National Institute for Research in Tuberculosis (ICMR)

Annual Report
2013-14

WHO Collaborating Centre for Tuberculosis Research & Training
International Centre of Excellence in Research

NATIONAL INSTITUTE FOR RESEARCH IN TUBERCULOSIS

Research Activities

April 2013 – March 2014

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PREFACE

The year under review at NIRT saw continued activity in all aspects of tuberculosis research – clinical and socio-behavioural, bacteriological, epidemiological and basic science. The clinical trial on treatment shortening has yielded important interim results, which will be disseminated shortly. Similarly, the trial of daily versus intermittent treatment in HIV-infected TB patients has begun to show trends that need to be confirmed with larger numbers. Both these trials have important programmatic implications. The main constraint has been rapid enrolment of patients, due mainly to the strict inclusion and exclusion criteria. This will be overcome by expanding the number of clinical sites recruiting patients into our trials and collaborating with more institutes.

Many other clinical studies have been completed, with important results that have translational value. Pharmacokinetic studies of TB drugs in adults and children with and without HIV co-infection have thrown up unexpected findings, which need to be explored in greater depth. It is clear, however, that there are multiple factors that influence TB drug levels in patients and larger studies are needed to elucidate all the biological, environmental and pharmacogenetic factors associated with variations in drug levels. Further work will also be needed in the area of pharmacokinetic modeling in relation to MICs of the circulating clinical strains, in order to establish optimal drug dosages of first and second line anti-TB drugs.

Operational research supported by the WHO/USAID funded Model DOTS Project is covering areas from evaluation of newer diagnostics in children to smoking cessation strategies in TB patients. The results are expected to feed into the program and find answers to questions that arise as the program faces new challenges. These operational research studies attempt to answer questions related to diagnosis, treatment and program implementation. An example is a randomized trial of behavioural interventions for alcohol intake cessation in an attempt to reduce default and improve TB treatment outcomes. All these studies are undertaken in close collaboration with state and district level TB officers of the RNTCP.

A monograph has been published that attempts to capture the work done, lessons learnt and findings published between 1999 and 2014 from the Model DOTS project. Many of the findings have had an impact on TB control policy and practice in India and globally. The experience brings out the critical importance of operational research and the need to continue, expand and enrich the scope of research within programmatic settings.

In the area of basic science research, we have made progress with elucidating the immune responses in TB patients with and without diabetes mellitus and are examining local immune responses at the site of pathology from lymph node tissues, using advanced flow cytometric techniques. Work on virulence factors of M.tuberculosis as well as interpretation of whole genome sequence data has been initiated. Elucidation of protective epitopes against TB disease and identification of antigens

that can be used to differentiate latent from active TB is another area of work. The role of Vitamin D receptor polymorphisms in recurrent TB is being explored.

A number of workshops, seminars and training programs were conducted. New collaborations – both national and international – have been established and Memoranda of Understanding signed. A number of extramural project grants were received and studies are under way, including two large cohorts of TB patients to study risk factors for TB disease progression and treatment outcomes.

In the coming year, focus will be on evaluation of indigenous diagnostics for detection of TB as well as drug resistance and initiation of clinical trials with new TB drugs and adjunctive therapies. We have to work towards reaching the goal of zero TB deaths and a significant reduction in incidence by the year 2025. This will require team work and collaboration of all the various stakeholders to achieve universal coverage and provide quality care to all TB patients. I wish to place on record my gratitude and appreciation to all my colleagues in NIRT, as well as the front-line workers often working in very challenging conditions, for their continued endeavour to control TB in India.

Soumya Swaminathan, MD, FASc, FNASc, FAMS
Director
National Institute for Research in Tuberculosis, ICMR

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DISTINGUISHED VISITORS/
GUEST LECTURES

S.No.	Date of Lecture	Name of the Speaker	Affiliation of the speaker	Topic of the Lecture
1	May 09, 2013	Dr Alena Srinivasan	Wellcome Trust Fellow, ICGEB, New Delhi	How protective CD4 T-cell responses to *M. tuberculosis* are generated
2	July 17, 2013	Dr. Kedar Narayan	Post-doctoral Fellow, NCI, NIH	3D Cellular Imaging
3	July 18, 2013	Mr Shyam Manisastry	Cleveland State University, 2121, Euclid Ave. KB1409, Cleveland, Ohio 44115. USA.	GCP, GCLP SOPs and their application in NIRT clinical trials
4	July 29, 2013	Dr. Nerges Mistry	Director, The Foundation for Medical Research, Mumbai	Mumbai fact file on drug resistant TB strains
5	Aug. 5, 2013	Dr Sanjib Bhakta	Director of ISMB-Mycobacteria Research Laboratory and University Reader in Molecular Microbiology at Birkbeck, University of London and UCL, U.K	Tackling drug resistance and persistence in *Mycobacterium tuberculosis* - Integrated approach in novel therapeutic intervention
6	Sept. 12, 2013	Dr James Shepherd	Medical Officer, Clinical and Operations Research, World Health Organization, Geneva	The Rifaquin Trial results
7	Sept. 18, 2013	Dr. Vishwanath Venkataraman	Associate Professor of Microbiology/Immunology, Western University School of Health Sciences, Pomona, CA Campus, 309 E. Second St. Pomona, CA	Host immune responses to TB
8	Dec. 02, 2013	Prof Kirk R. Smith	Professor of Global Environmental Health and Associate Director for International Programs, University of Berkeley, USA	Air Pollution and Health
9	Dec. 12, 2013	Ms. Peggy Coulter	Senior International Laboratory QA/QC Coordinator, Patient Safety Monitoring in International Laboratories (SMILE), Maryland, USA.	GCLP & Bio Safety practices in a TB Laboratory

ABBREVIATIONS

AFB	Acid fast bacilli
ARR	Acquired rifampicin resistance
ART	Anti-retroviral treatment
ATT	Anti-TB treatment
AP	Auramine-O phenol
AUD	Alcohol use dependence
BSA	Bovine serum albumin
CS	Contact specific
CFA	Culture filtrate antigen
CFP	Culture filtrate proteins
DBS	Dried blood spots
DKO	Double knock-out
DSMB	Data and safety monitoring board
DST	Drug susceptibility testing
EB	Empirical Bayesian
EMB	Ethambutol
FCS	Fetal calf serum
FGDs	Focus group discussions
FLD	First line drugs
FNAC	Fine needle aspiration cytology
Fqs	Fluoroquinolones
FWB	Far western blotting
GMPS	Guanosine monophosphate synthase
HCs	Healthy controls
HCS	Healthy control subjects
HCP	Health care providers
HHC	Healthy household contacts
HPLC	High performance liquid chromatography
IGRAs	IFN-γ Release Assays
IPT	Isoniazid preventive therapy
IRIS	Immune reconstitutiton inflammatory syndrome
LTBI	Latent TB infection
LJ-SP	LJ with sodium pyruvate
LJ	Lowenstein Jensen
LAL	Limulus amebocyte lysate
LAM	Lipoarabinomannan
LED	Light-emtting diodes
LRP	Luciferase reporter phage assay
Lx	Levofloxacin
MCMC	Markov chain Monete Carlo
MDR-TB	Multi-drug resistant TB
MOX	Moxifloxacin
MSM	Men having sex with men
NC	Number of codons
NSP	New sputum smear positive
NVP	Nevirapine
OFX	Ofloxacin

PBMC	Peripheral blood mononuclear cell
PBPs	Penicillin binding proteins
PCA	Principal component analysis
PCR-RFLP	Polymerase chain reaction based restriction fragment length polymorphism
PhAS	Phenol ammonium sulphate
PLHIV	People living with HIV
PMDT	Programmatic management of drug-resistant TB
QFT-GIT	Quantiferon TB gold assay
RMP	Rifampicin
ROC	Feceiver operating characteristic
RT-PCR	Real-time polymerase chain reaction
RT-PCR	Reverse transcription polymerase chain reaction
SKM	Selective Kirchner's medium
SLD	Second line drugs
SOM	Self organizing maps
SHG	Self help group
SNP	Sodium nitroprusside
STPK	Serine / threonine protein kinases
TLR	Toll like receptor
TST	Tuberculin skin test
2D-LPE	Two dimensional-liquid phase electrophoresis
VDR	Vitamin D receptor
XDR-TB	Extensively drug resistant TB
ZN	Ziehl-Neelsen

CLINICAL STUDIES

DEPARTMENT OF CLINICAL RESEARCH

STUDIES IN PROGRESS:

CL-1: A randomized controlled clinical trial comparing daily vs. intermittent 6 – month short course chemotherapy in reducing failures & emergence of acquired rifampicin resistance in patients with HIV and PTB

Principal Investigator	:	Dr. G. Narendran (email:nareng@nirt.res.in)
Co-PI	:	Dr. Soumya Swaminathan
Collaborators	:	Govt. Hospital of Thoracic Medicine, Tambaram; Govt. Rajiv Gandhi General Hospital, Chennai; NJIL and OMD, Agra; Govt. Stanley Hospital, Chennai; Govt. Otteri Hospital, Chennai; Govt. Vellore Medical College and Hospital, Vellore; Govt. Rajaji Hospital, Madurai
Source of funding	:	USAID (Model DOTS Project)
Study period	:	2009-2015
Trial Registry No.	:	476/09, NCT No. 933790

Background: HIV-TB is an important dual infection in India demanding attention from programme managers and clinicians with focus on duration of treatment and schedule of administration. We undertook this study to investigate the schedule of therapy, sputum conversion, radiological clearance, immune reconstitution inflammatory syndrome (IRIS), treatment emergent adverse drug reactions and drug levels.

Aims: (i) Primary: to compare daily vs. intermittent therapy of anti-TB treatment (ATT) in reducing failures and emergence of acquired RMP resistance (ARR) and

(ii) Secondary: to examine sputum conversion, IRIS, emergence of ARR, radiological improvement, plasma rifampicin (RMP) concentrations and toxicity profile with respect to dosing schedule

Methods: HIV-infected patients with culture positive TB are randomized to one of three regimens, namely (1) Daily regimen ($2EHRZ_7/4HR_7$), (2) part daily ($2EHRZ_7/4HR_3$) and (3) a fully intermittent regimen ($2EHRZ_3/4HR_3$), given for 6 months duration and followed up for a further period of one year, stratification is based on CD4 counts and sputum smear grading. Blood samples at 2-hr post dosing is being collected at months 2 and 6 of ATT. Toxicity is monitored using modified CTC and DAIDS criteria. Unfavourable responses in each regimen during treatment and follow-up are compared. Both intent to treat analysis and per protocol analysis was performed.

Results: A total of 247 patients have been enrolled so far. (Centre-wise case enrolmen: Tambaram TB sanatorium - 54, Government

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Rajiv Gandhi Hospital, chennai -83, TRC -14, Vellore Govt general hospital -20 and Government Rajaji Hospital, Madurai- 76). The baseline parameters are given in Table 1. At the end of intensive phase of the 179 available cases, smear conversion in the daily and intermittent arms were 63% and 51% respectively, while culture conversion was 93% in the daily arm compared to 82% in the intermittent arm (p=0.04). Whether this early response through speedier culture conversion would reflect the final outcome, is being evaluated.

