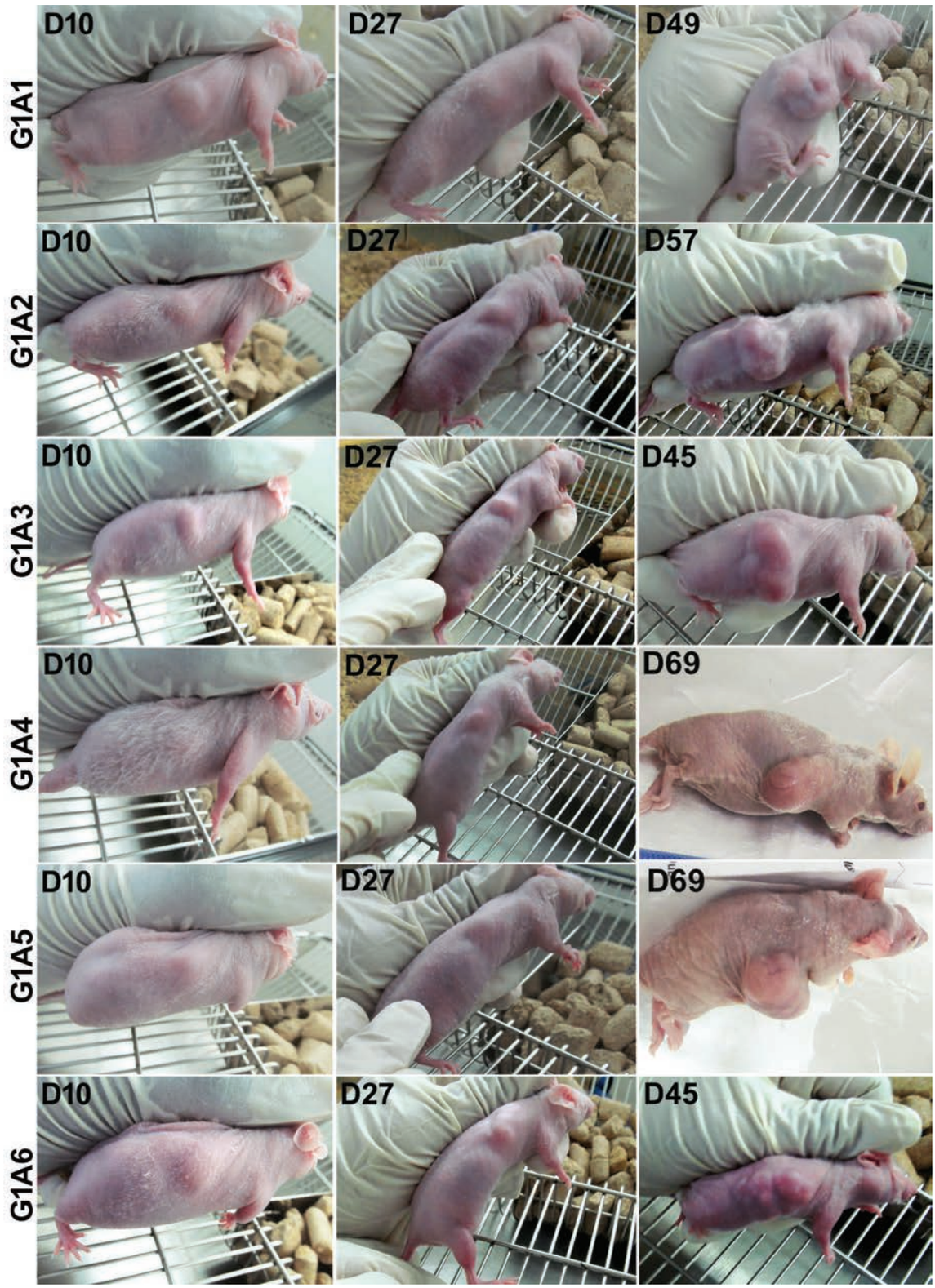


Fig. 2: Appearance of control nude mice injected with HEK293 cells in Matrigel at various time points. The images in the right column were shot on the day of necropsy.

Significant results:

All six mice receiving HEK293 cells formed tumors at the site of injection at different post-injection time points. The progress in tumor volume and the day of necropsy are represented in Figure 1 and 2. In the keratinocyte group, mice numbered G2A1 & G2A2, the nodule caused by the residual Matrigel resolved completely by day 49 while in mouse G2A3 and G2A5 it resolved by days 59 and 91, respectively (Figure 3). In the remaining four mice, the nodule showed initial rapid regression but a small and soft nodule remained unresolved. One such mouse, numbered G2A8, was sacrificed on Day 91 along with those showing complete resolution. As the dissection of the injection site showed only the residual Matrigel (Figure 4) and histopathology of the nodule and the internal organs revealed no tumor cells the remaining mice (G2A4, G2A6 and G2A7) were also sacrificed on day 108. None of these mice revealed tumor cells either in the residual nodule or in internal organs.

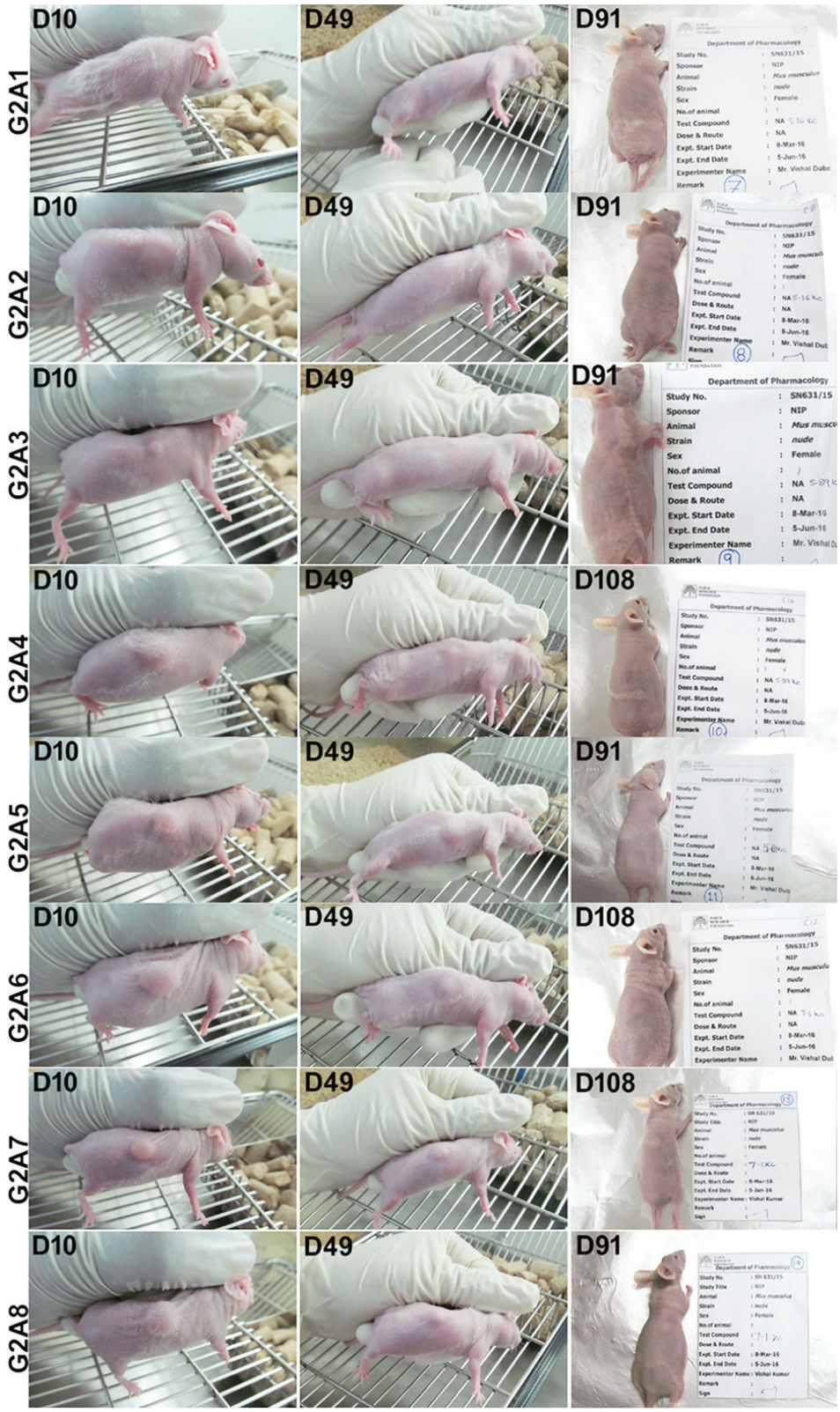


Fig. 3: Nude mice injected with cultured keratinocytes in Matrigel at various time points. The images in the right column were shot on the day of necropsy.

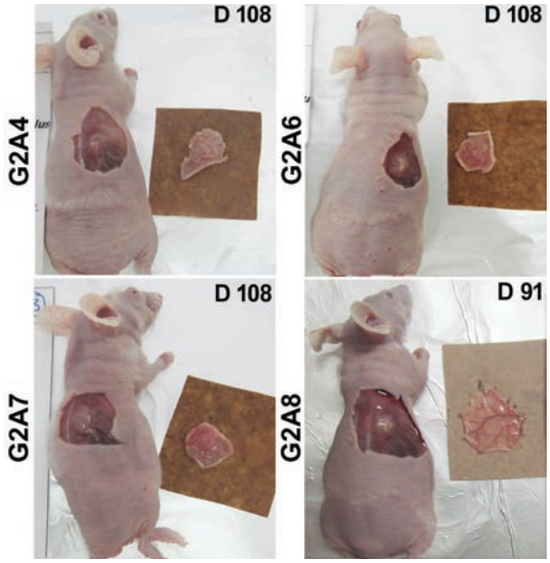


Fig. 4: Residual nodule of Matrigel with cultured keratinocytes. The day of necropsy is indicated on the respective image.

III. Chromosomal stability in the cultured keratinocytes

Methods:

Preparation of chromosomal spreads: The sub-confluent keratinocytes at P5 were exposed to 0.1 µg/ml demecolcine for 4 hours followed by trypsinization to first remove the feeders and finally detach keratinocytes. The cells were incubated in hypotonic solution of 0.075M KCl for 40 minutes and fixed in cold methanol-acetic acid (3:1) fixative. The cell suspension was then slowly dropped over acid clean slides and air-dried. Slides stained in a final working solution of 0.08% Giemsa stain containing 5% glycerol and methanol in PBS were used for chromosome counts. For the assessment of structural aberrations G-banding was adopted that involved overnight incubation at 60^{o}C, digestion in 0.25% trypsin in PBS for 2 minutes and staining in Giemsa for 15 minutes. International System for Human Cytogenetic Nomenclature 2005 was followed for scoring.

Significant results:

The G-banded Karyotyping in fifth passage cultured keratinocytes revealed normal chromosomal number with XY configuration (Male patient) and no structural alterations were noted (Figure 5).

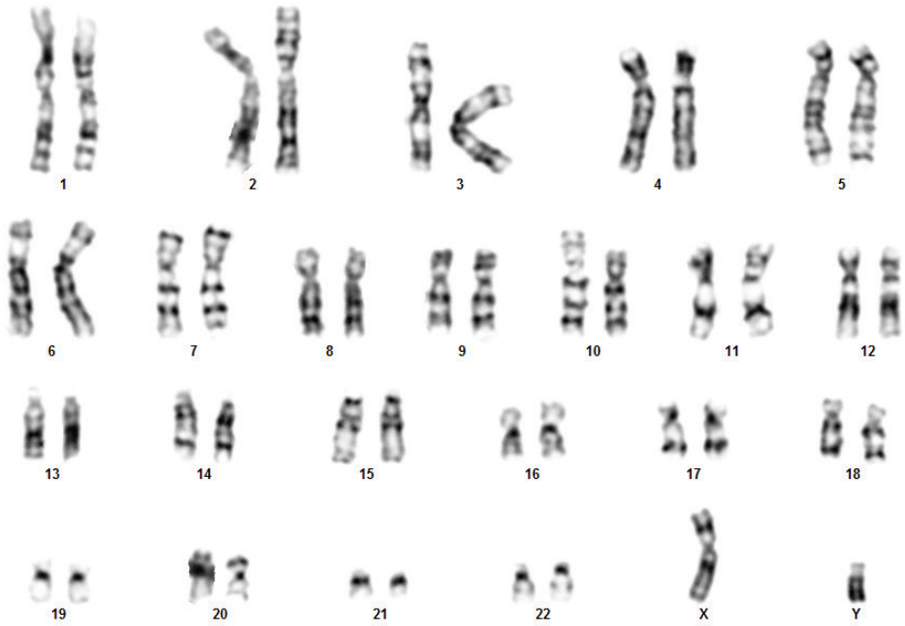


Fig. 5: G-banded chromosomes of cultured keratinocytes (P5).

Conclusion:

The culture process showed no detectable Karyotype abnormalities in keratinocytes; therefore, may not pose serious concerns from the clinical application viewpoint.

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. A concept proposal for the GIA scheme of DHR for the proposed clinical trial was submitted to the Department of Health Research. Additionally, efforts will also be made to identify a suitable industry.