Table 1: Baseline parameters of trial participants

Demographics	Daily	Part daily	Intermittent
Total randomized	85	82	90
Age (yrs)	37 ± 8	40 ± 10	39 ± 9
Body weight (kg)	43.1 ± 8.6	42.5 ± 8.3	43.5 ± 6.5
Hemoglobin (g/dl)	9.7 ± 2.4	9.4 ± 2.0	10.0 ± 2.1
Hemotocrit (%)	28.5 ± 7.0	27.3 ± 5.6	28.9 ± 7.2
Viral load log 10 (copies/ml)	4.9 ± 1.1	4.8 ± 1.1	4.9 ± 1.1
ATT-ART interval* [days]	15	14	15
CD4 count/mm^{3} **	132 (71 – 241)	144 (81 – 258)	145 (74 – 332)

Above values are mean$\pm$SD; *denotes median; **denotes median (IQR)

CL-2: **Evaluation of different strategies (pharmacologic intervention versus enhanced motivation vs. standard motivation) for smoking cessation in TB patients under treatment in the revised national TB Programme – A cluster randomized effectiveness trial**

Background: Smoking prevalence among males (both rural and urban) has increased in India (NFHS III Vs NFHS II). Higher relapse, increased morbidity and mortality among smokers with TB has been observed. A pilot study conducted at NIRT, Madurai, showed that at month 1, 35-45% of TB patients quit smoking when counseling was given by a doctor / medical social worker. We proposed to evaluate different smoking cessation strategies in TB patients treated in the programme.

Aim: To compare the feasibility, acceptability and effectiveness of pharmacologic therapy (Bupropion SR) versus enhanced counselling package in smoking cessation among TB patients initiating treatment

Methods: Study Design: Cluster randomized effectiveness trial.

Study Procedure: DMCs from two districts - Villupuram and Kanchipuram were randomly selected (cluster randomization) to receive (1) T1 – Bupropion SR along with standard counseling, or (2) T2 – enhanced counseling arm including provisions of Educative materials on smoking cessation, flip charts presentation, posters display, video presentation or (3) C – standard routine counseling/Control arm. Smoking cessation is assessed by self reporting and confirmed by Carbon monoxide monitors, done at 0, 2 and 6th month of ATT. TB outcome is recorded at 6th month for these patients. The total sample size has been calculated to be 1200 patients, 400 in each arm. So far, 58 patiens have been recruited (details given in Table 2). The study is ongoing.

Table 2: Recruitment details of study subjects

District	No. screened	Arms			
		Drug	Enhanced	Std	Total
Villupuram	229	5	6	20	31
Kancheepuram	121	8	5	14	27
Total	357	13	11	34	58

CL-3: Randomized clinical trial to study the efficacy and tolerability of 3- and 4-month regimens containing moxifloxacin in the treatment of patients with sputum positive PTB – Study XXIV

Principal Investigator : Dr.V.V. Banu Rekha
(email: banurekha@nirt.res.in)
Co-Principal Investigator : Dr.M. Makesh Kumar
Source of funding : Intramural
Study period : 2007-2014
CTRI Registration No. : PROVCTRI/2008/091/000024

Background: The currently recommended 6-month regimen for the treatment of newly diagnosed PTB patients has been in use since the 1970s. This regimen, though highly effective, poses challenges for patients and providers due to the long duration. Shortening the duration of ATT will therefore be an important contribution to TB control and is recognized as a research priority. To address this issue a randomized clinical trial is being conducted by the NIRT in Chennai, Madurai and Vellore.

Aim: (i) to study the efficacy and tolerability of the standard 4-drug TB regimen supplemented with moxifloxacin

(MFX) in PTB patients with the aim of shortening treatment duration

Methodology: Patients with newly diagnosed sputum positive, HIV sero-negative PTB are randomly allocated to 3-month or 4-month MFX regimens, or a 6-month control regimen (Table 3). Treatment is directly observed and response to treatment is assessed by clinical evaluations and with sputum examinations. The patients are also closely monitored for adverse drug reactions. Patients with successful treatment outcome are followed up for 24 months after completion of treatment with monthly evaluations for assessing recurrence of TB.

The study regimens are described in Table 3.

Table 3: Study regimens

Regimen	Intensive phase	Continuation phase	Duration (mths.)
Test regimen 1	3 RHZEM daily		3 (intake stopped)
Test regimen 2	2 RHZEM daily	2 RHM daily	4
Test regimen 3	2 RHZEM daily	2 RHM thrice weekly	4
Test regimen 4	2 RHZEM daily	2 RHEM thrice weekly	4
Control regimen	2 RHZE thrice weekly	4 RH thrice weekly	6

R – rifampicin; H – isoniazid; Z – pyrazinamide; E – ethambutol; M - moxifloxacin

Results: A total of 931 patients have been enrolled in the study. The baseline characteristics of these patients are shown in Table 4. A majority of the patients were male, had advanced disease evidenced by strongly positive sputum cultures and radiological involvement of more than two lung zones.

Table 4: Baseline characteristics of 931 patients enrolled in study

Regimen	Test Reg. 1 (n = 112)	Test Reg. 2 (n = 212)	Test Reg. 3 (n = 210)	Test Reg. 4 (n = 210)	Control Reg. (n = 187)	Total (n = 931)
Sex						
Male	89 (79%)	163 (77%)	158 (75%)	145 (69%)	148 (79%)	703 (75%)
Female	23	49	52	65	39	228
Age						
<35 years	58 (52%)	99 (47%)	113 (54%)	116 (55%)	101 (54%)	487 (52%)
≥35 years	54	113	97	94	86	444
Initial sputum culture						
1+	3	12	17	17	9	58
2+ or 3+	109 (97%)	200 (94%)	193 (92%)	193 (92%)	178 (95%)	873 (94%)
Extent of Initial X-ray involvement (zones)						
≤ 2	25	45	42	49	42	203
> 2	87 (78%)	167 (79%)	168 (80%)	161 (77%)	145 (78%)	728 (78%)

A salient finding of this study is that the proportion of patients who became sputum culture negative after the initial two months of treatment was significantly higher (94%) in the MFX arm (consolidated for all four test regimens) compared to the control arm (77%). This observation which was made earlier (Annual Reports 2009-2010, 2010-2011, 2011-2012, 2012-2013) is sustained even with a larger population. Fig. 1 illustrates the proportion of patients with negative sputum cultures at 15, 30, 45 and 60 days of treatment. This is a significant finding as it shows that patients treated with the MFX regimens become less infectious earlier and to a greater degree compared to those treated with the control regimen.

Fig.1: Sputum culture conversion with treatment in 931 patients
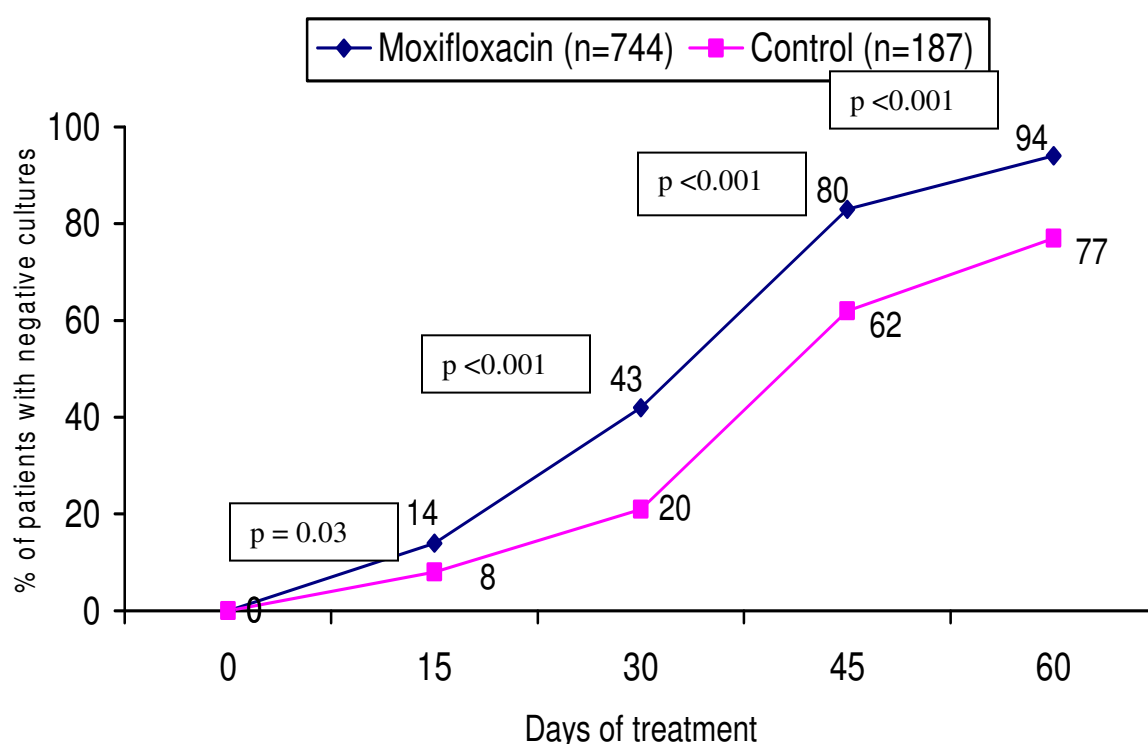
Table 5 describes the results at the end of treatment in 770 patients, and recurrence of TB among those who had a favourable response at the end of treatment. Of the patients treated with MFX regimens, 92% had negative sputum cultures at the end of treatment compared to 81% in the control regimen (intent to treat analysis).

Of the 689 patients with successful outcome at the end of treatment, 56 had recurrence of TB during post-treatment follow-up. TB recurrence was significantly higher in the

Test Regimen 1 (3-month MFX regimen) compared to the 4-month MFX regimens and the control regimen. Based on this information, the Data and Safety Monitoring Board (DSMB) had earlier recommended temporary cessation of intake to this regimen (Annual Report 2010-2011), pending a more detailed review. Following an interim review of the data, the DSMB had 3 yrs ago recommended the cessation of recruitment to this regimen. Intake to the other regimens is continuing.

Table 5: Response at the end of treatment and TB recurrence during 24 months follow-up

Regimen	Patients	Response at end of treatment			TB recurrence*
		Favourable	Unfavourable	Lost to flu	
Test regimen 1 (intake stopped)	112	103 (92%)	2	7	20 (19%)
Test regimen 2	166	152 (92%)	8	6	9 (6%)
Test regimen 3	166	152 (92%)	6	8	12 (8%)
Test regimen 4	166	152 (92%)	7	7	9 (6%)
Control regimen	160	130 (81%)	12	18	6 (5%)

* in those with favourable response at the end of treatment (column 3)

CL-4: Randomized clinical trial to study the efficacy and tolerability of a 4-month regimen containing ofloxacin compared to the standard 6-month regimen in the treatment of patients with superficial lymph node TB

Principal Investigator	:	Dr. D. Baskaran
		(email: baskar.d@nirt.res.in)
Collaborators	:	Govt. Stanley Hospital and Gov t. Rajaji Gandhi Medical College Hospital, Chennai
Source of funding	:	Intramural
Study period	:	2013-2016
Trial Registry No.	:	CTRI/2013/03/003481

Background: TB lymphadenitis is the most common presentation of extra-PTB, accounting for 30–40% of cases in reported series. Under the Revised National Tuberculosis Control Programme (RNTCP), patients with TB lymphadenitis are currently treated with a thrice weekly regimen (Category-I) with 4 drugs (RMP), Isoniazid (INH), Ethambutol (EMB) and Pyrazinamide (PZA) for the first two months followed by 2 drugs (RMP and INH) for the next four months. A study done at the NIRT has shown that even less intensive regimens, viz. a 6-month regimen of $2RHZ_2/4RH_2$ and a 6-month regimen of RH daily were highly successful in patients with biopsy confirmed lymph node TB in Madurai, south India. Despite its efficacy, the regimen must be administered for at least 6 months to be fully effective. The delivery of TB chemotherapy in the field would be much easier if the duration of therapy could be shortened without sacrificing efficacy. This requires the development of agents with potent bactericidal and/or sterilizing activity against *M. tuberculosis*, which allows shortening the treatment duration.

The current study is comparing the efficacy and tolerability of a 4-drug regimen consisting of RMP, INH, PZA and Ofloxacin (OFX) daily during an intensive phase of two months, followed by a 3 –drug regimen of RMP, INH and OFX thrice weekly during the continuation phase with the 6-month thrice weekly control regimen - thrice-weekly regimen of RMP, INH, EMB and PZA for 2 months followed by RMP and INH for 4 months.

OBJECTIVES:

Primary objectives:

To compare the efficacy of the two regimens in terms of

a. Response at the end of treatment

Secondary objectives:

(ii) to compare the incidence of

a. "Paradoxical reaction" during treatment and follow-up

b. Adverse drug reactions in newly diagnosed superficial lymph node TB patients treated with 4-month OFX containing regimens, with the same in those treated with the 6-month control regimen

Study design:

Design: Prospective, randomized (open-label) parallel arm, controlled clinical trial

Drug Dosages:

All doses are as tablets or capsules are given once daily or thrice weekly as per the regimen under direct supervision.

OFX – 600mg; (800mg for patients weighing more than 45Kg)

INH – 300mg (daily); 600mg (thrice-weekly);

RMP – 450mg (600 for patients weighing 60 kg. or more);

PZA – 1500mg daily and thrice weekly

EMB – 1200mg thrice weekly

Study population:

Patients attending the surgical, medical clinics of Govt.. Stanley Medical College Hospital and Govt. Rajiv Gandhi Medical College Hospitals in Chennai with Fine

Study progress:

The study started in November, 2012. As on March 31 2014, 500 patients were screened,

needle Aspiration Cytology (FNAC) proved superficial TB lymphadenitis or clinical evidence of lymph nodes enlargement are considered for the study. Patients are randomly allocated to either the test or control regimen.

All patients undergo clinical evaluation every month including an assessment of the lymph node in the clinic. All patients are followed up for a period of 24 months after completion of treatment.

Study outcome:

The following outcome measures will be compared beween the test and control regimens:

Primary outcome

a) Favourable, probably favourable but biopsy recommended, unfavourable at the end of treatment

b) Relapse during follow-up in those with favourable response at the end of treatment

Secondary outcome measure

a) Paradoxical reactions

b) Adverse reactions to anti-TB drugs

The study is being conducted in Chennai. The estimated sample size for this trial is 320 patients.

of whom 33 have been recruited to the trial. Details are available for 26 patients (Table 6).

Table 6: Patient details

	Regimens	
	2EHRZ$_3$ / 4RH$_3$	20HRZ$_7$ / 20HR$_3$
	n = 12	n = 14
Sex		
Females	9	7
Males	3	7
No. of nodes		
Multiple	9	14
Single	3	-
Baseline lymphnode culture		
Neg	5	1
Pos	75	13

CL-5: Evaluation of newer diagnostic tools and feasibility of consensus case definition in the diagnosis of intrathoracic TB in children

Principal Investigator	:	Dr. Soumya Swaminathan (email: soumyas@nirt.res.in)
Co-Principal Investigator	:	Dr. V.V. Banu Rekha
Collaborators	:	Govt. Stanley Hospital (GSH), Chennai; Institute of Child Health (ICH), Chennai; Christian Medical College (CMC), Vellore; Govt. Vellore Medical College (GVMC), Vellore and Govt. Rajaji Hospital (GRH), Madurai
Source of funding	:	USAID (Model DOTS Project)
Study period	:	2013-2016

Background: The lack of a gold standard for diagnosis of childhood TB is a major obstacle to accurately quantifying the true burden in children which is probably both over and under-diagnosed among children in different settings. The need for improved TB

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diagnostics in children is consistently acknowledged. Promising novel techniques (Xpert® MTB/RIF, urine LAM) that have been developed for the diagnosis of TB need to be tested and validated in children. Xpert® MTB/RIF (Cepheid, Sunnyvale, USA) is an automated user-friendly real-time PCR assay designed for the rapid and simultaneous detection of *M. tuberculosis (M.tb)* and RMP resistance. Lipoarabinimannan (LAM) is a structurally important 17.5kD heat-stable glycolipid found in the cell wall of *M.tb*. Detection of LAM antigens in urine has several potential advantages, as urine samples are simple to collect and process.

A group of international experts have developed a consensus reference standard and case definition for PTB in children, for use in research and clinical settings. This study provides an ideal opportunity to test the feasibility and clinical relevance of this consensus case definition.

Aims: (i) to determine the diagnostic accuracy of Xpert® MTB/RIF (Cepheid, Sunnyvale, USA) in the diagnosis of intra-thoracic TB in children and to study the feasibility of utilizing the newly developed consensus case definition

(ii) to compare the yield of *M.tb* from different specimen collection methods (expectorated / induced sputum, gastric lavage) in various age groups and to evaluate urine LAM, in the diagnosis of intra-thoracic TB

Methodology: All children aged < 15 yrs attending the paediatric out-patient department with any of the following are screened for the study - (a) cough (b) weight loss/ failure to thrive (c) persistent unexaplained fever (d) persistent, unexplained lethargy or reduced playfulness. Symptom screening, a detailed general and clinical evaluation is done. Chest X-ray, tuberculin skin test (TST), collection of gastric lavage / induced / electroporated sputum for Xpert® MTB/RIF, AFB smear, culture (and drug susceptibility testing (DST) if culture positive) are done.

In addition, in infants (i.e. aged < 1yr), stools are collected for two consecutive days which are examined by Xpert® MTB/RIF, for AFB smear, culture and DST, if culture positive. Urine for LAM, blood investigations and FNAC are done, if needed. TB diagnosis in children is made and classified into the following groups based on smear result, chest radiograph and TST as confirmed TB, Probable TB and others. Follow up is done at 2 weeks, 4 weeks, 8 weeks, and end of treatment.

Sample size: 2800 children with presumptive TB

Current status: The study was initiated in August 2013. The study is currently enrolling children from Govt. Stanley Hospital (GSH), Chennai; Institute of Child Health (ICH), Chennai; Christian Medical College (CMC), Vellore; Govt. Vellore Medical College (GVMC), Vellore; and Govt. Rajaji Hospital (GRH), Madurai.

As of March 2014, a total of 581 children have been enrolled in this study and the study is ongoing (Table 7).

Table 7: Site-wise recruitment details of children

	GSH	ICH	GVMC	CMC	GRH	Total
Number of children screened	311	248	42	105	73	779
Number of children enrolled	253	242	27	13	46	581
Number of children on assessment	15	8	2	2	5	32

CL-6: Predictors and immunologic characterization of TB-associated Immune Reconsitution Inflammtory Syndrome (IRIS) in HIV-TB patients started on antiretroviral therapy

Principal Investigator	:	Dr. G. Narendran (email: nareng@nirt.res.in)
Co-Principal Investigator	:	Dr. Soumya Swaminathan, Dr. Sudha Subramanyam, Mr.S. Anbalagan
Source of funding	:	NIH Intramural to India grant 2008 and ICMR adhoc grant from 2012
Study period	:	2009-2014

The study was initated to detect early predictors of TB-associated IRIS among HIV/TB patients initiating anti retroviral treatment (ART) so that effective preventive strategies could be put in place. This would also help to predict IRIS, which has so far remained a clinical diagnosis.

Methodology: HIV/TB co-infected patients with and without IRIS manifestations were prospectively followed through progressive

stages of immune restoration and clinical and immune predictors were serially recorded. Both univariate and multivariate analysis was carried out for evaluation of predictors, using various clinical and immunological markers.

Results: A total of 48 HIV positive patients with newly diagnosed culture confirmed PTB who were ATT and ART naïve were recruited. Fig. 2 shows the sequence of IRIS events from start of ATT to subsidence of IRIS in a single patient. Fig. 3 shows the diversity of IRIS manifestations among study cases. We found that IL-6 and CRP combination could successfully predict IRIS. Univariate analysis showed that low CD4, high viral load, opportunistic infection, miliary TB, presence of extrapulmonary focus, low hemoglobin and shorter ATT-ART interval were associated with IRIS. Hemoglobin and time interval between ATT-ART remained significant in multivariate regression analysis. Paradoxical TB-IRIS was characterized by high plasma levels of inflammatory biomarkers related to monocytes.