BIO
INFORMATICS

BIOINFORMATICS

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

Scientific Staff : Dr. Arun Kumar Jain, Dr. Dibyabhaba Pradhan, Dr. Shweta Aggarwal, Mr. Arnab Nayek

Technical staff : Mr. Prince Gautam

Duration : Five years (01 April 2013 to 31 Mar 2018)

Aims, Objectives and Background:

National Institute of Pathology is committed to improve patientcare through better disease diagnosis. Therefore, Biomedical informatics centre, NIP, has identified its major research focus as "**to implement/develop biomedical informatics techniques for assisting disease diagnosis and therapies at the point of patient care**", through the major objectives sketched by ICMR for the taskforce project. Biomedical informatics centre constantly striving to promote and support informatics in biomedical research through National level workshops, training and research facility for biomedical scientists, research scholars and students, since its inception from 1/4/2013.

The achievements and ongoing research in this directions are outlined below as per objectives drawn by ICMR for the taskforce project.

Work done during the year:

ACTIVITIES OF THE CENTRE TO MEET PRIMARY OBJECTIVES OF THE TASK-FORCE PROJECT

A. TO IDENTIFY GENETIC LOCI ASSOCIATED WITH DISEASES

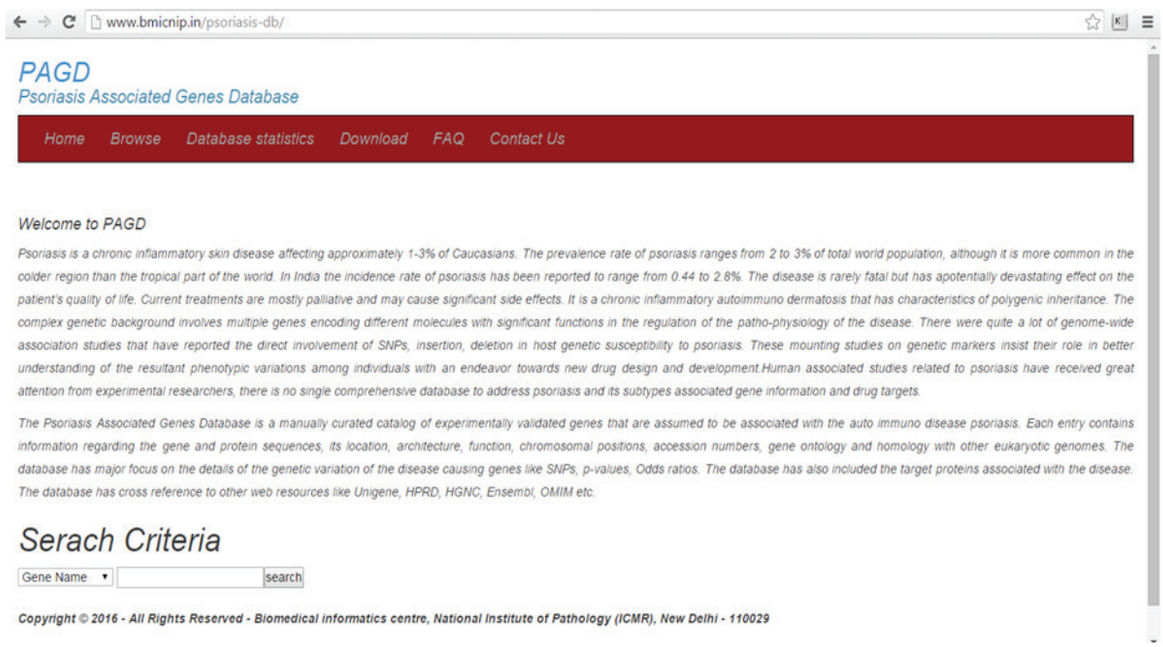
i) Psoriasis Associated Gene Database (http://www.bmicnip.in/psoriasis-db)

Outline: Psoriasis is a chronic, immune-mediated inflammatory skin disease which affects both children and adults. The prevalence rate of psoriasis ranges from 2 to 3%

of total world population (Lu et al., 2013), although it is more common in the colder region than the tropical part of the world. Prevalence rate of psoriasis in Indian scenario is 0.44% - 2.8% (Chandran et al., 2010). The disease is rarely fatal but has a potentially devastating effect on the patient's quality of life. Current treatments are mostly palliative and may cause significant side effects.

Psoriasis is generally thought to be a genetic disease which is triggered by environmental factors (Menter et al., 2008). The complex genetic background involves multiple genes encoding different molecules with significant functions in the regulation of the pathophysiology of the disease. Genome-wide association (GWAS) studies have reported direct involvement of SNPs, insertion, deletion in host genetic susceptibility to psoriasis. These mounting studies on genetic markers insist their role in better understanding the resultant phenotypic variations among individuals with an endeavour towards new drug design and development.

Although human associated studies related to psoriasis have received great attention from experimental researchers; there is no single comprehensive database to address psoriasis associated gene information. We have developed a web based interface called Psoriasis Associated Gene Database (PAGD) which provides detailed information for analysing the involvement and association of human genome in psoriasis.



The current version of database contains 165 genes, with around 600 genetic markers involved in different types of psoriasis collected from 199 published research articles through PubMed. The database compiled basic information on genes associated with psoriasis, cross-references to OMIM, HGNC, NCBI-UNIGENE, ENSEMBL, PharmaGKB, KEGG, VEGA, Homologene, HPRD database and the protein databank. The genetic variant details such as SNPs, Population/Ethnicity, Psoriasis type, p value, Odds Ratio with CI, of the 169 genes were compiled in a graphical user web interface.

The database is unique in the sense; it includes a comparative analysis tool wherein researcher can interactively verify if a genomic sequence encompasses known mutations that are likely to induce psoriasis upon suitable environmental trigger.

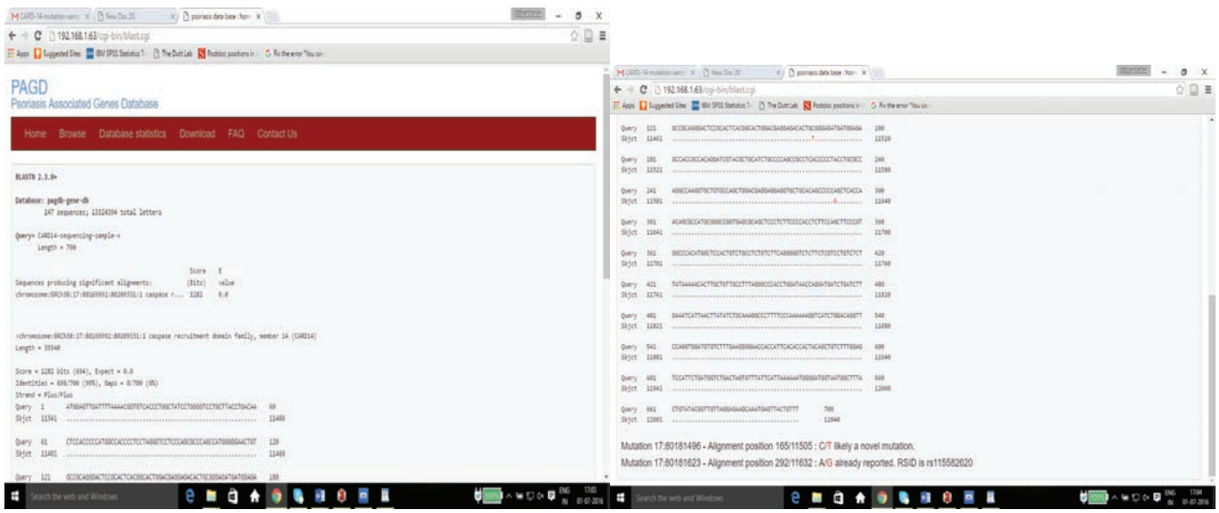


Fig. PAGD comparative genomics tools to detect psoriasis susceptible mutations in an individual's genes sequence

ii) Whole exome sequencing of psoriasis

Outline: We have initiated a pilot study to identify genetic loci associated with psoriasis. Whole exome sequencing for plaque psoriasis has been carried out to get insight on genomic signature of psoriasis patient. The exome sequencing data were compared with genetic markers identified through PAGD database. The project is proposed to be conducted with large scale samples alongside RNA-seq, proteomics and metabolomics studies to understand pathogenesis of psoriasis. The potential biomarkers and therapeutic targets expected to identified through this project would form basis for the next objective to develop solutions for communicable disease.

iii) Esophageal Cancer Database

Outline: Esophageal cancer (EC) is a disease in which malignant cells form in the tissues of the esophagus. The most common types of esophageal cancer are squamous cell carcinoma and adenocarcinoma. It is the 6th leading cause of death from cancer and the 8th most common cancer in the world. Epidemiological researches have demonstrated that both environmental factors (eg. Alcohol consumption) and genetic factors contribute to the risk of EC development. Researchers worldwide have completed many genomics, transcriptomics and proteomics studies regarding the pathophysiology of esophageal cancer. These investigations have generated vast amounts of data and the speed of these investigations is accelerating with the rapid growth of cancer knowledge, decreased costs of detection and computation, and spread of the Internet. There is a need of single comprehensive database to address esophageal cancer associated genomics, transcriptomics and proteomics information. Therefore, Biomedical Informatics Centre, NIP have initiated a project to construct an integrated database that catalogs gene, transcripts and proteins-related facts associated with esophageal cancer which have been accumulated in the biomedical literature and may appear in the future, and that delivers filtered and highly relevant data to both scientists and clinicians.

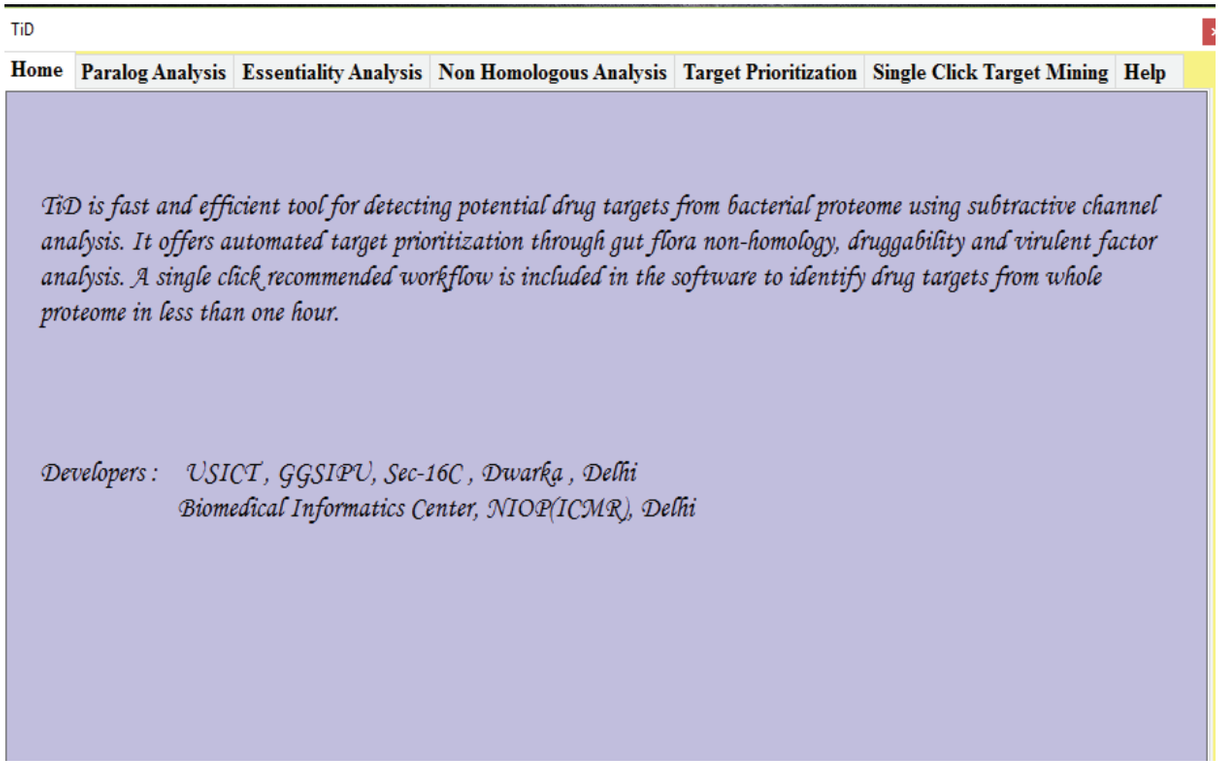
B. TO DEVELOP SOLUTIONS FOR CONTROLLING COMMUNICABLE AND NON-COMMUNICABLE DISEASES

i) TiD: Standalone software for mining putative drug targets from bacterial genome

Outline: Target identification (TiD) is a standalone software for identification potential drug targets from bacterial genome through automated subtractive genomic analysis with human genome and helpful Gut flora. The standalone application is developed using Bio Python v2.7 and Visual studio 2015 community edition. It provides an intuitive interface to be used by microbiologist interested in therapeutic target discovery against infectious disease. The automated subtractive analysis takes ~1 hour to scan putative targets from a bacterial genome which is faster and accurate then ~2-3 weeks' efforts required in manual curation for the same. The downstream target prioritization can be performed through drug target annotation of putative

target tab which provide links to pathway analysis, network analysis, sub cellular localization and functional annotations resources. The software is tested successfully on published articles used subtractive channel analysis for drug target identification. Therefore, TiD would be a useful tool for microbiology research laboratories.

TiD is available to download at http://bmicnip.in/TiD



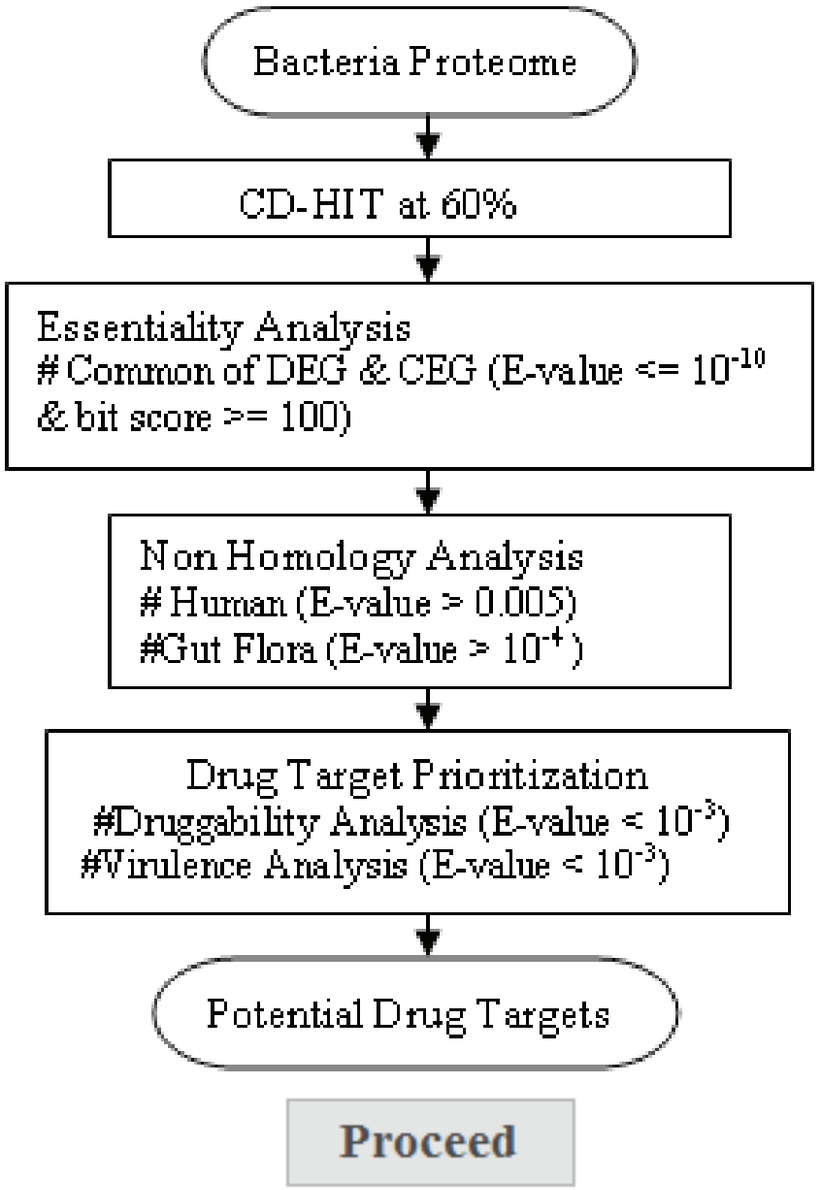


Fig. TiD home page and single click target mining workflow

ii) Probing Binding Mechanism of Interleukin-6 and Olokizumab: *In silico* Design of Potential Lead Antibodies for Autoimmune and Inflammatory Diseases

Outline: Computer aided antibody engineering has been successful in design of new biologics for disease diagnosis and therapeutic interventions. Interleukin-6 (IL-6), a well-recognized drug target for various autoimmune and inflammatory diseases such as rheumatoid arthritis, multiple sclerosis and psoriasis was investigated *in silico* to design potential lead antibodies. Here, crystal structure of IL-6 along with monoclonal antibody olokizumab was explored to predict antigen-antibody (Ag-Ab) interacting residues using DiscoTope, Paratome and PyMOL. Tyr56, Tyr103 in heavy chain and Gly30, Ile31 in light chain of olokizumab were mutated with residues Ser, Thr, Tyr, Trp and Phe. A set of 899 mutant macromolecules were designed and binding affinity of these macromolecules to IL-6 were evaluated through Ag-Ab docking (ZDOCK, ClusPro and Rosetta server), binding free energy calculations using Molecular Mechanics/Poisson Boltzman Surface Area (MM/PBSA) method and interaction energy estimation. In comparison to olokizumab, eight newly designed theoretical antibodies demonstrated better result in all assessments. Therefore, these newly designed macromolecules were proposed as potential lead antibodies to serve as a therapeutics option for IL-6 mediated diseases (Verma et al. 2016 J. Recept. Signal Transduct. Res. 1–16. doi:10.3109/10799893.2016.1147584).

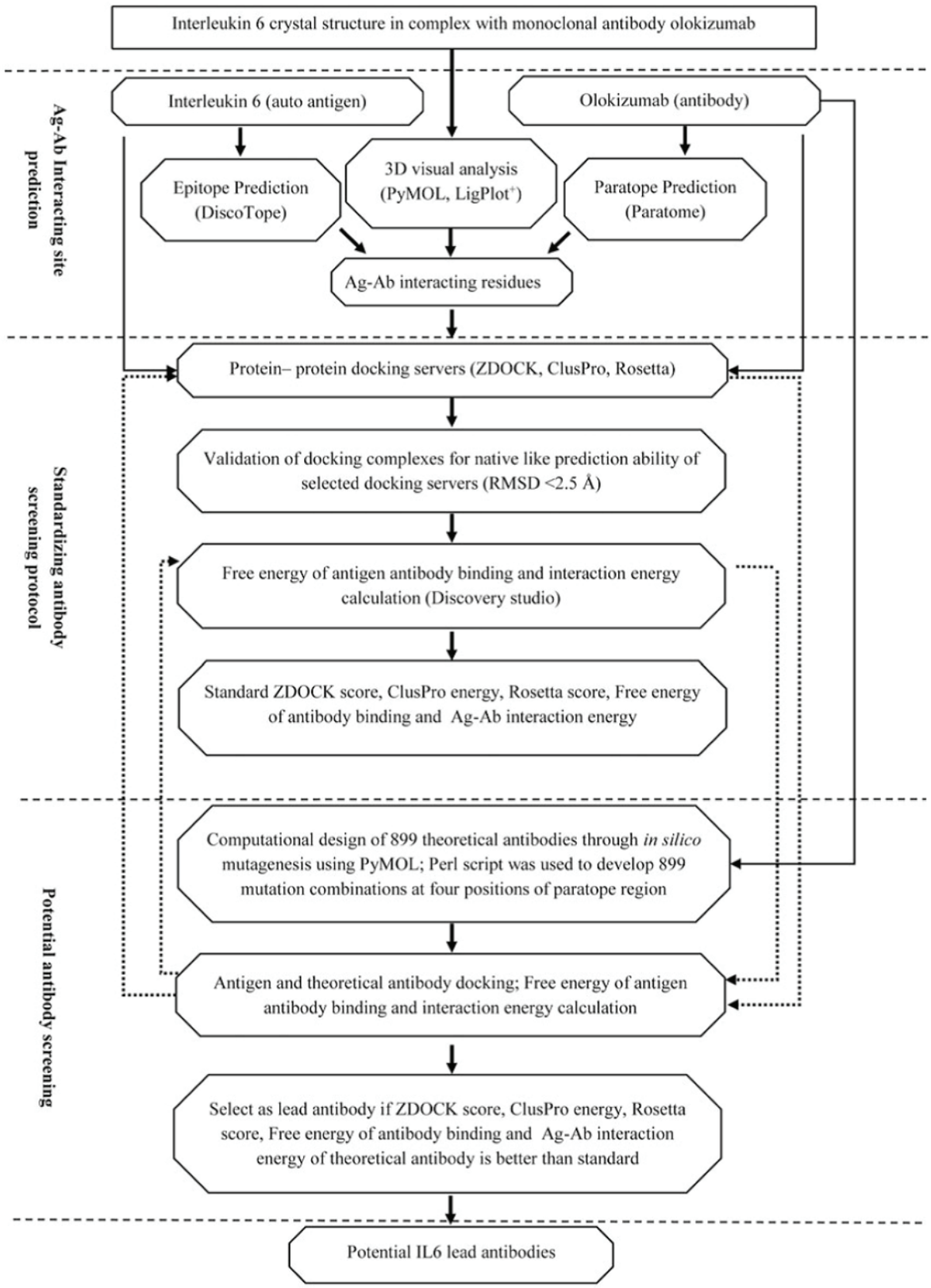


Fig: Protocol of IL6 antibody design

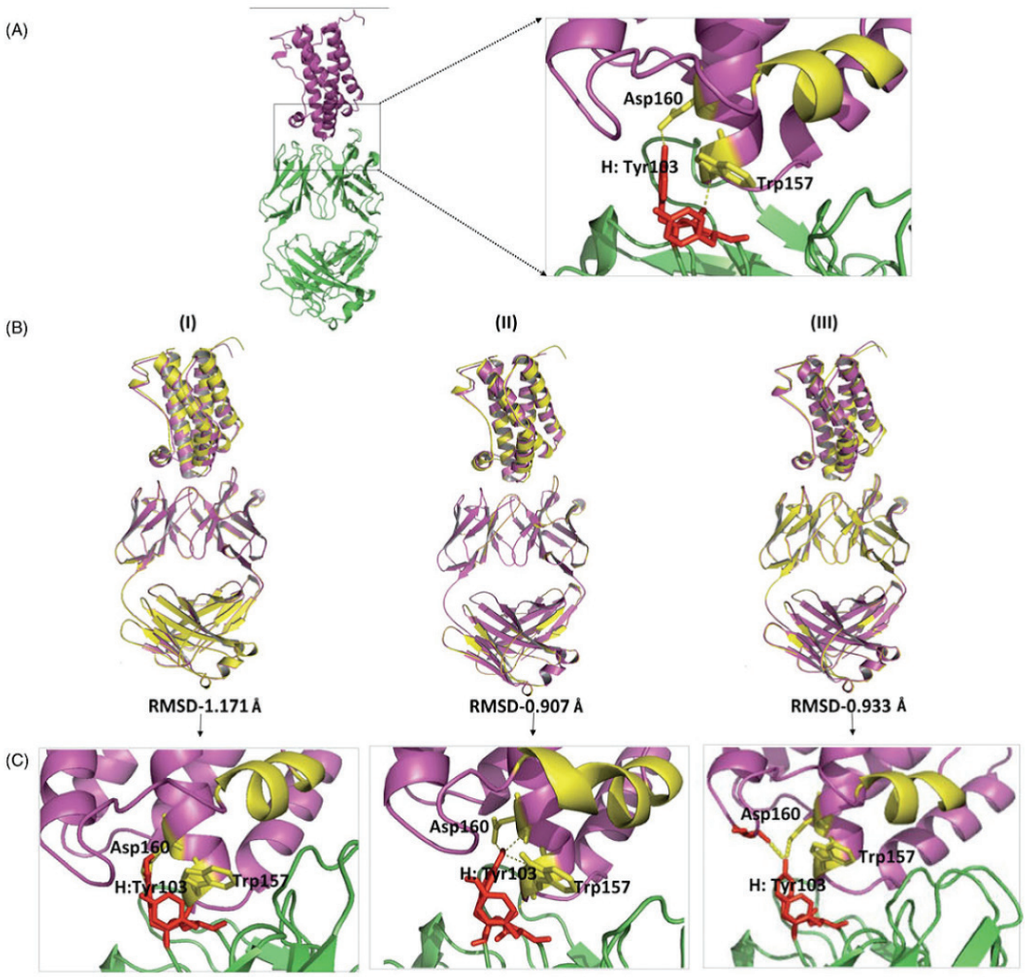


Fig. Three protein-protein docking software (ZDOCK, ClusPro and Rossetta) reproduce experimental binding orientations.

Table: Eight proposed lead monoclonal antibodies with better binding affinity compared to olokizumab

Table 1. Docking score/energy of proposed eight lead monoclonal antibodies and olokizumab.