Fig. 2: Chest skiagrams of a MILIARY TB patient showing improvement with ATT and paradoxical worsening with ART and again clearance after steroids

A- PRE-ATT   B- PRE-ART   C- AT IRIS   D- AFTER STEROIDS

A	B	C	D
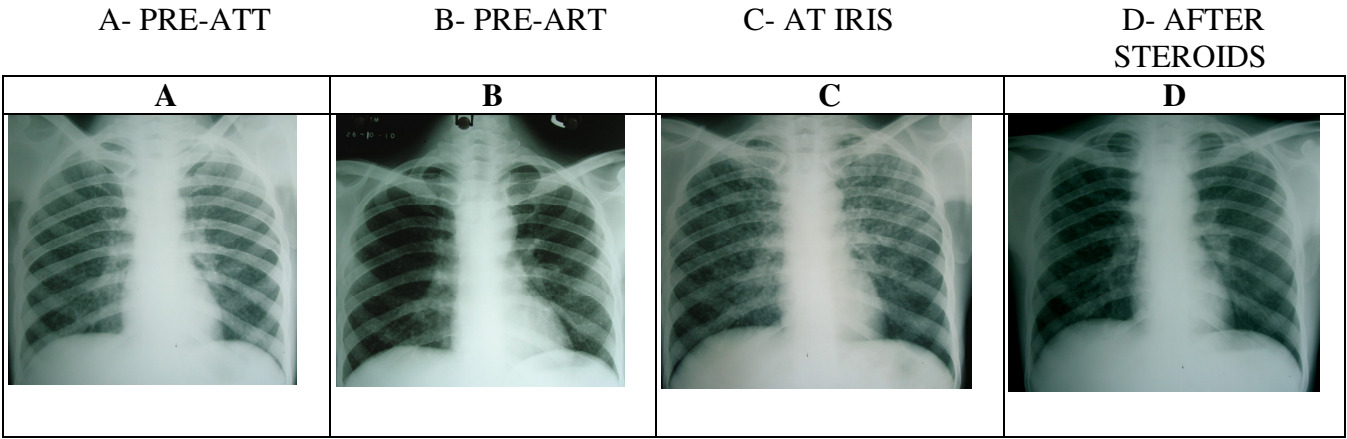
Fig.3: Diverse manifestations of IRIS in different patients

A	B	C	D
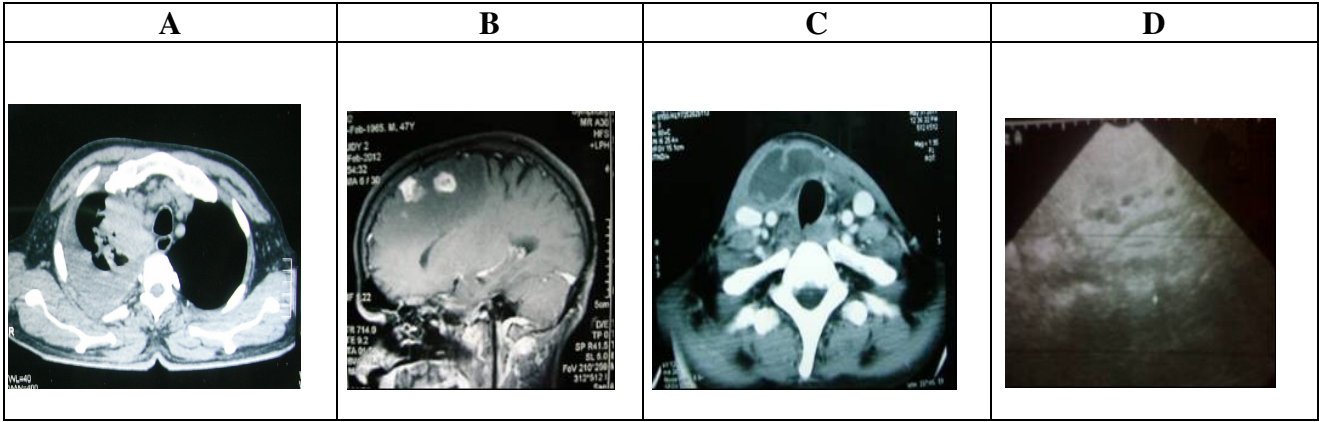
A- Massive para tracheal nodes presenting as strider pleural effusion present
B- Multiple tuberculoma brain
C- Cold abscess mimicking a thyroid swelling
D- Peri-portal chain of lymph nodes

CL-7: **A prospective study to determine the Incidence of TB among patients with type 2 diabetes mellitus**

Principal Investigator	:	Dr.M. Makeshkumar (email: makeshkumar.m@nirt.res.in)
Co-Principal Investigator	:	Dr. Soumya Swaminathan
Collaborators	:	Dr. Tazibha Hussain (RMRC, Bhubaneswar), Dr. Vijay Viswanathan (MV Hospital for Diabetes, Chennai), Dr.P. Dharmarajan (Institute of Diabetology, Rajiv Gandhi Govt. General Hosiptal, Chennai), Dr.E. Subbiah (Dept. of Diabetology, Govt. Rajaji Hospital, Madurai)
Source of funding	:	WHO through Model DOTS Project
Study period	:	2013-2017

Background: The diabetes epidemic has a major impact on the epidemiologic dynamics of TB and poses several challenges to the control of TB in a resource-poor country like India. diabetes/TB burden can be brought under control by timely diagnosis of TB among diabetics by intensified case finding, followed by adequate and effective treatment of detected cases and possibly preventive therapy. Given the serious threat posed by the diabetes epidemic on control of TB, and the current gaps in knowledge related to diagnosis, prevention and treatment of TB among diabetes persons in the Indian population, we have initiated a cohort study in a representative population to establish the incidence of TB in Type 2 diabetes patients.

Aims:

Primary objective:

To determine the incidence of TB among people with Type 2 Diabetes Mellitus

Secondary objectives:

(i) to identify risk factors for TB among people with Type 2 Diabetes Mellitus

(ii) to study the diagnostic accuracy of sputum smear for diagnosis of TB among people with Type 2 Diabetes Mellitus.

(iii) to correlate clinical and radiographic features of TB with severity of Type 2 diabetes

(iv) to evaluate the diagnostic accuracy of Gene Xpert MTB/RIF among Type 2 Diabetes Mellitus patients with suspected TB

Methods: This is a multicentric prospective cohort study among Type 2 diabetic patients to study the incidence of TB. Study participants are recruited from patients who

attend Diabetic OPD at Government General Hospital Chennai, Government Rajaji Hospital Madurai, and MV Hospital for Diabetes, Royapuram, Chennai and Capital Hospital, Bhubaneshwar and District Hospital, Khurdha, Odhisa.

So far, 128 patients have been recruited against a targeted sample size of 6000 patients. Their mean age, body weight and glycosylated hemoglobin values were 50 years, 61 kg and 8.1 respectively. There were 94 females and 34 males. The study is ongoing.

CL-8: HIV-associated lipodystrophy syndrome in children: Role of nutrition, ART and genes

Principal Investigator	:	Dr. Soumya Swaminathan (email: soumyas@nirt.res.in)
Collaborators	:	Dr. Christine Wanke, Dr. Alice Tang, Tufts University, Dr. Anita Shet, St. John's National Academy of Health Sciences, Bangalore
Source of funding	:	National Institute of Health (5RO1 A1084390)
Study period	:	2011-2015

This is a prospective multi-centric observational study undertaken at NIRT, Chennai and Madurai and St. John's National Academy of Health Sciences, Bangalore, to determine the incidence and risk factors for dyslipidemia, abnormalities in glucose tolerance and body shape abnormalities, in HIV-infected children between the ages of 2 and 12 years after initiating ART, as well as to determine the role of genetic factors in the development of fat redistribution, insulin resistance and dyslipidemia.

The study, initiated in June 2011, plans to enrol 440 children with HIV infection. As on 31st March 2014, 277 children between the age group of 2-12 years have been recruited to the study. Details about their demographics, clinical and dietary history (Food security questionnaire, 24 hour dietary recall), physical examination, anthropometric measurements are collected. Baseline data is shown in the Table 8. These children are followed every 3 months upto 24 months after initiation of ART. Blood investigations to measure lipid

profile, peripheral insulin resistance, C-reactive protein, hematology, CD4 cell counts and viral load measurements are done at baseline and every 6 months till 24 months of follow-up.

Table 8: Baseline demographics of HIV-infected children enrolled to the study

S. No.	Characteristics (n=218)	Mean (SD)	Range
1.	Age in years	8 (3)	2 – 12
2.	Body weight in kgs	18 (6)	7 – 41
3.	Height in cms	111 (18)	64 – 148
4.	CD4 cell count in cells/ mm^3	540 (471)	2 – 3312
5.	Viral load in copies/ml	6,21,775	
6.	Total Cholesterol (mg/dl)	129 (35)	51- 269
7.	HDL-c (mg/dl)	29 (11)	7 – 63
8.	LDL-c (mg/dl)	78 (28)	16 – 196
9.	Serum Triglycerides (mg/dl)	141 (71)	30 – 393
10.	Blood glucose (mg/dl)	84 (14)	29 – 153
11.	Serum Insulin (mg/dl)	8.3 (12)	1 – 94
12.	Serum C-reactive Protein	2.3 (3)	0.1 -14.7

As part of genotyping studies, the association between PLIN gene polymorphisms and lipodystrophy was studied. Results were analysed using Gene Mapper software (ABI). The variant allele frequency of the PLIN gene in the study population is shown in Table 9.

Table 9: Variant allele frequencies of PLIN gene

S. No.	PLIN SNP	No. of subjects	Allele frequency	
1	PLIN_rs1052700 (14995A>T)	224	0.64 (A)	0.36 (T)
2	PLIN_rs2289487 (6209T>C)	223	0.57 (C)	0.43 (T)
3	PLIN_rs894160 (11482G>A)	226	0.67 (G)	0.33 (A)
4	PLIN_rs2304795 (13041 A > G)	221	0.54 (G)	0.46 (A)

Preliminary findings suggest that variant alleles of PLIN gene are present in significant numbers in our population.

The role of these polymorphisms in HIV- associated Lipodystrophy Syndrome in HIV-infected south Indian children who are on ART is yet to be analyzed. The study is ongoing.

CL-9: High density lipoprotein cholesterol and gene polymorphisms among HIV- infected south Indians on first line antiretroviral therapy

Principal Investigator	:	Dr.C. Padmapriyadarsini (email: padmapriyadarsinic@nirt.res.in)
Source of funding	:	Fogarty International (NIH)
Study period	:	2013-2014

This is a cross-sectional study to determine whether HDL-cholesterol gene polymorphisms (single nucleotide polymorphisms in ABCA1, CETP, LIPC, LPL and APOC3 genes) are associated with unfavorable blood HDL-cholesterol levels, in HIV- infected adults in south India, after 12 – 15 months of Nevirapine (NVP) based ART regimen.

The study outcome will be looking for the presence of single nucleotide polymorphism in ABCA1, CETP, LIPC, LPL and APOC3 genes, in individuals with low HDL-c after 12 – 15 months of NVP based first line ART. The estimated sample size for this outcome is 300. The study was initiated in January 2013 and as on 31st March 2014, 275 patients have been recruited. Further, recruitment of patients is continuing.

CL-10: Study on the effectiveness and feasibility of TB preventive therapy for people living with HIV in India - adults and children

Principal Investigators	:	Dr.C. Padmapriyadarsini, Dr.P.K. Bhavani, Dr. Soumya Swaminathan (email:padmapriyardarsinic@nirt.res.in/ soumyas@nirt.res.in/bhavanipk@nirt.res.in)
Collaborators	:	ART MOs of study sites, NACO, CTD
Funding Agency	:	USAID (Model DOTS project)
Study period	:	2013-2015

This is a prospective multicentric study with phased implementation, looking at the feasibility and effectiveness of TB preventive therapy for HIV-infected adults and children attending ART centres in several states.

Primary aim: To assess the effectiveness of isoniazid TB preventive therapy (IPT) in people living with HIV (PLHIV) (at different CD4 counts and both pre-ART and on ART)

Secondary aims: (i) to assess the effectiveness of simple algorithms to exclude active TB prior to IPT initiation

(ii) to assess the feasibility of providing IPT for PLHIV attending ART centers.

(iii) to measure number needed to screen and number needed to treat to prevent one case of TB

The study consists of two phases – Phase I: Enhanced TB surveillance (all sites, all ART centre attendees) for all HIV-infected adults and children.

Phase II: Provision of IPT: After 6 months (following the completion of Phase I), Phase II will begin at that ART centre and all eligible patients attending that ART centre will be administered IPT.