	ClusPro		ZDOCK	Rosetta		Free energy (kcal/mol)			
Designed mutation	Central energy	Minimum energy	Total score	Docking energy	Interface score	Free energy	Interaction energy	Electrostatic energy	VDW energy
1. H: Y56F,Y103W L: G30Y, I31T	−605.1	−738.7	1484.7	−570.7	−7.8	−66.67	−248.9	−180.0	−68.9
2. H: Y56F,Y103W L: G30Y,I31S	−602.8	−735.4	1478.6	−571.4	−7.7	−55.81	−249.1	−180.7	−68.3
3. H: Y103W L: G30F, I31T	−643.2	−766.2	1511.5	−571.6	−8.0	−43.75	−227.8	−154.9	−72.9
4. H: Y103W L: G30T, I31F	−580.5	−729.8	1398.5	−571.1	−7.1	−41.00	−208.8	−146.8	−62.0
5. H: Y56F, Y103W L: G30Y	−595.0	−742.7	1525.6	−570.9	−7.3	−36.73	−262.9	−193.5	−69.4
6. H: Y56F, Y103W L: G30F	−611.8	−745.6	1572.7	−572.9	−8.9	−30.32	−221.4	−146.8	−74.6
7. H: Y103W L: I31Y	−608.4	−716.8	1499.8	−570.6	−7.1	−24.82	−206.5	−141.1	−65.3
8. H: Y103W L: G30Y, I31T	−629.2	−763.7	1449.2	−569.2	−6.9	−23.19	−248.6	182.6	−65.9
Olokizumab	−578.0	−716.3	1393.1	−567.2	−6.8	−17.89	−204.6	−144.8	−59.8

Out of 899, eight designed mAb(s) were showed better binding affinity in all evaluations. First column indicates chain: standard residue (position of residue) mutated residue such as [H: Y56F] means Tyr at 56th position of heavy chain was mutated with Phe.

iii) Computer aided antibody design for IL23 and TNF-alpha

Outline: Similar approach as discussed in (ii) was followed to identify potential lead antibodies for IL23 and TNF-alpha. In this studies, FoldX free energy analysis was used additionally to generate theoretical antibodies those are likely to show favorable binding affinity towards their respective drug targets.

iv) Discovery of potential inhibitors of BMX non-receptor tyrosine kinase through e-pharmacophore based virtual screening

Outline: BMX is expressed in bone marrow, granulo/monocytic cells and arterial endothelium. The stimulated production of IL-6, an important mediator of autoimmunity, has been shown to be dependent on BMX in human fibroblast-like synoviocytes by the use of siRNA knockdown. IL-6 over expression is known to play critical role in progression of psoriasis (Saggini et al., 2014). BMX has been also shown to be overexpressed in human prostate cancer. This signifies discovery of potential BMX inhibitors would represent promising therapies for autoimmune disorders and decipher potential anti-cancer applications. Two E-pharmacophores were designed based on two crystal structure of BMX in complex with inhibitors

(3SXR and 3SXS) available at Protein databank. Five potential BMX inhibitors were proposed through virtual screened based on two pharmacophore models (Pradhan et al., 2015 J. Biomol. Struct. Dyn. 33 Suppl 1, 118–120).

v) Potential small molecule inhibitors of TNF-alpha, PDE4 and JAK2

Outline: TNF-alpha (tumor necrosis factor-α), PDE4b and JAK2 are well validated targets of many inflammatory and autoimmune diseases including psoriasis. Therefore, potential lead molecules for these three targets were identified through a structure based virtual screening protocol. These works have been carried out as part of student's dissertations.

vi) T-cell epitope driven subunit vaccine screening from whole proteome of Zika Virus

Outline: The recent outbreak of Zika virus in 23 countries and territories in the America has raised attention to the possible devasting effects of the virus on human population. The patients infected with Zika virus were mainly managed through supportive care as no specific antiviral treatment or vaccine is available so far. Hence, Epitope-driven vaccine design for Zika virus remains a top priority because a mere progress has been made in this regard. In the current study, an immunoinformatics driven whole proteome based stratagy was implemented to identify 14 epitopes as T-helper cell mediated immunogens and 14 epitopes as Cytotoxic T-cell mediated immunogens. Seven antigenic peptides contained overlapping HLA-I and HLA-II binding motifs hence may elicit protective T-cell immune response against Zika virus. Conservancy and population coverage analysis were also performed on proposed CD4+ and CD8+ T-cell epitopes to identify their efficiencies over different strains and variable populations. These *in silico* screened antigenic peptides definitely unlock a new vision which may be the prospective mean in Zika virus research towards subunit vaccine design with further experimental validations.

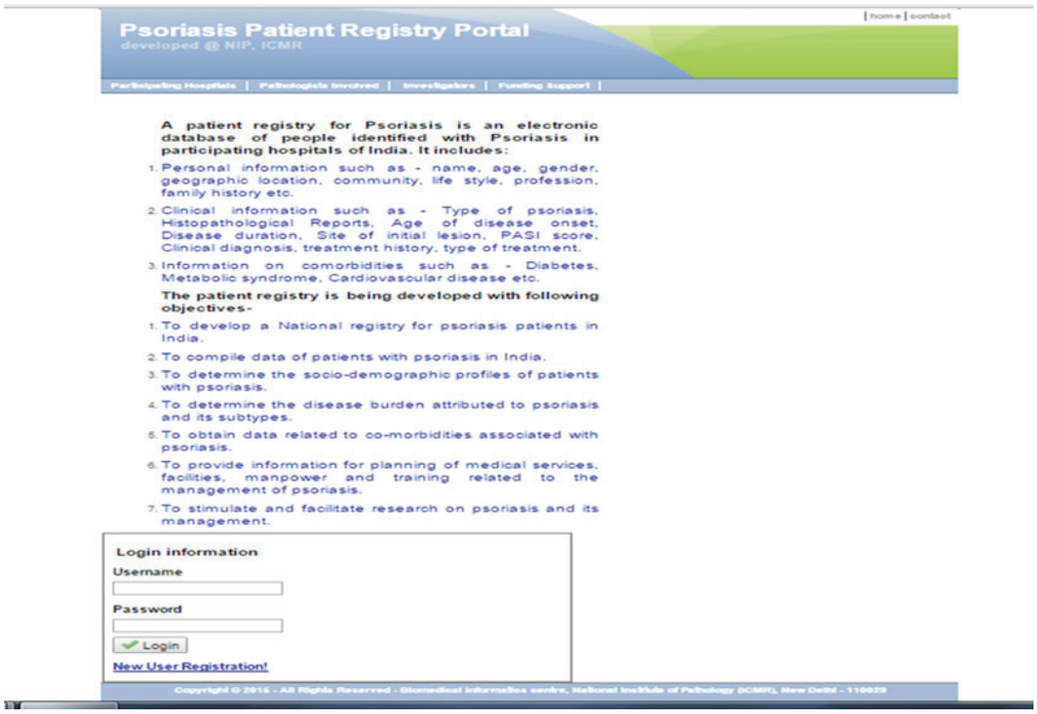
C. TO DEVELOP A NATIONAL REPOSITORY OF CLINICAL INFORMATION/ DATA, HIGH-THROUGHPUT DATA, GENOTYPE AND PHENOTYPE

i) Psoriasis patient registry Portal

Outline: A National Psoriasis patient registry is under development by Biomedical informatics centre, NIP in collaboration with Dermatology Dept., Safdarjang hospital. The major objectives of the patient repository are

- To compile data of patients with psoriasis in India.
- To determine the socio-demographic profiles of patients with psoriasis.
- To determine the disease burden attributed to psoriasis and its subtypes.
- To obtain data related to co-morbidities associated with psoriasis.
- To provide information for planning of medical services, facilities, manpower and training related to the management of psoriasis.
- To stimulate and facilitate research on psoriasis and its management.

Data has been collected from National Institute of Pathology for the year 2012-2014. Registry data provides information on following attributes: Demographics: Name, Age, Gender, Occupation, House Address & Phone no.; Clinical details: Family history, Disease duration, Age of onset, PASI score, Site of initial lesion, Affected area, Clinical notes, Clinical diagnosis, Co-existing conditions, Seasonal variation, Subsequent visit, Treatment, Hospital name, Referred by; Biopsy details: Biopsy no., Date of receipt, Site of biopsy, Gross specimen reviewed by, Gross description, Histopathological report.



Upon completion the patient registry would be available in NIOP server.

ii) Electronic histopathology report

BIC also working on developing an electronic histopathology record in collaboration with Histopathology Laboratory. The histopathology image repository would be useful in increasing precision on cancer diagnosis at the point of care as well as a resource for teaching medical graduates.

iii) Tools to predict pollutants from patient samples is developed

A Perl script is developed to predict possible pollutant contaminations in human blood sample. The tool was developed based on known RT values and spectra data of selected 63 pollutants. Combination of RT, Maxima and minima spectra led to predict probable presence of these 63 pollutants in a given sample with ~94% accuracy.

D. TO PROMOTE APPLICATIONS OF CUTTING-EDGE TECHNOLOGIES IN MEDICAL RESEARCH

1) National workshop - 02

i) A workshop on 'Bioinformatics tools for Biomedical Research' was organized by the centre during March 24-27, 2015 to promote cutting-edge technologies in medical research. 21 PhDs, 3 MDs and 7 M.Sc. students were trained from different states of India were trained.

ii) 2nd National workshop on Next Generation Sequencing in disease diagnosis and therapeutic target discovery was organized from 14-18th March 2016. The five-day training program included two days of wet lab experiment on next generation sequencing using ion torrent followed by three days' data analysis using open source bioinformatics software. The workshop covered guest lectures by renowned experts of the topic alongside approximately five hour hands on training on each day. Twenty candidates (MDs, PhD and M.Sc.) were selected from 62 applications received from different states of India.

2) Long-term training

Six M.Sc. students completed 4th semester dissertation using the Biomedical informatics centre facilities in the year 2015-16. Two M.Sc. dissertation students were currently being trained by BIC. Alongside three students also continuing as post-M.Sc. trainee in the Biomedical Informatics center.

Six M.Sc. students completed six-month dissertation training in 2015-16 are

- Ms. Monika Yadav, M.Sc. Biochemistry, Jamia Millia Islamia University, Delhi
- Ms. Rashi Verma, M.Sc. Biochemistry, Jamia Millia Islamia University, Delhi
- Ms. Noor Saba Khan, M. Sc. Biotechnology, Invertis University, Bareilly
- Ms. Afreen Khan, M. Sc. Biotechnology, Invertis University, Bareilly
- Ms. Manisha Ray, Dept. of Bioinformatics, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha
- Ms. Prajna Parimita Kar, Dept. of Bioinformatics, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha

3) Biomedical Informatics center is also assisting and providing training to PhD and MD students leading to complete their respective thesis work.

4) Biomedical Informatics center also set up Offline Galaxy server for NGS data analysis for researchers of host institute and regional institutes.

3) Paper presentation/Invited lecture delivered: 02

- Dr. Dibyabhaba Pradhan, Scientist-II, delivered an invited lecture along with hand on session entitled “Streamlining NGS data analysis and molecular modelling for precise disease diagnosis and targeted therapeutic design in 18th Workshop on Molecular Modelling and Drug – Protein Interactions” organized by Bioinformatics Centre, Mahatma Gandhi Institute of Medical Sciences, Sevagram during January 11 - 12, 2016.
- Mr. Arnab Nayek, Research Assistant of Biomedical Informatics Centre, NIP presented the paper title “ADSETMEAS: Automated Determination of Salt-bridge Energy Terms and Micro Environment from Atomic Structures using APBS method, version 1.0” in “The 29th Annual Symposium of The Protein Society” conference held at Barcelona, Spain from July 22nd to July 25th, 2015.

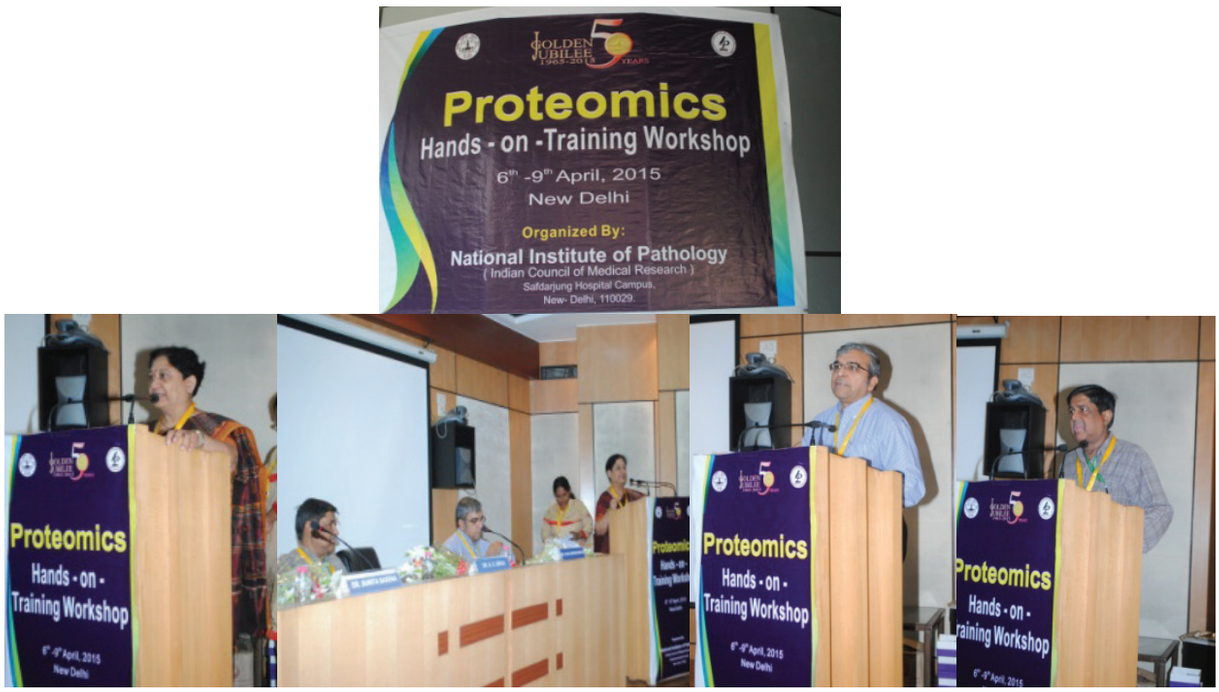

ACADEMIC
ACTIVITIES

INSTITUTIONAL ACTIVITIES

- 2nd National Workshop on "Next Generation Sequencing in Disease Diagnosis and Therapeutics Target Discovery" from 14th -18th March 2016.