The study was initiated in a phased manner at five ART centers in Tamil Nadu, two in Karnataka, one each in Andhra Pradesh and New Delhi. The sample size required for the study is 6000 adults and 1800 HIV-infected children. As on 31st March 2014, 4000 adults and 1300 children have been recruited to the study and the study is ongoing. Results of phase I of the pediatric and adult studies are shown in Figs. 4 & 5.
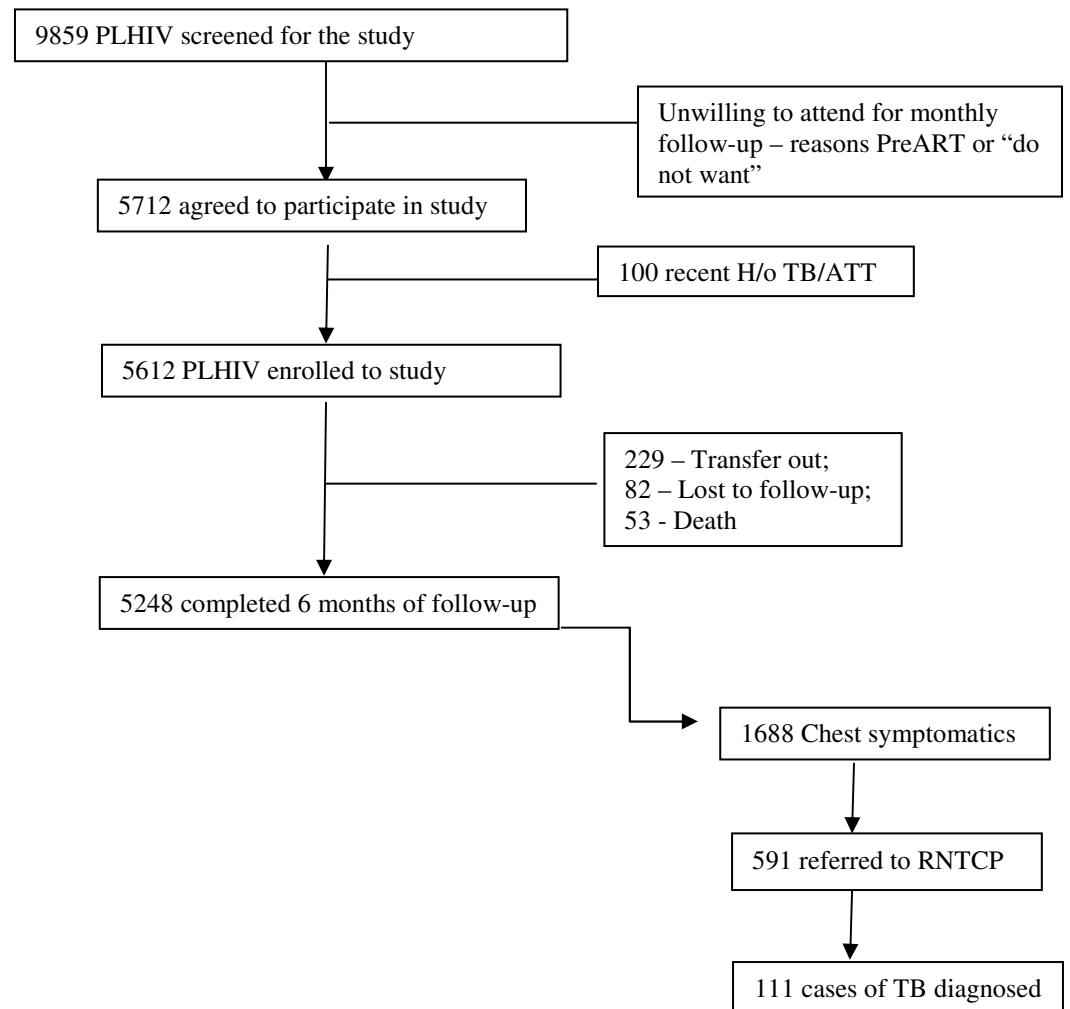
Fig.4: Study scheme in the pediatric population
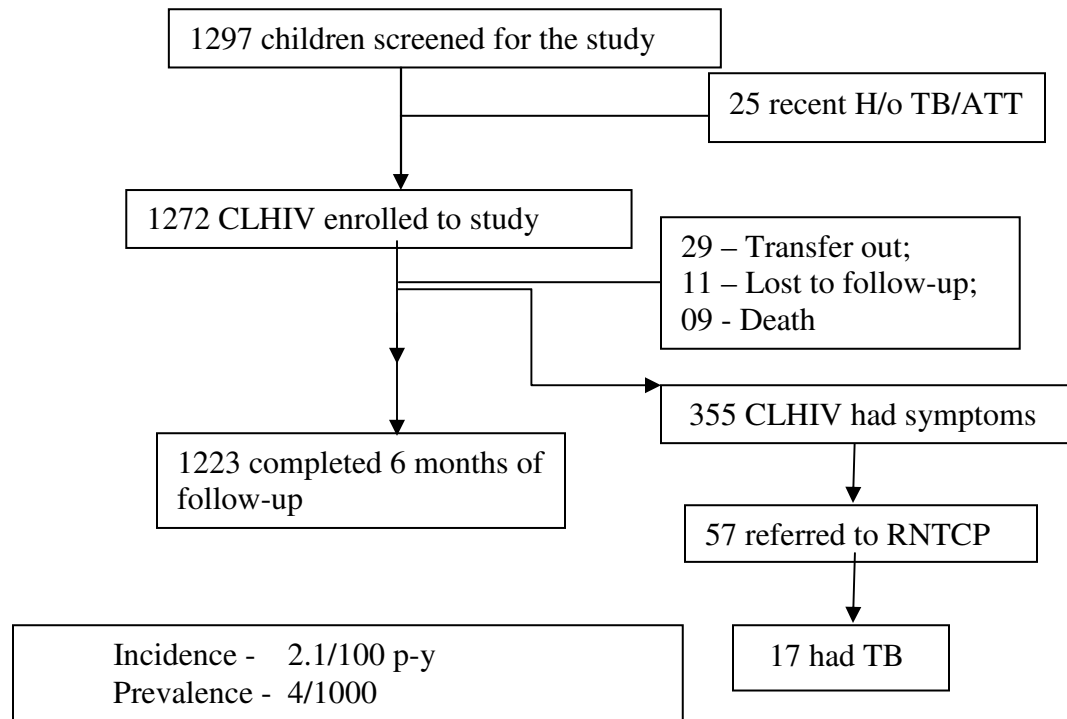
Interim findings: Of the 1272 HIV-infected children enrolled into the study, 17 had TB; the incidence and prevalence were 2.1/100 person years and 4/1000 respectively.

Fig.5: Study scheme of adults attending ART centre

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        ┌─────────────────────────────────────┐
        │  9859 PLHIV screened for the study   │
        └─────────────────────────────────────┘
                        │
                        │          ┌──────────────────────────────────┐
                        │          │ Unwilling to attend for monthly  │
                        ├─────────▶│ follow-up – reasons PreART or "do│
                        │          │ not want"                        │
                        ▼          └──────────────────────────────────┘
        ┌─────────────────────────────────────┐
        │  5712 agreed to participate in study │
        └─────────────────────────────────────┘
                        │
                        │          ┌──────────────────────────────────┐
                        ├─────────▶│ 100 recent H/o TB/ATT            │
                        ▼          └──────────────────────────────────┘
        ┌─────────────────────────────────────┐
        │  5612 PLHIV enrolled to study        │
        └─────────────────────────────────────┘
                        │
                        │          ┌──────────────────────────────────┐
                        │          │ 229 – Transfer out;              │
                        ├─────────▶│ 82 – Lost to follow-up;          │
                        │          │ 53 - Death                       │
                        ▼          └──────────────────────────────────┘
        ┌─────────────────────────────────────┐
        │ 5248 completed 6 months of follow-up │
        └─────────────────────────────────────┘
                                 │
                                 ▼
                        ┌─────────────────────────┐
                        │ 1688 Chest symptomatics  │
                        └─────────────────────────┘
                                 │
                                 ▼
                        ┌─────────────────────────┐
                        │ 591 referred to RNTCP    │
                        └─────────────────────────┘
                                 │
                                 ▼
                        ┌─────────────────────────┐
                        │ 111 cases of TB diagnosed│
                        └─────────────────────────┘
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CL-11: **C-TRIUMPH: Cohort for TB research by the Indo-US medical paertnership multicentric prospective observational study**

Principal Investigator	:	Dr. Soumya Swaminathan (email:soumyas@nirt.res.in)
Funding Agency	:	DBT
Study period	:	2013-2018

This is a prospective multi-centric observational cohort study at two sites, Chennai and Pune, which will enroll TB patients and their household contacts to study host and microbial risk factors associated with progression from TB infection to disease, TB disease progression, treatment outcomes, and transmission. A repository of biological specimens will be created, which can be used for future basic science research including biomarker discovery, and be made available to investigators of this Centre Partnership on request. After all regulatory approvals, the study received funding in later part of 2013. Staff recruitment and preparatory work to initiate the study happened during early 2014. We propose to initiate enrollment to the study by May 2014.

CL-12: **Inhibition of host-induced mycobacterial efflux pumps as a novel strategy to counter drug tolerance and virulence of PTB**

Principal Investigator	:	Dr. Soumya Swaminathan (email:soumyas@nirt.res.in)
Funding Agency	:	DBT
Study period	:	2013-2015

This is a phase 2 single-centre, open-label verapamil dose-finding pharmacokinetic study of verapamil given in conjunction with RMP. The goal of this study is to determine the contribution of the efflux pump-mediated tolerance mechanism in delayed or incomplete sterilization in active PTB. It is hypothesized that verapamil added to standard TB therapy will accelerate sputum clearance of M.tb. The study objectives are:

1. Determine the compensatory increase in verapamil dose that can

offset the increased metabolism of verapamil when it is co-administered with RMP.

2. Confirm the safety and tolerability of verapamil in patients with TB without underlying cardiac disease.

Funding for the study was received in later part of 2013. Preparing the study dossier for DCGI submission and for Institutional Ethics committee submission was in progress during this period.

CL-13: EDOTS - Effect of diabetes on TB severity

Principal Investigator	:	Dr. Pradeep Menon (email:menonpa@nirt.res.in)
Funding Agency	:	DBT
Study period	:	2014-2017

A multicentric study has been started to determine the effect of diabetes on TB severity. In Chennai, the collaborating centres are NIRT and MV Diabetes Centre. The study is being conducted at Pullianthope and Tondiarpet RNTCP centres.

The estimated sample size is 300 TB patients (150 with DM and 150 without DM).

Of the 63 eligible cases, 18 were known DM cases (KDM), 15-New DM cases, 16-Pre DM, 14-Non DM. Total DM 33 (KDM+NEW DM) were enrolled to the study. One patient was found to be Pre-DM after enrolment, so excluded from the study. Sputum smear and culture reports have been received for 23 patients, of which one was found to be culture negative and was excluded, according to the study criteria. One patient was started on a MDR-TB regimen.

The study is in progress.