- Workshop on" Research Methodology for Medical and Biomedical Scientists" from 22nd-24th April 2015.

- Proteomic Hands-on Training workshop from 6th – 9th April 2015.

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DR. K. K. SINGH

- National 'Swacchta Diwas' was celebrated on 2nd October 2015 and oath was taken by all NIP staff.

- 15th Smt. Pushpa Sriramachari Foundation Day Oration was organised by NIP on 1st May 2015 and the guest oration titled, 'Improving health of rural population by advances in technology', was delivered by Dr. Kiran Katoch.

- *Dr. S. Sriramachari Young Scientist Award 2015 was given to Ms. Vanila Sharma,* for her work on "*Leishmania donovani* specific Ubiquitin related modifier-1 (LdUrm1): an early endosone associated post translational modification".

AWARDS & HONOURS/PATENTS

1. **Dr. Poonam Salotra** conferred TWAS fellowship at the 26th General Meeting of The World Academy of Sciences (TWAS) at Vienna, Austria, in Nov. 2015.

2. **Dr. Sangita Rastogi** awarded Travel grant by ICMR for participation in 'ESCCAR International Congress on Rickettsia & other Intracellular Bacteria' at Laussane, Switzerland from June 13th-16th, 2015.

3. **Dr. Usha Agrawal** awarded HRD fellowship for short-term foreign fellowship in "Quality control and Quality assessment" in Biobanking at BC Cancer Agency, Victoria, BC, Canada from Dec. 1st, 2015 to Jan 31st, 2016.

4. **Dr. AP Singh** awarded 'Dr Bishnupriya Devi award' for best original article published in Indian Journal of Dermatology, Venereology and Leprology for the year 2015 by IADVL.

5. **Dr. Ruchi Singh** received "Bill & Melinda 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).

PATENTS

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

2. Genetic Markers for Diagnosis of Tuberculosis caused by *Mycobacterium tuberculosis.* Inventors: Hasnain SE, Rahman Syed Asad, Thornton Janet M, Ahmad J, Ehtesham NZ. European Patent application no. PCT/IB2014/066469/ 2015.

EXTRAMURAL PROJECTS

NEW PROJECTS

1. Investigation on Paromomycin resistance in *Leishmania donovani* using molecular and biochemical tools.
   Dr. Poonam Salotra, ICMR (2015-18).
2. Genome wide analysis of genetic alterations and gene expression profile in hormone sensitive and hormone refractory prostate cancer.
   Dr. Anju Bansal, ICMR (2015-18).
3. Identification and characterization of Artemisinin resistance associated genes in *Leishmania*
   Dr. Ruchi Singh, ICMR (2015-18).
4. Virtual Centre of Excellence on multidisciplinary approaches aimed at interventions against *Mycobacterium tuberculosis* (Phase II).
   Dr. Nasreen Ehtesham, DBT (2015-19).
5. Role of long non-coding RNA MEG3 in breast carcinogenesis
   Dr. S.A. Raju Bagadi, DST (2016-19).
6. Study of HLA-DR3 and/ or DQ2 restricted CD4+T cells in Type 1 diabetes in North India.
   Dr. Neeraj Kumar, DST (2016-19).
7. Autoantibody response and identification of tumor-associated antigens in gall bladder carcinoma-immunoproteomics approach.
   Dr. Poonam Gautam, DST (2016-19).

ONGOING PROJECTS

1. Study on micro RNA signature associated with breast cancer stem cells (CSCs) and their role in drug response.
   Dr. Sunita Saxena, Dr. SA Raju, ICMR (2012-16).
2. Targetted sequencing of breast cancer specific genes in early-onset breast carcinoma.
   Dr. Sunita Saxena, Dr. SA Raju, ICMR (2013-16).
3. Understanding the role of micronutrients in *Mycobacterium tuberculosis* infection using guinea pig as a model.
   Dr. Nasreen Ehtesham, ICMR (2013-17).
4. Differential protein profile for identification of markers in recurrent urothelial cancer.
   Dr. Usha Agrawal, ICMR-SRF (2014-16).

COMPLETED PROJECTS

1. Understanding the role of androgen receptor signaling in breast cancer.
   Dr. Sunita Saxena, Dr. Jatin Mehta, ICMR-PDF project (2013-2015).
2. Study of characterization of TMPRESS2: ERG and PCA3 as prostate cancer biomarkers in Indian.
   Dr. Sunita Saxena, Dr. Anju Bansal, Dr. Shalu Jain, ICMR-PDF project (2013-15).
3. Virtual Centre of Excellence on multidisciplinary approaches aimed at interventions against Mycobacterium tuberculosis (Phase I).
   Dr. Nasreen A. Ehtesham, DBT
4. Development of new live-attenuated vaccines candidates for kala-azar.
   Dr. Poonam Salotra, DBT (2011-15).
5. Immunopathogenesis of Reactive Arthritis induced by Chlamydia trachomatis
   Dr. Sangita Rastogi, Mr. Praveen Kumar, ICMR-SRF project (2010-15).
6. Understanding the role of chemokines and their receptors in growth and development of glioblastoma
   Dr. Avninder Singh, Dr. Sunita Saxena, Ms. Ira Sharma (2014-16).

ICMR POST-DOCTORAL FELLOWSHIP PROJECTS

1. Molecular mechanism of drug resistance in Acute Myeloid Leukemia (AML): Role of ATP Binding Cassette (ABC) transporters.
   Dr. Pradeep Kr. Chauhan Dr. Sunita Saxena (2015).
2. Understanding the role of androgen receptor signaling in breast cancer
   Dr. Jatin Mehta, Dr. Sunita Saxena
3. Expression of aldo-ketoreductase family 1B10 (AKR1B10) gene in breast carcinoma: The effects on drug and tobacco exposure.
   Dr. Mishi Wasan, Dr. Sunita Saxena
4. Proteomic analysis of *Leishmania donovani* membrane components involved in host-parasite interaction.
   Dr. Sandeep, Dr. Poonam Salotra
5. Functional characterization of RipA (Rv1477), an invasion associated and secretion protein and its impact on virulence.
   Dr. Manish Bhuwan Dr. Nasreen Z. Ehtesham

PUBLICATIONS

1. Gannavaram S, Bhattacharya P, Dey R, Ismail N, Avishek K, **Salotra P,** Selvapandiyan A, Satoskar A, Nakhasi HL. Methods to evaluate the preclinical safety and immunogenicity of genetically modified live-attenuated *Leishmania* parasite vaccines. ***Methods Mol Biol.*** 1403:623-38 (2016).

2. Kaushal H, Bras-Gonçalves R, Avishek K, Deep DK, Petitdidier E, Lemesre JL, Papierok G, Kumar S, Ramesh V, **Salotra P.** Evaluation of cellular immunological responses in mono- and polymorphic clinical forms of Post Kala-Azar Dermal Leishmaniasis in India. ***Clin Exp Immunol.*** 185(1):50-60 (2016).

3. Sharma V, Sharma P, Selvapandiyan A, **Salotra P**. Ubiquitin related modifier-1 (LdUrm1): an early endosome associated ubiquitin like conjugation in *Leishmania donovani. **Mol Microbiol.*** 99(3):597-610 (2016).

4. Gupta P, Sharma R, Chandra J, Kumar V, **Singh R**, Pande V, Singh V. (Clinical manifestations and molecular mechanisms in the changing paradigm of vivax malaria in India. ***Infect Genet Evol.*** 39:317-324 (2016).

5. Ramesh V, R. Singh, Kumar A, Verma A, Deep DK, Verma S and **Salotra P**. Decline in clinical efficacy of oral miltefosine in treatment of post kala-azar dermal leishmaniasis (PKDL) in India. ***PLoS Negl Trop Dis*** 22; 9(10):e0004093. (2015).

6. Ramesh V, Kaushal H, Mishra AK, Singh R and **Salotra P**. Clinico-epidemiological analysis of Post kala-azar dermal leishmaniasis (PKDL) cases in India over last two decades: a hospital based retrospective study. ***BMC Public Health*** 15:1092 (2015).

7. Khubaib M, Sheikh JA, Pandey S, Srikanth B, Bhuwan M, Khan N, Hasnain SE and **Ehtesham NZ** (2016) Mycobacterium tuberculosis Co-operonic PE32/PPE65 Proteins Alter Host Immune Responses by Hampering Th1 Response. ***Frontiers in Microbiology***. 7:719.

8. Bhuwan M, Arora N, Sharma A, Khubaib M, Pandey S, Chaudhuri TK, Hasnain SE, **Ehtesham NZ**. (2016) Mycobacterium tuberculosis Virulence Factor RipA with Chaperone MoxR1 Is Required for Transport through the TAT Secretion System. ***mBio***. pii: e02259

9. Pandey S, Khubaib M, Tripathi D, Sharma A, Choudhary T. K., Hasnain SE, **Ehtesham NZ** (2016). Mycobacterium tuberculosis peptidyl-prolyl isomerases also exhibit chaperone like activity *in-vitro* and *in-vivo*. ***PLos One*** e0150288

10. Grover S, Gupta P,. Kahlon PS, Goyal S, Grover A, Dalal K, Ali. S, **Ehtesham NZ**, Hasnain SE (2016) Analyses of methyltransferases across the pathogenicity spectrum of different Mycobacterial species point to an extremophile connection ***Journal of Molecular Biosystems.*** *12: 1615*

11. Rasheedi S, Suragani M, Raviprasad P, Ghosh S, Suragani RN, Ramaiah KV, **Ehtesham** NZ. (2015). Functional characterization of PeIF5B as eIF5B homologue from Pisum sativum. ***Biochimie.*** 118: 36-43

12. Rahman SA, Singh Y, Kohli S, Ahmad J, **Ehtesham** NZ, Tyagi AK, Hasnain SE. (2015) Reply to '"Mycobacterium indicuspranii" is a strain of Mycobacterium intracellulare': "M. indicuspranii" is a distinct strain, not derived from M. intracellulare, and is an organism at an evolutionary transition point between a fast grower and slow grower. ***mBio.***6pii: e00352-15.

13. Kohli S, Singh Y, Sowpati DT, **Ehtesham** NZ, Dobrindt U, Hacker J, Hasnain SE (2015) Human mesenchymal stem cells: New sojourn of bacterial pathogens.**.** ***Int J Med Microbiol***. 305:322-326.

14. Hasnain SE, O'Toole RF, Grover S, **Ehtesham** NZ. (2015) Whole genome sequencing: a new paradigm in the surveillance and control of human tuberculosis. ***Tuberculosis*** (Edinb). 95:91-4.

15. Singh A, Suragani M, **Ehtesham** NZ, Krishna A (2015) Localization of resistin and its possible roles in the ovary of a vespertilionid bat, Scotophilusheathi. ***Steroids***.; 95:17-23.

16. Kumar P, Bhakuni DS, **Rastogi S** (2015). Do IgA antibodies to *Chlamydia trachomatis* have protective role in humoral immunity: A study in reactive arthritis patients. ***Microbes Infection***, 17(11-12): 806-10.

17. Verma R, Yadav M, Pradhan D, Bhuyan R, Aggarwal S, Nayek A**, Jain AK** . Probing Binding Mechanism of Interleukin-6 and Olokizumab: In silico Design of Potential Lead Antibodies for Autoimmune and Inflammatory Diseases. ***Journal of Receptor and Signal Transduction***. 10.3109/10799893.2016.1147584, 2016.

18. Pradhan D, Priyadarshini V, Aggrawal S, Pradeep N, Nayek A, **Jain AK**, Umamaheswari A (2015) Discovery of potential inhibitors of BMX non-receptor tyrosine kinase through e-pharmacophore based virtual screening. ***Journal of Biomolecular Structure and Dynamics***; 33(supplement):118-120.

19. Kumar SN, Telang AG, Patil RD. Singh KP, **Jain AK**, Sharma R (2015). Study on combined effects of ochratoxin A and endosulfan on antioxidant enzymes in rats. ***J. Environ. Biol.*** 36(3) : 601-605.

20. Kumar SN, Telang AG, Singh KP, Bastia B, **Jain AK** (2015). Toxic manifestations of endosulfan and ochratoxin-A in adult male rats. ***MOJ Toxicol***. 1(3): 00012.

21. Kumar SN, Bastia B, Telang AG, Singh KP, Singh R, **Jain AK** (2015). Combined toxicity of endosulfan and ochratoxin-A in rats: Histopathological changes. ***J. Histol. Histopathol***. 2: doi: 10.7243/2055-091X-2-22.

22. Prasad P, Singh N, Das B, Raisuddin S, Dudeja M, **Rastogi S** (2016). Differential expression of superoxide dismutases in early aborters infected with *Chlamydia trachomatis*. ***International Journal of Infectious Diseases***, 45: P203-4.

23. Singh N, Prasad P, Kumar P, Singh LC, Das B, **Rastogi S** (2016). Expression of prostaglandin receptors in *Chlamydia trachomatis*-infected recurrent spontaneous aborters. ***Journal of Medical Microbiology***, DOI:10.1099/ jmm 0.000256.

24. Singh N, Prasad P, Das B, **Rastogi S** (2016). Recurrent Spontaneous abortion: Significance of early non-invasive detection of *Chlamydia trachomatis* infection. ***International Journal of Infectious Diseases***, 45: P47.

25. Kumar P, Bhakuni DS, **Rastogi S** (2016). Is there a correlation between *Chlamydia trachomatis* detection and development of disease in reactive arthritis/ undifferentiated spondyloarthropathy patients. ***International Journal of Infectious Diseases***, DOI: 10.1016/ j.ijid.2016.02.464.

26. **Siraj F,** Kaur M, **Agrawal U.** Clear cell odontogenic carcinoma of maxilla: A diagnostic challenge. ***Clin Cancer Investig J*** 2016;5:256-8.

27. Agrawal BK, Marwaha S, Bhatnagar M, Parry SA, **Agrawal U**. Association of systemic lupus erythematosus and beta thalassaemia trait- a case report. ***Journal of Clinical and Diagnostic Research*** 2016, 10:OD03-OD04.

28. Agrawal BK, Manchanda B, Garg A, Mittal A, Mahajan NC, **Agrawal, U.** (2015). Mean platelet volume in acute myocardial infarction: a case-controlled study. **Journal of Cardiovascular Research**, 1:4

29. Kumari N, Vasudeva P, Kumar A, **Agrawal U**. Adenocarcinoma of urinary bladder: A report of two patients. ***J Cancer Res Ther***. 2015 Oct-Dec;11(4):1033. doi: 10.4103/0973-1482.148722.

30. Kumari N, Saxena S, **Agrawal U**. Exosomal protein interactors as emerging therapeutic targets in urothelial bladder cancer. ***J Egypt Natl Canc Inst***. 2015 Jun;27(2):51-8. doi: 10.1016/j.jnci.2015.02.002. Epub 2015 Feb 25.

31. Agnihotri SK, **Agrawal U**, Ghosh I. Brain most susceptible to cadmium induced oxidative stress in mice. ***J Trace Elem Med Biol***. 2015 Apr;30:184-93. doi: 10.1016/j.jtemb.2014.12.008. Epub 2015 Jan 3.

32. Chugh RM, Chaturvedi M, **Yerneni LK** (2015). An evaluation of the choice of feeder cell growth arrest for the production of cultured epidermis. ***Burns*** http://dx.doi.org/10.1016/j.burns.2015.08.011. ***Burns*** 41: 1788-1795

33. Chugh RM, Chaturvedi M, **Yerneni LK** (2016). Exposure cell number during feeder cell growth-arrest by Mitomycin C is a critical pharmacological aspect in stem cell culture system. ***Journal of Pharmacological and Toxicological Methods*** 80: 68-74.

34. Chugh RM, Chaturvedi M, **Yerneni LK** (2016). Efficient and safe feeder cell processing strategy using mitomycin C as a substitute to irradiation. ***Cytotherapy*** 18:S75. DOI:10.1016/j.jcyt. 2016.03.150.

35. **Kumar N,** Kaur G, Kanga U, Mehra NK, Neolia SC, Tandon N, Zucman SC. CTLA4+49G allele associates with early onset of type 1 diabetes in North Indians. ***Int J Immunogenet*** 2015 Dec; 42(6): 445-52.).

36. Sharma I, **Singh A**, Mishra AK, Singh LC, Ramesh V, Saxena S. Is CXCL10/CXCR3 axis overexpression a better indicator of leprosy type 1 reaction than inducible nitric oxide synthase? ***Indian J Med Res.*** **2015;142**:**681-9**

37. Singh V, Singh LC, **Singh A**, et al. Status of epigenetic chromatin modification enzymes and esophageal squamous cell carcinoma risk in northeast Indian population. ***American J Cancer Res*** **2015;5:979-99**.

38. Saxena AK, Jain S, Ramesh V, **Singh A**, Capoor MR. Chromoblastomycosis: demonstration of abundant microorganisms on microscopy of a scaly crust following intralesional corticosteroids. ***J Eur Acad Dermatol Venereol*** **2015**;**29:189-90**

39. **Singh A**, Ramesh V, Ramam V. Histopathological characteristics in post kala-azar dermal leishmaniasis: a series of 88 cases. ***Indian J Dermatol Venreol Leprol*** **2015;81:29-34**

40. Jha A, Taneja J, Ramesh V, **Singh A**. Annular epidermolytic icthyosis: a rare phenotypic variant of bullous congenital icthyosiform erythroderma. ***Indian J Dermatol Venreol Leprol*** **2015;18:194-97.**

41. **Siraj F**, Ansari MK, Sharma KC, **Singh A**. Chordoid meningioma: a diagnostic dilemma. ***J Cancer Res Therap*** **2015**;11:663-65

42. **Siraj F**, Dalal V, Kaur M, Suri K. Radiation induced Leiomyosarcoma of the Oral Cavity: A Rare Occurrence Detected on 18F-FDG PET/CT. ***World J Nucl Med*** (Ahead of print).

43. **Siraj F,** Dalal V, Khan AA, Jain D. Hepatosplenic T-cell lymphoma with coexistent hepatitis B infection: a rare clinicopathologic entity. ***Tumori***. 2015 Oct 15;0(0):0. doi: 10.5301/tj.5000445. [Epub ahead of print].

44. Khan AA, Jain D, **Siraj F**, Aggarwal S. Spontaneous regression of orbital lymphoma. ***Indian J of Cancer*** 2016; 53 : 11-12 .DOI 10.4103/0019-509X.180841.

45. Mallya V, **Siraj F**, Singh A, Sharma K C. Giant cell glioblastoma with calcification and long-term survival. ***Indian J Cancer*** 2015;52:704-5.

46. Dalal V, Kaur M, **Siraj F, Singh A, Bansal A.** Primary testicular lymphoma with solitary cutaneous nodule as the initial presentation. ***Can Urol Assoc J.*** 2015 Sep-Oct;9(9-10):E744-7. doi: 10.5489/cuaj.3065. Epub 2015 Oct 13.

47. Sharma I, **Siraj F**, Sharma KC, **Singh A.** Immunohistochemical expression of chemokine receptor CXCR3 and its ligand CXCL10 in low-grade astrocytomas and glioblastoma multiforme: A tissue microarray-based comparison**. *J Can Res Ther*** [Epub ahead of print][cited 2015 May 5].

48. Narula V, **Siraj F**, **Bansal A.** Renal leiomyosarcoma with soft tissue metastasis: An unusual presentation. ***Can Urol Assoc J.*** 2015 Mar-Apr;9(3-4):E139-41. doi: 10.5489/cuaj.2396.

49. Tandon M, Panwar P, Garg P, Chintamani, **Siraj F**. Neurofibromatosis with male breast cancer-risk factor or co-incidence? Report of two rare cases. ***Breast Dis.*** 2015;35(1):29-32.

50. **Bansal, A., Siraj F**, Grover SB, Bhatnagar A, **Saxena, S**. Mesenteric lymphangioma: A rare intraabdominal finding in a pregnant woman. ***Onc Gas Hep Rep*** 2015;4:46-8.

SCIENTIFIC ACTIVITIES

DR. SUNITA SAXENA

1. Ethical Committee Meeting at VMMS & SJH on 8th December 2015
2. SAC Meeting at NIRRH, Mumbai on 16th December 2015
3. PDF Meeting & Review Committee Meeting at ICMR on 22nd & 23rd December 2015
4. Meeting regarding University status for ICMR at Gurgaon on 23rd December 2015
5. Meeting on "CHAMPS" i.e. Child Health and Mortality Prevention Surveillance in the Conference Hall of NIOP on 11th January 2016
6. Meeting regarding University status for ICMR at ICMR on 15th January 2016
7. ICMR Award Function Ceremony at JLN Auditorium, AIIMS on 19th January 2016
8. Attended MRHRU Meeting at DHR on 19th January 2016
9. Advisory Member of University Research Advisory Board-Swami Vivekanand University, Meerut on 22nd January 2016
10. Presentation regarding University Status for ICMR before the Executive Committee on 28th January 2016
11. Pathology Workshop organized by Deptt. of Pathology, VMMC & SJH and NIP, ICMR-Lecture by Dr.Sunita Saxena on "Understanding and Applications of Cancer Genomics on 29th January 2016
12. Annual Medical Students Conference 2016 at VMMC, New Delhi on 29th January 2016
13. Review Committee Meeting University Status for ICMR at NIOP on 3rd February 2016

14. Meeting of RAC of MRHRU Punjab held at Parivar Kalyan Bhawan, Chandigarh on 4th February 2016

15. Ethics Committee Meeting in the Committee Room of MS Office, SJH, New Delhi to discuss fresh Projects on 9th February 2016

16. Attended the International Symposium on "Roe of Herbals in Cancer Chemoprevention and Treatment at JNU Convention Centre, New Delhi on 9th & 10th February 2016

17. SAC Meeting at ICPO, Noida on 11th March 2016

18. CHAMPS (Child health and Mortality Prevention Surveillance) meeting at NIP on 11th March 2016

19. Invited for Inauguration of Festival Innovation at President's Estate, Sports Ground, New Delhi on 12th March 2016

20. Workshop on NGS and Bioinformatics Organized by NIP from 14th to 18th March 2016

21. Diagnostic Biosystem (India) one day CME on Molecular Diagnostics at Hyatt Regency, New Delhi on 18th March 2016

22. National Workshop to prepare guidelines on use of Anti-cancer drugs held at AIIMS, New Delhi on 21st March 2016

23. Selection Committee Member for the Award of PDF of ICMR at ICMR Hqrs, New Delhi on 22nd March 2016

24. Meeting with the Ethics Committee of VMMS to discuss fresh projects held at MS Office, SJH on 29th March 2016

25. Meeting for Academy at ICMR on 29th March 2016

Dr. Poonam Salotra

1. Invited member of Technical Specification Committee meeting regarding Kala-azar elimination program held at Nirman Bhawan, New Delhi, April 2015.

2. Attended ECD-PRC meetings at ICMR in May and Sep 2015.

3. Member of Technical Advisory Committee Meeting at Chennai and Hyderabad for BIRAC project June, 2015.

4. Participated and presented work on drug resistance at 5th Central European Symposium on Antimicrobials and Antimicrobial Resistance (CESAR) 2015, at Sibenik, Croatia in Sep, 2015.

5. Invited participant at the Conference on "New R&D Pathways to Address Neglected Diseases' Needs in the Indian Sub-continent" organized by DNDi, New Delhi at India International Centre, New Delhi in Oct 2015.

6. Invited participant at the 13th General Conference and 26th General Meeting of The World Academy of Sciences (TWAS) at Vienna, Austria, in Nov 2015.

7. Invited participant at Consultative meeting of ICMR and DNDi, PHD house, Delhi Dec 2015.

8. Member of Technical Advisory Committee Meeting at Hyderabad for BIRAC project Dec, 2015.

9. Attended meeting for monitoring progress of Post Doctoral Fellowship projects at ICMR at Dec 2015.

10. Attended Scientific Advisory Committee (SAC) meeting of RMRI Patna in Jan, 2016.

11. Attended meeting on "Monitoring drug susceptibility in VL cases" with DNDi and Kalacore at PHD house, New Delhi Jan 2016.

12. Presented work on "Diagnostics for VL and PKDL" at "Brainstorming meeting of Vector Borne Diseases Science Forum" held at ICMR, Delhi in Feb, 2016.

13. Continued as Co-ordinator of weekly Journal Club/ Seminars at National Institute of Pathology.

14. Reviewer for projects submitted for funding to DBT and DST.

15. Continued to serve as Associate Editor for the journal BMC Infectious Diseases.

16. Appointed reviewer of manuscripts submitted to various journals including Br J Dermatol., PLoS Neglected Tropical Diseases, Antimicrob Agents & Chemotherapy, J of Antimicrobial Chemotherapy, PloS One, Journal of Clinical Microbiology, Parasites and Vectors.

Dr. Nasreen Z. Ehtesham

1. Elected as an Executive Council member of Kashmir Central University as President's nominee.
2. Member Secretary of Institute Biosafety Committee.
3. Reviewer for Short Term Research project for ICMR.
4. Member of Institute Animal Ethics Committee.
5. Chairman, Institute Ethics Committee at IIT, Indore.
6. Reviewed several research projects submitted to DBT.
7. Attended meetings for selection of Post Doctoral Fellowship at ICMR in March 2016.
8. External Expert and Doctoral Committee member for PhD students at Kusuma School of Biological Scineces, IIT Delhi.
9. Reviewed research papers submitted to Plos ONE, FEMS Microbiology Letters, Oncotarget, Cytokines etc.

DR. SANGITA RASTOGI

1. Participated in ESCCAR International Congress on Rickettsia and other Intracellular Bacteria held at Laussane, Switzerland during 13th-16th June 2015 Title of presentation: '*Do IgA antibodies to Chlamydia trachomatis have protective role in humoral immunity: a study in reactive arthritis patients*'.