CL-14: Multi-centric cohort study of recurrence of TB among newly diagnosed sputum positive PTB patients treated under RNTCP

Principal Investigator	:	Dr. Soumya Swaminathan (email:soumyas@nirt.res.in)
Collaborators	:	National Tuberculosis Institute, Bangalore, Lala Ram Swarup Inst. of TB & Respiratory Diseases, New Delhi, RMRCT, Jabalpur, Thiruvananthapuram Medical College, Thiruvananthapuram, Mahatma Gandhi Inst. of Medical Sciences, Wardha
Funding Agency	:	Central TB Division
Study period	:	2014-2016

Background: In the RNTCP, newly diagnosed smear - positive PTB patients are treated with a 6-month thrice-weekly regimen, consisting of an initial intensive phase (IP) of isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E) for two months followed by a continuation phase of H and R for four months ($2H_3R_3Z_3 E_3 / 4 H_3R_3$), given under direct observation, fully during the IP and partly during the continuation phase. The country reports around 87% 'cure' among smear-positive patients treated with this regimen. A TB recurrence rate of 10-12% has been reported from localized studies. There is little information on the proportion and predictors of patients who develop recurrent TB among those patients who have had a successful outcome at the end of treatment and on proportion of recurrent TB due to re-infection or to endogenous reactivation. This knowledge would have a bearing on the efficacy of the regimen.

Study Objective

Primary Objective: To estimate the recurrence of TB among newly diagnosed sputum positive PTB patients who have been successfully treated under RNTCP

Secondary Objective: To distinguish between *relapse* and *re-infection* among those who have recurrence of TB. To identify risk factors for unfavourable treatment outcomes (treatment failed, lost to treatment follow-up and died) and recurrent TB

Methodology

This is a prospective, multi-centric cohort study. conducted by the following six institutes; NIRT, Chennai, NTI,

Bangalore, National Institute of Tuberculosis and Respiratory Diseases (NITRD), New Delhi, Regional Medical Research Centre for Tribals (RMRCT), Jabalpur, Thiruvananthapuram Medical College, Thiruvananthapuram (TMCT) and Mahatma Gandhi Institute of Medical Sciences (MGIMS), Wardha. New smear positive PTB patients treated under RNTCP are enrolled. They will be followed up till treatment completion, and those with successful treatment outcome will be followed up for a period of 12 months after completing treatment. These patients will be subjected to the following procedures: Structured interview, sputum examination for TB smear, culture, DST and genotyping and blood tests for diabetes mellitus and HIV infection.

The study was initiated in May 2014 and is ongoing.

Retrospective data analysis:

Smear positive, culture negative phenomenon in newly diagnosed PTB patients during treatment follow-up

Principal Investigator	:	Dr. S. Ramesh Kumar (email: ramesh@nirt.res.in)
Source of funding	:	International Union against TB and Lung Diseases (The Union)
Study period	:	2012-2013

Background: The World Health Organization (WHO) guidelines recommend that TB patients whose treatment has failed (based on positive smear results at five months or later) should be started on an empirical multi-drug resistant (MDR) TB regimen. There have been concerns about this recommendation, as many of the smear positives during follow-up may be culture negative. We aimed to assess the extent of the 'smear positive culture negative' (S+C-) phenomenon in patients receiving regimens with RMP throughout the course of treatment.

Methods: Subjects included in this analysis were culture-confirmed new smear positive PTB patients who had participated in four of the randomized controlled clinical trials at the NIRT from January 2000 to August 2012 and initiated on a six month RMP – containing, thrice weekly intermittent regimen [Category I, $2EHRZ_3/4HR_3$; H-

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Isoniazid (INH); R-RMP; Z-Pyrazinamide (PZA); E-Ethambutol (EMB)]. We reviewed their records and extracted follow-up smear (using fluorescent microscopy) and culture (using Lowenstein Jensen media) results. The proportion of follow-up smear positives that were culture negative was calculated, disaggregated by month of follow-up examination, pre-treatment drug susceptibility status, HIV status and pre-treatment smear grading. Entry and analysis was done using EpiData software.

Results: Of 520 patients, that included 176 HIV-infected patients, 199, 81, 47 and 43 were smear-positive at months 2, 4, 5 and 6 respectively; of these 138 (69%), 62 (75%), 32 (68%) and 27(63%) respectively were culture-negative. The S+C- phenomenon was more pronounced among '1+positive' patients than among 2+ or 3+ positive patients and in 'pan-susceptible' patients than in those with any resistance, and did not vary by HIV status. Also the S+C-phenomenon was more frequent in follow-up smear results graded 1+, followed by 2+ and 3+.

Conclusion: Among new smear and culture positive TB patients, nearly two thirds of treatment follow-up positive smears were culture negative. This throws a doubt on the reliability of positive smear microscopy results during follow-up in guiding patient management. Starting MDR-TB treatment empirically based on smear results, even in resource-limited settings, is incorrect and can have hazardous consequences. There is an urgent need to revisit the WHO recommendation concerning empirical MDR-TB treatment.

Department of Socio Behavioral Research

STUDIES COMPLETED:

(i)   A study on psycho-social issues facing MDR-TB patients to design appropriate intervention strategies to promote drug adherence

Principal Investigator   :   Dr.E. Thiruvalluvan
                                            (email: thiruvalluvane@nirt.res.in)
Source of funding   :   Intramural
Study period   :   2013-2014

Background: The magnitude of the MDR-TB problem in India is becoming difficult to manage. Studies found MDR-TB levels of about 3% in new cases and 12%-17% in RT cases. The main challenges of dealing with MDR-TB are limited access and poor adherence to treatment as only a small fraction of diagnosed patients is receiving appropriate care. Treatment compliance depends upon the psycho-social behavior of the patient, which is deeply influenced by practices and beliefs ingrained in the culture in which the person is brought up. There is a need therefore, to understand psycho-social and behavioral determinants that would probably lead to better insights into MDR-TB control strategies.

Specific aims:

(i) to understand the psychosocial issues facing MDR-TB patients

(ii) to gain insight on the challenges faced by HCP (Health care providers) in managing MDR-TB patients

Methods: This is a qualitative study that is part of a larger experimental study to design an acceptable intervention for MDR-TB patients.The study has been approved for the qualitative phase which included focus group discussions (FGDs) and interviews.

Four FGDs, 68 in-depth interviews among patients and 16 interviews with HCPs (Doctors, Health visitors, Sector Health Nurse, Lab technicians and Treatment supervisors) have been completed. The findings from this phase will be used to develop an intervention programme that is feasible and acceptable for MDR patients which will be tested through an experimental design. Content analysis was done and interpreted in themes.

Themes

Past history of TB: All the participants had previous history of TB treatment, nearly one – third of the participants had past history of TB in their family. Almost all the participants were irregular for treatment.

31

Knowledge of MDR: Most of the participants did not know they had MDR-TB, why it was caused and why they needed long duration of treatment.

"I was informed that I need to take 12 tablets per day for two years with 6 months injection daily. I did not understand why… (Female, 38yrs old)

Reaction to diagnosis: Almost all the participants expressed concerns over the number of tablets and pain associated with injection. The reactions towards MDR-TB diagnosis are varied; some of them are fear, suicidal thought, hopelessness, stigma, confusion and guilt.

"I felt like killing myself. How was I going to take tablets for 2 years? ……I do not even have proper food" (Male, 36 yrs old)

"Who will marry me; since I am taking MDR- TB treatment, I am worried about my future" (Female, 19 yrs old)

"I got this disease because of irregular treatment and my habit of alcohol use. Now I am suffering for it (Male 27 yrs old)

Discrimination: Dissatisfaction with HCPs was expressed by nearly one-fourth of the participants and few participants expressed concerns over lack of privacy and confidentiality.

"I am treated differently as compared to another TB patient attending the centre. I am told to move away and comments are made. I do not know why I am different from other TB patient ……." (Male, 34 yrs old)

Challenges faced by HCPs: HCPs expressed their challenges in treating MDR-TB patients. They were unable to spend adequate time to provide care and support for MDR-TB patients due to their work load – more number of TB patients, maintaining register, stock, supply, frequent meetings, delay in getting LPA result, transporting of the drugs to the health centres, lack of knowledge to deal MDR-TB patients, addressing issues with regard to alcohol and smoking.

The study findings have brought out the psychosocial concerns of MDR patients that need to be addressed. The need for need based intervention by skilled counselors to deal with these issues to ensure treatment compliance is highly recommended.

STUDIES IN PROGRESS:

SB-1: **HIV prevention through mobile phone technology among male sex workers in India - Indo-US collaborative study**

Principal Investigator	:	Dr. Beena E. Thomas (email: beenathomas@nirt.res.in)
Collaborators	:	Dr. Mathew Mimiaga, Harvard Medical School / Fenway Institute, USA, Dr. Steve Saffren, MGH (Massachusetts General Hospital)
Source of Funding	:	Indo-US Joint working group on HIV
Study Period	:	2011 -2014

Background: This proposal is an outcome from a collaborative intervention study done in NIRT to address psychosocial needs of Men having sex with men (MSM). Individual and group inrterventions which required that MSM come to the intervention sessionswere studied. This was a challenge and we felt that there was need to reach out to them in a better way. Our prior formative work revealed that the vast majority of male sex workers in this region have mobile phones and use these to network with "pimps" (individuals who manage male sex workers (MLSWs) and mediate client interactions), other sex workers, and to schedule sex work clients. It was therefore felt that interventions for MSM could be delivered using mobile phone technology.

Aims: (i) to fully develop an HIV risk reduction counselling intervention for MLSWs in India, using mobile phone technology

(ii) to examine the feasibility, acceptability and potential impact of the proposed intervention in a pilot randomized controlled trial

(iii) to assess if potential mediators of intervention differentially change in the intervention group and if these changes are associated with the primary outcome (reduced sexual risk taking)

Methods: The study was initiated in 2011 and will be done in phases.

Phase 1: Intervention Development (completed)

Conducted formative qualitative interviews (N = 40) and focus groups (4 groups with 6-8 participants) to develop the intervention with community and participant inputs. This phase helped to further develop all study procedures, and helped finalize the intervention manual.

Phase 2 (Open Pilot)

Ten participants were enrolled and consented to participate. Two of them did

not come after the first individual session as they left Chennai and no plans of returning in near future. Eight of the remaining participants completed all intervention sessions (individual and mobile phone). Interventions included face to face individual counseling initially followed by counseling through mobile phone and motivational text/voice messages sent daily to serve as reminders in the first 4 weeks. Mobile phone sessions were done in the 6th and 8th week and messages sent thrice a week till 12 weeks of intervention. This phase helped to establish the feasibility of the intervention, identify possible barriers and finalize the intervention. After completion of this phase the intervention was further modified. The data collection instruments were finalized and this was placed for ethics approval for initiation of Phase 3.

Phase 3 Randomized control trial

So far, 97 participants have been recruited (having completed written informed consent, baseline questionnaire and HIV testing) and randomized 47 into the control arm and 50 to the intervention arm. Intervention is in progress. The 3rd month follow-up is completed for 44 participants. The 3rd and 6th month follow-up are in progress.