2. Assisted in conducting '*End-user program on nature.com & subscribed electronic journal– Nature*' through ICMR E-Journal Consortium at National Institute of Pathology, New Delhi on 15th September 2015.

3. Attended lecture on "*Expansion of Biopharmaceutical Innovation and New Vaccine Development (NVD)*" delivered by Dr. Julie Gerberding, Executive Vice President—Global Public Policy, Merck & Co. Inc. on 2nd November 2015 at ICMR (Hq), New Delhi.

4. Invited to attend Symposium titled, '*Innovate or copy paste'- A debate on innovation and intellectual property in the health sector* at India International Centre, New Delhi on 17th November 2015.

5. Convened Institutional Animal Ethics Committee (IAEC) meeting at National Institute of Pathology, New Delhi on 24th November 2015.

6. Attended lecture titled, '*Implementation research for addressing the NCD burden*' delivered by Prof. Brian Oldenberg, Chair of Non-Communicable Disease Control and Director of the Centre for Health Equity in the Melbourne School of Population and Global Health, University of Melbourne, Australia on 22nd December 2015 at ICMR (Hqs.), New Delhi.

7. Attended talk titled, '*On nobody's word: Evidence and modern science*' by Sir Venkatraman Ramakrishnan, Deputy Director of the Medical Research Council Laboratory of Molecular Biology, Cambridge organized at KK Birla Auditorium, FICCI, New Delhi on 8th January 2016.

8. Attended meeting held for DG, ICMR's media interaction at ICMR Hqs., New Delhi on 14th January 2016.

9. Attended Science Circle lecture on '*Science and Society- Infectious Diseases as an example*' by Professor Jorg Hacker, President, German National Academy of Science, Leopoldina at German House, New Delhi on 4th February 2016.

10. Participated in 17th International Congress on Infectious Diseases (ICID-2016) during 2nd-5th March 2016 at Hyderabad.

Dr. Usha Agrawal

Served as external examiner for DNB Pathology Practicals in

- September 2015
- April 2016
- June 2016

Evaluated DNB Thesis for NBE in November 2015

Dr. LK Yerneni

Delivered a talk entitled "Keratinocyte Culture" during the Training Programme for Burns Specialists 09th-11th March, 2016, under the session IV "Recent Advances in Burn Management"

Dr. Ruchi Singh

1. Invited reviewer for manuscript submitted to Journal of Clinical Microbiology, JOVE, Antimicrob Ag Chemoth, Journal of Vector Borne Diseases.

2. Member of Institutional ethics committee.

3. Faculty for Pre-PhD course work at NIP for Symbiosis University, Pune

4. Reviewer for short-term studentship projects of ICMR (2015-16).

5. Participated and presented work in 56th Conference of Association of microbiologists of India at JNU Delhi

6. Presented research work in 17th International Conference of Infectious Diseases organized by ISID at Hyderabad (2016).

Dr. Fouzia Siraj

1. Presented guest lecture entitled " Overview of In- situ- Hybridization" at Update on Advances in Cancer Research and Hands-on Workshop on Molecular Biology Techniques organized by Cancer Research Foundation, India and DBT Centre for Molecular Biology and Cancer Research, BBCI in collaboration with National Institute of Pathology (ICMR), New Delhi . Held at Dr B. Borooah Cancer Institute, Guwahati 10th September, 2015.

2. Poster presentation at Clinico pathological Conference on Gastro-Hepato-Pathology. A case based discussion. Fortis Escort Heart Institute, Okhla New Delhi. 21st February, 2016
   - **Rare Collision tumor of rectum-**A case of malignant melanoma and squamous cell carcinoma

3. Poster presentation at 31st Annual Conference of Delhi Chapter IAPM (DAPCON) : V.P.Chest Institute, organized by UCMS and GTB Hospital.New Delhi, 28th February, 2016
   - **Expression and clinico-pathological significance of Androgen receptor in Triple negative breast cancer…. (Best poster award)**
   - Recurrence and Malignant transformation in Pigmented Villonodular Synovitis: A Rare Event
   - Solitary Langerhans cell histiocytosis of hard palate: A diagnostic pitfall

4. Attended **Lymphoma Update 2016**. Essentials and beyond. Rajiv Gandhi Cancer Institute, New Delhi, 14-15th March, 2016

5. Other events:
   - **Swach Bharat Abhiyan. Awareness Programme and Poster Competition.** Held at National Institute of Pathology, ICMR,7th October, 2015
   - **Received 2nd prize in poster competition for staff**

Dr. Neeraj Kumar

Delivered lecture entitled "HLA Typing techniques and Analysis" in International Workshop on "The role of MHC Complex in Biology and Medicine" held at Miranda House college, University of Delhi on March 15, 2016.

Dr. Poonam Gautam

1. Attended **Sciex NextGen Proteomics Workshop** held from 15th-16th December, 2015 at Centre of Excellence, Sciex, Gurgaon, India.

2. **Invited talk** entitled "Proteomic Approaches in Disease Biomarker Discovery – Applications and Challenges" **An Update on Advances in Cancer Research and Hands-on Workshop on Molecular Biology Techniques** held from 10^{th} -12^{th} September, 2015 at Dr. B. Borooah Cancer Institute, Guwahati.

STUDENTS ACTIVITIES

Dr. Dibyabhaba Pradhan

- Dr. Dibyabhaba Pradhan, Scientist-II, delivered an invited lecture along with hand on session entitled "Streamlining NGS data analysis and molecular modelling for precise disease diagnosis and targeted therapeutic design in 18th Workshop on Molecular Modelling and Drug – Protein Interactions" organized by Bioinformatics Centre, Mahatma Gandhi Institute of Medical Sciences, Sevagram during January 11 - 12, 2016.

- Mr. Arnab Nayek, Research Assistant of Biomedical Informatics Centre, NIP presented the paper title "ADSETMEAS: Automated Determination of Salt-bridge Energy Terms and Micro Environment from Atomic Structures using APBS method, version 1.0" in "The 29th Annual Symposium of The Protein Society" conference held at Barcelona, Spain from July 22nd to July 25th, 2015.

Mr. Rishi Man Chugh

Submitted Ph.D. thesis on "A Study on Differential Growth-Arrest of 3T3 Fibroblasts used as Feeders for Epidermal Stem Cell Propagation" in October 2015.

Mr. Praveen Kumar

1. Participated in international conference and presented poster titled, '*Is there a correlation between Chlamydia trachomatis detection and development of disease in reactive arthritis/ undifferentiated spondyloarthropathy patients*'. 'ICID-2016, Hyderabad' (2nd - 5th March 2016)

2. Submitted Ph.D. thesis on "Studies on the role of *Chlamydia trachomatis* infection in the pathogenesis of reactive arthritis/undifferentiated spondyloarthropathy" in December 2015.

Ms Namita Singh

1. Received **Bill and Melinda Gates Foundation Award** for *Young Investigator from India & South-East Asia* from International Society of Infectious Diseases at 17th International Congress of Infectious Diseases, Hyderabad (2nd - 5th March 2016).

2. Oral presentation titled, "*Recurrent spontaneous abortion: Significance of early non-invasive detection of Chlamydia trachomatis infection*" at 17th International Congress on Infectious Diseases, held at Hyderabad, India (2nd - 5th March 2016).

Ms Priya Prasad

1. Participated in international conference and presented poster titled, '*Differential expression of superoxide dismutases in early aborters infected with Chlamydia trachomatis*'. ICID-2016, Hyderabad' (2nd - 5th March 2016).

2. Participated in hands-on training on "Gene Expression Microarray" organized by National Institute of Pathology (ICMR), New Delhi (21st - 22nd April 2016).

Ms Vanila Sharma

Awarded Dr. S. Sriramachari Young Scientist Award for the year 2015 held in May, 2015 at National Institute of Pathology (ICMR), New Delhi for her work on "*Leishmania donovani* specific Ubiquitin related modifier-1 (LdUrm1): an early endosone associated post translational modification".

Dr. Sandeep Verma

Presented work entitled *'Identification of Leishmania donovani membrane proteins interacting with macrophages by 2-Dimensinal gel Electrophoresis and Mass Spectrometry'* in 2nd International Conference on "New Challenges in Biotechnology and Molecular Biology in the Context of 21st Century from 27-29, Feb. 2016 at St. John's College (Agra).

Mr. Javeed Ahmad

Javeed Ahmad was awarded the EMBO fellowship to work for 3 months in MRC, Cambridge, UK

Ms Deepti Bajpai

Deepti Bajpai was awarded the Post-Doc fellowship from ICMR to work with Dr. Nasreen Z. Ehtesham.

Mr Manjunath P

Manjunath P. was awarded the DST INSPIRE fellowship.


SCIENTIFIC
ADVISORY
COMMITTEE

SCIENTIFIC ADVISORY COMMITTEE

1. **Dr. Subrata Sinha** — **Chairperson**
   Director,
   National Brain Research Centre (NBRC),
   Near NSG Campus,
   Nainwal Mode, Manesar,
   Gurgaon - 122 050,
   Haryana

2. **Dr. Shubhda Chiplankar**
   Director,
   Advance Center for Treatment,
   Research & Education in Cancer,
   Kharghar,
   Navi Mumbai – 410 210

3. **Dr. Dhananjaya Saranath**
   601-B, Kalpak Gulistan,
   9A Perry Cross Road,
   Bandra West,
   Mumbai - 400 050

4. **Dr. N.K. Mehra**
   C1/10, AIIMS Campus,
   All India Institute of Medical Sciences,
   Ansari Nagar,
   New Delhi – 110 029

5. **Dr. Chitra Sarkar**
   Professor,
   Department of Pathology,
   All India Institute of Medical Sciences,
   Ansari Nagar,
   New Delhi – 110 029

6. **Dr. Kiran Katoch**
Ex-Director, JALMA
C/o Dr. Rohini Katoch Sepat, IPS,
Suprintendent of Police,
Near 1st Cross Degree College,
Ooragaum,
Kolar Gold Fields,
Karnataka – 563 117

7. **Dr. Syed Kalbey Raza**
Director,
Institute of Pesticides Formulation Technology,
Sector – 20, Udyog Nagar,
Gurgaon – 122 018

8. **Dr. Pooja Sakhuja**
Professor & Head,
Department of Pathology,
G.B. Pant Hospital,
Jawahar Lal Nehru Marg,
New Delhi – 110 002

9. **Dr. Sudha Bhattacharya**
Professor & Dean,
Department of Environmental Sciences,
Jawahar Lal Nehru University,
New Delhi

10. **Dr. J.K. Batra**
Scientist VIII & Dy. Director,
National Institute of Immunology,
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11. **Prof. Jaya S. Tyagi**
Professor,
Department of Biotechnology,
All India Institute of Medical Sciences,
New Delhi – 110 029

12. Dr. Ravi Sirdeshmukh
Distinguished Scientist & Asso. Director,
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Hyderabad – 500 039

13. Dr. Ashwini Kumar
Industrial Toxicology Research Centre,
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Lucknow – 226 001

14. Dr. Ashok Mukhopadhyay
Scientist VI,
National Institute of Immunology,
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New Delhi-110067

15. Dr. Ravi Mehrotra
Director,
Institute of Cytology & Preventive Oncology,
Research-cum-Clinical Complex,
1-7, Sector-39, Near Degree College,
Opposite City Centre,
Noida-201 301 (U.P.)

16. Dr. Vijay Kumar
Scientist 'G' & Head,
Division of B.M.S.,
Indian Council of Medical Research,
Ansari Nagar,
New Delhi – 110 029


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ANIMAL ETHICS
COMMITTEE
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Scientist 'F', NIP (ICMR), New Delhi

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Dr. Om Singh
Retd. Scientist, NII, New Delhi

LINK CPCSEA NOMINEE
Dr. D N Rao
Professor & Head, Dept. of Biochemistry, AIIMS, New Delhi

SCIENTIST MEMBER
Dr. Vijay Pal Singh
Veterinarian (STO), Animal House Facility, IGIB (CSIR), Delhi

SOCIAL SCIENTIST MEMBER
Dr. R Gopinath (IFS)
Dy Conservator of Forests North, Delhi

VETERINARY CONSULTANT
Dr. P K Yadav
Sr. Veterinary Officer, Experimental Animal facility, AIIMS, New Delhi

NIP MEMBERS

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Scientist 'F', NIP (ICMR), New Delhi

Dr. Laxman Kumar Yerneni
Scientist 'E', NIP (ICMR), New Delhi

MEMBER SECRETARY
Dr. Sangita Rastogi
Scientist 'F', NIP (ICMR), New Delhi

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NIP, New Delhi

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Director-Professor
Department of Burns, Plastic and Maxillofacial Surgery
VMMC & SJH, New Delhi

EXTERNAL MEMBER

Dr. V. Ramesh, MD
Professor and HOD of Dermatology
VMMC & SJH, New Delhi

Dr. Sachin Manocha, MD
Asst. Professor of Pharmacology
VMMC, Safdarjang Hospital, New Delhi-29

Dr. Goutam Bhattacharya, M.Sc, Ph.D, LLB
K & S Partners, Gurugram

Ms. Bhavna Mukhopadhyay
Executive Director, VHAI, New Delhi

Dr. Achal Bhagat, MD, MRC Psych.
Chairperson, Saarthak (NGO), New Delhi

INTERNAL MEMBER

Dr. Usha Agrawal, MD
National Institute of Pathology, ICMR, New Delhi

Dr. Ruchi Singh, MSc, Ph.D
National Institute of Pathology, ICMR, New Delhi

MEMBER SECRETARY

Dr. L.K. Yerneni, M.Sc. Ph.D
National Institute of Pathology, ICMR, New Delhi


REFERRAL
SERVICES

REFERRAL SERVICES

DIAGNOSTIC DIVISION (HISTOPATHOLOGY AND CYTOLOGY)

In the year under report, more than 4000 samples were received in the histopathology laboratory for routine diagnosis and immunohistochemistry panel was also performed in more than 2000 cases as required. In addition research projects, both Institutional and non-Institutional, were supported and both processing and interpretation was offered to the research scholars. Numerous cases were also received for second opinion and report was given in these cases also.