SB-2: **An experimental study to enhance treatment adherence in TB patients with alcohol use dependence**

Principal Investigator	:	Dr. Beena E. Thoms (email: beenathomas@nirt.res.in)
Source of funding	:	USAID through MDP
Study Period	:	2013-2014

Background: This study is an outcome of a previous pilot study conducted during 2009-10 in Chennai Corporation to explore the frequency of alcohol use among TB patients. 29% of the 490 TB patients (all male) consumed alcohol, the prevalence of alcohol use dependents (AUD) among them being 52%. The qualitative component of the study highlighted the need of an intervention among TB patients with AUD and a study to test the feasibility and the acceptability for such an intervention. This randomized experimental intervention study has been

planned to enhance treatment adherence in TB patients who consume alcohol.

Aims: (i) to enhance treatment adherence of TB patients who consume alcohol by reducing the default rate through intervention strategies

(ii) to evaluate the impact of intervention strategies on alcohol intake by comparing of TB patients who consume alcohol in the experimental group with those TB patients in the control group

Methods: This study was initiated in January 2009 to January 2010 and reported in last years report as an ongoing study. This study had to be stopped due to lack of funding. The study has been initiated again in August 2013

An experimental research design was adopted for this study. The study sites included 4 of the 10 corporation zones in Chennai. All TB patients above 18 years of age diagnosed during the period of were screened for the study. The study is done in phases.

Phase 1: Qualitative phase

This phase included FGDs, in-depth interviews with patients, family members and health providers. The baseline data on total number of patients on different regimens, cure rates, defaulter rates, reasons for default and the number of patients who consume alcohol was obtained from the study sites. Based on the findings, from the situational analysis, an intervention module was designed for the intervention among the TB patients.

Phase 2: Quantitative phase

Patients who consume alcohol and willing to be part of the study above 18 years of age were considered as eligible participants. Patients registered from the month of August 2013 to January 2014 were screened for the study in the 4 zones and eligible participants enrolled. Assessments were at baseline and 6th month. For the experimental zone the interventions were at 0, 2nd month, 4th month and 6th month.

Control zones

480 participants out of 549 patients registered were enrolled and 185 participants were eligible and all were willing to be a part of the study and recruited after obtaining their consent. The 6th month follow-up is completed for 33 participants till April, 2014.

Experimental zones

392 participants out of 431 patients registered were enrolled and 114 participants were eligible and 112 participants were willing to be a part of the study and 2 participants were unwilling to participate in the study.

The zero month intervention has been completed for 112 participants, 2nd month intervention for 108 participants, 4th month intervention for 84 participants and 6th month intervention for 38 participants till April 2014.

Outcome Measures

The following outcome measures will be compared between the experimental and control zones:

Primary: Default rate of the TB patients will be compared from the treatment records of the patients in intervention and control zones.

Secondary: Comparison of alcohol intake of TB patients' pre and post intervention at the baseline and end of treatment in the intervention group.

The study is in progress.

SB-3: **A community based approach in designing a model TB sensitization programme for self help groups - A study from Tiruvallur district, Tamil Nadu**

Background: A model TB sensitization programme was designed for self help groups (SHGs) who were living in Tiruvallur District, Tamil Nadu, by adopting a community based approach.

Primary aim: To design a model sensitization programme on TB for SHGs based on participatory action approach which would facilitate their involvement in the RNTCP

Secondary aims:

based on participatory action approach which would facilitate their involvement in the RNTCP

Secondary aims:

(i) to promote awareness on TB among SHGs

(ii) to ascertain the feasibility and acceptability of SHG members in the community for identifying chest symptomatic, referring them for TB investigations and being DOT providers

(iii) to assess the challenges SHGs face (if any) in being involved in the RNTCP programme

Methods: This is an experimental study on a cohort of SHG representatives randomly selected in Tiruvallur district of Tamil Nadu. This study was initiated on May 2012 and is being done in phases.

Phase 1:*1a. Situational analysis* - In this phase, the number of SHGs in the area, the number of members in each group, the geographic distribution of SHGs and the profile of SHGs members with respect to their socio demographic details were enlisted.

1b. Formative phase - This phase included FGDs and interviews to find out the involvement, of the SHGs, in health programmes and to ascertain their level of awareness of TB, areas which need to be addressed in TB awareness campaigns and their experiences (if any) with TB control programme. This phase used a community based participatory approach. The intervention manual and the format of the intervention was finalised in this phase.

Phase 2: A cohort of 1400 SHG representatives from 2 blocks, representing various areas in the district were randomized to an experimental and control group. 1560 participants have been recruited (764 in the experimental and 796 in the control group). The data analysis is in progress.

Outcome measures:

The outcome measures are the number of SHG member involved in TB awareness programme, the number of chest symptomatic identified and referred by SHG member and SHG member who volunteered to be DOTS provider. The study is in progress.

LABORATORY STUDIES

Department of Bacteriology

STUDIES COMPLETED:

(i)   Viability and retrieval of *M. tuberculosis* from selective Kirchner's medium using bovine albumin serum instead of fetal calf serum

Principal Investigator   :   Dr. Gomathi Sekar (email: gomathis@nirt.res.in)
Source of funding   :   ICMR-Intramural
Study period   :   2012-2017

Background: Selective Kirchner's medium (SKM) is a liquid medium with small amount of casein and calf serum as supplement (FCS); it is used for the isolation of *M. tuberculosis* from extra pulmonary specimens, where the bacilliary load is low. Bovine serum albumin (BSA) is evaluated as a substitute for fetal calf serum (FCS) in SKM.

Aim: To substitute BSA for FCS in SKM for the isolation and recovery of *M. tuberculosis* from extra pulmonary specimens

Methods: The study included extra pulmonary specimens received from different part of Tamil Nadu for diagnosis of TB. FCS containing SKM was prepared as per NIRT-SOP. Another set of SKM was prepared similarly substituting BSA for FCS. The specimens were processed with 5% H_2SO_4 and inoculated into one SKM-FCS and one SKM-BSA medium. After 6 weeks of incubation, both media were processed by modified Petroff's method and sub cultured on Lowenstein Jensen (LJ) medium. Recovery of *M. tuberculosis* from the two media and their morphology on LJ were compared.

Results: 307 extra pulmonary specimens were used for the comparison and results analysed statistically. Culture positivity for SKM-FCS and SKM-BSA were found to be 5.9% and 7.1% respectively.

Conclusion: SKM supplemented with BSA was comparable with FCS in the recovery of *M. tuberculosis*. Cost effectiveness, commercial availability, ease of storage makes BSA an ideal enrichment source for use in SKM. It can be used for the recovery of *M. tuberculosis* as an alternate for FCS in SKM.

STUDIES IN PROGRESS:

B-1: **Surveillance of PZA drug resistance among new sputum smear positive patients**

Principal Investigator	:	Dr.N.S. Gomathi
		(email: gomathisharma@nirt.res.in)
Source of funding	:	USAID through MDP
Study Period	:	2013-2014

Background: PZA is a drug given to new smear positive patients under category I. Limited information is available on the prevalence of resistance to PZA globally and none from India. Surveillance studies on PZA resistance are crucial for making policy decisions on inclusion of the drug in treatment regimens for management of TB.

Aim: To estimate the prevalence of PZA drug resistance among new sputum smear positive (NSP) cases of *M. tuberculosis* using BACTEC MGIT 960 system

Summary and Progress: Clinical isolates from new smear positive patients from the Tamil Nadu Drug Resistance Surveillance are used for the study. About 700 cultures will be included subject to availability. The isolates stored at 4°C are recovered by subculture on to BBL MGIT 7ml tubes and DST to PZA is set up in BACTEC MGIT960 at a concentration of 100µg/ml as per the manufacturer's protocol. Currently, DST to PZA has been completed for 500 cultures and the study is ongoing.

B-2: **Determination of cross resistance among fluoroquinolones in clinical isolates of *M. tuberculosis* from new sputum smear positive cases**

Principal Investigator	:	Dr.N.S. Gomathi
		(email: gomathisharma@nirt.res.in)
Source of funding	:	USAID through MDP
Study Period	:	2013-2014

Background: Fluoroquinolones (Fqs) such as such as OFX, gatifloxacin, levofloxacin and MFX are anti-tubercular drugs included in the treatment of patients with MDR-TB. A recent study on drug resistance surveillance among NSP cases in the state of Tamil Nadu, showed a high level (10.4%) of resistance to OFX. This has been attributed to the common use of OFX for the treatment of common respiratory infections in the

community. Conventionally, drug susceptibility testing to any Fqs in the laboratories is done using OFX though it has been replaced by MFX or levofloxacin (Lx) in the actual treatment regimens. Earlier reports suggesting cross resistance among Fqs in *M. tuberculosis* are available. Hence, use of OFX alone for DST to Fqs is likely to yield false resistant results. The current study proposes to document any cross resistance between OFX and MFX in clinical isolates *M. tuberculosis* from NSP cases from the Tamil Nadu region.

Aim: To estimate and document cross resistance among Fqs in clinical isolates of *M. tuberculosis* from new sputum smear positive cases using BACTEC MGIT 960 system

Summary and Progress: OFX resistant clinical isolates from new smear positive patients from the Tamil Nadu Drug Resistance Surveillance are used for the study. It is estimated to include about 200 cultures subject to availability. The isolates stored at 4°C are recovered by subculture on to BBL MGIT 7ml tubes and DST to OFX and MFX are set up in BACTEC MGIT960 at a concentration of 2µg/ml for OFX and 0.5 and 2 µg/ml for MFX as per the manufacturer's protocol. Currently, DST for 100 cultures has been completed and the study is ongoing.

B-3: **Performance of light emitting diode microscope in two different settings for TB diagnosis: A multi-centric study**

Principal Investigator	:	Dr. Gomathi Sekar (email: gomathis@nirt.res.in)
Collaborators	:	Director, Institure of Thoracic Medicine, Chennai
Source of funding	:	ICMR - extramural
Study Period	:	2013-2014

Background: Early diagnosis and treatment is essential to prevent the transmission of the disease in the community. Conventional fluorescence microscopy is more sensitive than Ziehl-Neelsen (ZN) and takes less time, but its use has been limited by the high cost of mercury vapour light sources, the need for regular maintenance and the requirement for a dark room. Microscopes using light-emitting diodes (LED) have been developed that can be used for both bright field microscopy as well as fluorescence microscopy. It offers the benefits of fluorescence microscopy without the

associated costs and dark room. Recently WHO recommended LED microscope for use in TB diagnosis. There is a need to assess the performance of microscope in different settings.

Aim: To evaluate the performance of Primo Star iLED microscope as fluorescence microscope for TB diagnosis in different settings.