Scientific Staff : Dr Sunita Saxena, Dr Usha Agrawal, Dr Anju Bansal, Dr Avninder Pal Singh, Dr Fouzia Siraj, Dr Shruti Sharma, Dr Manveen Kaur, Dr Varsha Dalal

Technical Staff : Ms Karuna, Ms Krishna, Mr Jagdish Pant, Mr Satyapal Singh, Mr Madanlal, Mr Shiv Bahadur, Mr Anil Verma, Mr Shyam Sundar, Mr Raj Singh, Mr Sanjay and Ms E. Sharda.

DIGITAL IMAGING LAB

The digital imaging lab houses the Digital slide scanning system, the cytogenetics work station and the Tissue microarrayer. We are happy to note that the lab is seeing an increasing footfall as the researchers become more aware of the facility and are using the equipment in the achievement of their research objectives. There are two ongoing dissertations using the slide scanning facility and numerous TMAs are being prepared by the Institute researchers.

Staff : Dr. Usha Agrawal

MOLECULAR BIOLOGY LAB

A total of 105 clinical samples were received from VL patients (blood, bone marrow), PKDL patients (blood, tissue biopsy and slit aspirates) and CL patients (tissue biopsy). Samples were processed for detection and species identification of *L. donovani* / *L. tropica* infections by PCR, PCR-RFLP, LAMP, Q-PCR and rk39 strip test.

Staff: Dr Poonam Salotra, Dr Ruchi Singh, Mr. Sandeep Verma, Ms. Vanila Sharma, Mr. Himanshu Kaushal, Mr. Kumar Avishek, Mr. Deepak Kumar Deep, Ms. Aditya, Ms. Keerti Kaumudi Dixit, Ms. Kamlesh Sharma, Mr. Anish Saxena.

CENTRAL PROTEOMICS FACILITY

Central Proteomics Facility, NIP, Delhi, has set up for gel-based separation of proteins and its analysis including IEF Cell, SDS-PAGE (small, medium and large format), ChemiDoc MP Image scanner and 2-D gel analysis software (PD Quest Advanced Software version 8.0). We have set up for 1-D and 2-D immunoblotting, scanner for imaging for fluorescently labelled proteins. We are expanding the set up for liquid chromatography-based separation of proteins/peptides and procurement of high pressure liquid chromatography (HPLC) and other equipments for the facility is under process. The facility is being maintained and used by different scientific groups in the Institute.

Staff : Dr. Poonam Gautam

MICROBIOLOGY LABORATORY

The Microbiology laboratory at National Institute of Pathology focuses on research in chlamydial infection pertaining to genital chlamydiasis and *Chlamydia*-induced reactive arthritis. In the year under report, 20 urine specimens were received from recurrent spontaneous aborters for detection of *C. trachomatis* infection by PCR and of PCR assays.

Staff: Dr. Sangita Rastogi, Mr. Praveen Kumar, Ms. Namita Singh, Ms. Priya Prasad, Mr. Kamal Dev.

ANIMAL HOUSE FACILITY (REG. NO.: 102/ GO/ REBI/ S/ 99/ CPCSEA)

The Animal House at NIP is a central facility registered with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forests & Climate Change (Govt. of India) and provides technical service to the Institute's scientists for small animal experimentation as per the CPCSEA guidelines. During the reporting period, Institutional Animal Ethics Committee meeting was conducted on 24.11.2015 for ethical clearance of research projects while inspection of the facility was undertaken by the CPCSEA nominee on 03.12.2015.

Staff: Dr. Sangita Rastogi, Mr. Kuldeep Kumar Sharma, Mr. Bala Dutt.

STEM CELL BIOLOGY LAB

The Cell Biology lab has been involved with the development of a cost-effective but effective strategy of imparting growth-arrest in Swiss 3T3 feeder cells. The ultimate aim of this research aspect is the large-scale production of Cultured Epidermal Autografts using human epidermal keratinocyte stem cells to be sourced from a small biopsy. The lab so far has been successful in segregating a specific Mitomycin C sensitive variant population from the normal Swiss 3T3 cultures by conventional cell culture approaches. This population was used to identify an optimal feeder batch through testing by a novel arithmetic dose derivation approach using Mitomycin C. The superior feeder functionality enabled faster production of epidermal sheets which are free of tumorigenesis, chromosomal defects and no traces of Mitomycin C and ready for clinical translation in burns.

The Lab Group Leader:

Dr. Lakshamana K Yerneni, Scientist E

Group members:

Mr. Rishi Man Chugh, SRF
Mr. Bijender, Technician C
Mr. Manoj Sejwal, Technician A

Collaborator:

Dr. Karoon Agrawal
Director-Professor,
Department of Burns, Plastic & Maxillofacial Surgery,
Safdarjung Hospital and VMMC,
New Delhi

LIBRARY

During the reporting period, the library continued to cater to the needs of the Institute's scientific staff, administrative staff, DNB/ M.Sc./ Ph.D. students, project staff and trainees. The library further subscribed to **5** online journals through *Wiley* and **15** Indian journals. The facility continued to give services to its users including access to online journals and reprints through inter-library loan from Safdarjung hospital library, NIC, National Medical Library, INSDOC and others. Inter-library loan requests were received through email and photocopies were dispatched by post. Xerox facility was further continued for the benefit of scientists and students.

The Institute's library has more than **10,000** books, bound journals, CDs, thesis, annual reports, WHO and AFIP fascicles pertaining to pathology, cancer, computer science, immunology, infectious diseases, toxicology, statistics, electron microscopy, confocal microscopy, sub-branches of pathology and other specialized medical subjects; Hindi books were further added to the library collection. The library displayed recent scientific publications, annual reports, newsletters of other Institutes, newspaper clippings of scientific, technical and various government publications. It also provided other services like indexing and abstracting of books/ journals. Also, the publication of NIP Newsletter was continued during the year and it was distributed to various ICMR Institutes and medical institutes.

NML-ERMED, JCCC@ ICMR (consortium between NML - ICMR - AIIMS), Cochrane, ICMR consortia and other online services were further provided to the scientific/ technical staff of NIP. JCCC and J-Gate custom content for consortia through *Informatics India Ltd.* was used for resource sharing with other ICMR libraries. The Local Area Network (LAN) facility (six nodes terminal internet connection) installed in the library for internet browsing and e-mail access was further continued. Online journals subscribed from Science Direct, Wiley, ICMR Consortia, NML-ERMED were accessed by scientists on their desktop. The facility thus allowed its users greater flexibility in meeting their needs.

Staff: Dr. Sangita Rastogi, Mrs. Anita Sharma, Mrs. Sangeeta Batra, Mr. Dharmender.

Email ID: ioplibrary@rediffmail.com


STAFF
LIST

STAFF LIST

SL.NO.	SCIENTIFIC STAFF	DESIGNATION
1	Dr. Sunita Saxena, MD (Path)	Director
2	Dr. Poonam Salotra, Ph.D.	Scientist 'G' w.e.f 1st Sept.'15
3	Dr. Nasreen Z Ehtesham, Ph.D	Scientist 'G' w.e.f 1st Sept.'15
4	Dr. AK Jain, Ph.D	Scientist 'F'
5	Dr. Sangita Rastogi, M.Phil., Ph. D.	Scientist 'F'
6	Dr. Usha Agrawal, MD	Scientist 'E'
7	Dr. LK Yerneni, Ph.D.	Scientist 'E'
8	Dr. Anju Bansal, MD (Path)	Scientist 'E' w.e.f 1st Sept.'15
9	Dr. Avninder Pal Singh, MD (Path)	scientist 'E' w.e.f 1st Sept.'15
10	Dr. Ruchi Singh, Ph.D	Scientist 'E' w.e.f 1st Sept.'15
11	Dr. Saurabh Verma, Ph.D.	Scientist 'D'
12	Dr. Sapna Negi, Ph.D.	Scientist 'D' w.e.f 22nd July'15
13	Dr. Appala Raju Bagadi, Ph.D.	Scientist 'C'
14	Dr. Fouzia Siraj, MBBS, DNB, MNAMS	Scientist 'C'
15	Dr. Shruti Sharma, MBBS, DNB	Scientist 'C'
16	Dr. Neeraj Kumar, Ph.D)	Scientist 'C' w.e.f Feb.'15
17	Dr. Poonam Gautam, Ph.D	Scientist 'C' w.e.f 1st Sept.'15
18	Dr. Prakash K Sahoo, Ph.D	Scientist 'B' upto 21nd July'15

SL.NO.	ADMINISTRATIVE STAFF	DESIGNATION
1	Mrs. R. Saratha	Administrative Officer
2	Mr. Raja Ram	Accounts Officer
3	Mr. Yogender Kumar	Section Officer (up to April 2015)
4	Mrs. Sunita Ahuja	Section Officer
5	Mr. VS Rawat	Section Officer
6	Mr. Jagdish Pershad	Private Secretary
7	Mr. Dashrath G. Khambadkar	Section Officer (w.e.f. 28.5.15)
8	Ms. Anita Sharma	Assistant Library Information Officer (w.e.f. 11.9.15)
9	Mrs. Rekha Rani	Personal Assistant
10	Mr. Subhash Babu	Assistant
11	Mr. Mangey Ram	Assistant
12	Mrs E. Sharda	Personal Assistant
13	Mrs. Sushma Ralhan	Assistant
14	Mrs. Sharmila Kamra	Assistant
15	Mr. Sonia Khattar	Assistant (w.e.f 1st Oct.'15)
16	Mr. Ajay Joshi	U.D.C.
17	Mr. Rajesh Kumar	U.D.C.
18	Ms. Jyoti	U.D.C
19	Mr. Brijender Singh	U.D.C
20	Mrs. Archana	U.D.C

SL.NO.	TECHNICAL STAFF	DESIGNATION
1	Dr. L.C. Singh	Technical Officer 'A'
2	Mrs. Madhu Badhwar	Technical Officer 'A'
3	Mr. Shiv Prakash	Technical Officer "A'
4	Mrs. Seema Sharma	Technical Officer 'A'
5	Mrs. Anita Bhatia	Technical Officer 'A'
6	Mr. Chandi Prasad	Technical Officer 'A' (Superannuated on 30.06.2015)
7	Mrs. Karuna	Technical Assistant
8	Mrs. Krishna	Technical Assistant
9	Mr. Kuldeep Kumar Sharma	Technical Assistant
10	Mrs. Valsamma Mathew	Technical Assistant
11	Mr. Jagdish Pant	Technical Assistant
12	Mr. P.D. Sharma	Technician 'C'
13	Mr. Suresh Bhimrao Kamble	Technician 'C'
14	Mr. Pushp Raj	Technician 'C'
15	Mr. Satyapal Singh	Technician 'C'
16	Mrs. Sangeeta Batra	Jr. Librarian
17	Mr. Madan Lal	Technician 'C'
18	Mr. Shiv Bahadur	Technician 'C'
19	Mr. Manwar Singh	Driver
20	Mr. Puran Singh	Driver
21	Mrs. Santosh Deora	Receptionist-cum-Tele. Operator
22	Sh. Bijendra Kumar	Technician 'B'
23	Sh. Kamal Dev	Technician 'B'
24	Mr. Anil Kumar Verma	Technician 'B'
25	Mr. Ajit Singh Lehra	Technician 'B'
26	Mr. Shyam Sunder	Technician 'B'
27	Mr. Daya Sagar	Technician 'B'
28	Mr. Sajid Hussain	Multi Tasking Staff (Technical)
29	Mr. Bala Dutt	Multi Tasking Staff (Technical)
30	Mr. Jagdish Ram	Technician 'A'
31	Mr. Dharmendar Singh	Technician 'A'

SL.NO.	TECHNICAL STAFF	DESIGNATION
32	Mr. Ram Chander Das	Multi Tasking Staff (Technical)
33	Mr. Puran Chand	Multi Tasking Staff (Technical)
34	Mr. Rajendra Kumar	Technician 'A'
35	Mr. Manoj Kumar Sejwal	Technician 'A'
36	Mr. Anish Kumar Saxena	Technician 'A'
37	Mr. Raj Singh	Technician 'A'
38	Mr. Chandrika Prasad	Multi Tasking Staff (Technical)
39	Mr. Sanjay Dutt Upreti	Multi Tasking Staff (Technical)
40	Mrs. Charanjeet Kaur	Multi Tasking Staff (Technical)

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