Study site

The following institutes in India have been selected for study:

1. NIRT, Chennai.

2. RMRC, Bhubaneshwar.

Methods: The study will include 1350 sputum samples from consecutive symptomatic patients attending the RNTCP clinic of ITM, Chennai. Two direct smears and two deposit smears (after processing the sample using 4%NaOH) are taken from each sample. One smear from each set is stained using Auramine- O Phenol (AP) and the remaining smears are stained using ZN. The slides are randomized. The AP stained smears are read by both iLED microscope and the regular UV microscope and compared. In addition, the results are compared with ZN smears read using the conventional bright field microscope. Any discrepancy is resolved by an umpire reader. In addition, random blinded rechecking for smear microscopy was performed for FM smears. Once in 15 days, 10% of slides were selected systematically, coded, restained and re-read for external quality assurance. The study is ongoing.

Progress: Upto March, 2014, 499 sputum specimens have been received. Among them, 54 were positive by both AP and ZN for direct smears and the remaining 445 were negative. The agreement between (a) iLED-FM and conventional FM, (b) iLED-FM and ZN will be done at the end of the study.

B-4: **Modified 'pot – method' of staining acid fast bacilli using phenol ammonium sulphate basic fuchsin tablets**

Background: Modified 'pot staining' method is a rapid diagnostic test but it is different from the existing ZN staining method. With pot staining, the smear making is made easy resulting in uniform spread of sputum material on the glass slides, the background of the smear is clear and the Acid fast bacilli (AFB) are distinctly seen. Further, the method does not involve heating of carbol-fuchsin and is technician friendly because it gives an opportunity for the laboratory technician to make smears at a convenient time. Further work on this project was initiated to develop the stain in "tablet form" for staining sputum smears, which is being done in collaboration with Vel's university, Chennai.

Aim: To evaluate the tablet formulation for staining AFB directly in the sputum container

Progress: A total of 15 formulations were received from Vel's University, Chennai during 2014. One of them has been found to stain AFB even in low grade positives. Tablets with varying concentrations of the stain will be provided by Vel's University for standardization.

Future plan:

The formulation and stability of the tablet is being pursued at present with collaboration with Vel's University. This would take another 5-6 months. Once the formulation of the tablet is standardized, evaluation of this tablet form for pot staining could be completed in 6 months time.

Dept. of Biochemistry & Clinical Pharmacology

STUDIES COMPLETED:

(i)   Pharmacokinetics of anti-TB drugs in HIV-infected children with TB

Principal Investigator	:	Dr. Geetha Ramachandran (email: geethar@nirt.res.in)
Collaborators	:	Institute of Child Health, Chennai, Govt. Hospital of Thoracic Medicine, Chennai, Kilpauk Medical College & Hospital, Chennai, Govt. Rajaji Hospital, Madurai, SN Medical College, Agra and Indira Gandhi Institute of Child Health, Bangalore
Source of funding	:	ICMR Task force on Paediatric HIV
Study period	:	2009-2013

Background: HIV infection causes impaired absorption of anti-TB drugs leading to low anti-TB drug levels in adults. Not much is known about the pharmacokinetics of first-line anti-TB drugs in HIV-infected children with TB in India, or about the factors influencing drug levels.

Aim: To study the pharmacokinetics of RMP, INH and PZA, factors influencing the pharmacokinetics of these drugs and TB treatment outcome in HIV-infected children with TB treated with fully intermittent regimens in the RNTCP

Methods: HIV-infected (n = 77) children with TB aged 1 to 15 years from six hospitals in India were recruited. During the intensive phase of TB treatment, a complete pharmacokinetic study was performed, after directly observed administration of drugs.

Drug concentrations were measured by high performance liquid chromatography (HPLC). INH acetylator status was determined using saliva. Nutritional assessment was performed by calculating z scores for weight and height based on the child's age and gender using the EPI-INFO 2002 software. Treatment outcomes were taken from the RNTCP treatment card. Multivariable regression analysis was done to explore factors impacting drug levels and treatment outcomes.

Results: Children with HIV & TB had significantly lower RMP peak concentration (C_{max}) (2.6 vs. 5.1µg/ml; p<0.001) and exposure (AUC_{0-8}) (10.4 vs. 23.4µg/ml.h; p<0.001) than those with only TB (Fig. 6). Children below 5 years had lower C_{max} and AUC_{0-8} of INH (p = 0.024, 0.041) and PZA (0.061, 0.045) than those above 5 years. Cmax of RMP (1.0 vs. 2.8µg/ml; p = 0.002)

and PZA (31.9 vs. 44.4µg/ml; p = 0.012) were significantly lower in children with unfavourable than favourable outcomes. Among all factors studied, PZA C_{max} influenced TB treatment outcome (p = 0.012; AOR = 1.09; 95% CI: 1.02 – 1.17). The proportion of children with sub-therapeutic C_{max} of RMP, INH and PZA was 97%, 26% and 34% respectively.

Conclusions: Younger age and HIV infection had an adverse impact on drug levels. PZA C_{max} significantly influenced treatment outcome in HIV-infected children with TB. The findings have important clinical implications and suggest the need to increase anti-TB drug doses in children with HIV & TB.

Fig.6: Peak concentration and exposure in HIV-infected & uninfected children with TB
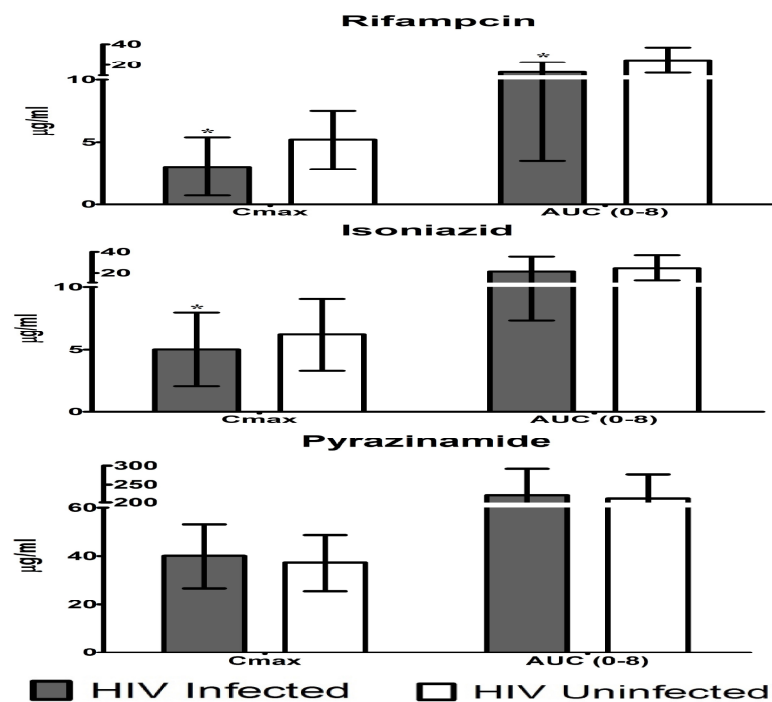
Above values are Mean; vertical bars represent standard deviation; denotes p < 0.001; Cmax – Peak concentration; AUC_{0-8} – Exposure

STUDIES IN PROGRESS:

BCP-1: Comparative pharmacokinetics of RMP during daily and intermittent dosing in HIV-TB patients

Principal Investigator	:	Dr.A.K. Hemanth Kumar (email: hemanthkumarak@nirt.res.in)
Collaborators	:	Govt. Hospital of Thoracic Medicine, Chennai and Govt. Rajaji Hospital, Madurai
Study period	:	2009-2013

Background: RMP forms the backbone of first-line ATT, particularly in HIV-infected persons, because of its rapid sterilizing action. However, emergence of resistance to RMP during ATT in patients with HIV infection remains a serious concern. RMP being a concentration-dependent killer of *M. tuberculosis* [6], intermittent dosing coupled with malabsorption due to HIV infection, increased tissue bacillary load and decreased immunity could cause ARR.

Aim: To compare the pharmacokinetics of RMP during daily and intermittent ATT in HIV-infected TB patients

Methods: HIV-infected patients with newly diagnosed PTB attending the NIRT clinics in Chennai and Madurai were recruited. Patients were randomized to receive either a daily, partly intermittent or totally intermittent ATT regimen for a period of six months. The pharmacokinetic study was undertaken during the intensive phase of ATT after patients had received a minimum of six doses of drugs. Serial blood samples (2ml) at pre-dosing and at 1, 2, 4, 6, 8, 12 and 24 hours after directly observed drug administration were collected. Plasma RMP concentrations were determined by HPLC according to a validated method.

A total of 41 patients (36 were males) have been recruited to the study. Analysis of data is in progress.

BCP – 2: **Effect of plasma MFX on treatment outcome in PTB patients treated with MFX-containing anti-TB regimens**

Principal Investigator	:	Dr. Geetha Ramachandran (email: geethar@nirt.res.in) ose
Source of funding	:	Intramural
Study period	:	2013-2015

Background: Among the newer generation of Fqs, MFX has a potential to shorten TB treatment. Studies in healthy subjects have shown that RMP co-administration reduces the blood levels of MFX. But it is not clear whether the decrease would affect the treatment efficacy of MFX. Prospective studies to relate MFX blood concentrations with TB treatment outcomes are required to understand the clinical relevance of the significant pharmacokinetic interaction between MFX and RMP.

Aim: To estimate plasma concentrations of MFX, RMP, INH and PZA and correlate with TB treatment outcome

Methods: This is a prospective study undertaken in PTB patients enrolled into a randomized controlled trial in which MFX is used along with other anti-TB drugs to treat TB. Blood samples at 1, 2 and 4 hours after drug administration are collected at one month and at end of treatment. Plasma concentrations of MFX, RMP, INH and PZA are estimated by HPLC. 126 patients have been recruited from Chennai and Madurai; of them, 79 have completed treatment. Further recruitment of patients is in progress.

BCP-3: **Pharmacokinetics of first-line anti-TB drugs in adult TB patients treated in the RNTCP**

Principal Investigators	:	Dr. Geetha Ramachandran, Dr.A.K. Hemanth Kumar (email:geethar@nirt.res.in/hemanthkumarak@nirt.res.in)
Collaboration	:	Chennai Corporation
Source of funding	:	USAID through MDP
Study period	:	2013-2015

Background: Low serum concentrations of anti-TB drugs have been associated with treatment

failure, relapse and acquired drug resistance in HIV-infected and non-HIV-infected TB

patients. A number of factors have been reported to influence anti-TB drug levels.

Aims: Primary aim:

(i) to study the influence of factors such as diabetes, HIV infection, malnutrition, old age (> 60 years), smoking, alcohol intake, gene polymorphisms, on 2-hour concentrations of RMP, INH and PZA

Secondary aims: (i) to determine 2-hour post dosing concentrations of RMP, INH & PZA in TB patients receiving treatment (Category I/II) under the RNTCP and (a) relate to TB treatment outcome (b) to determine cut-off levels for RMP, INH and PZA that would define sub-optimal treatment outcomes

(ii) to study the pharmacokinetics of RMP, INH & PZA in a sub-group of patients admitted to the study

Methods: A prospective cohort study is ongoing, in which adult TB patients with pulmonary or extrapulmonary TB started on category I or II ATT are recruited. All the patients are receiving ATT in the RNTCP treatment centres in Chennai Corporation. Each patient is investigated on two occasions, during the intensive phase and at end of treatment. During both occasions, blood at two hours after directly observed drug administration is done. Genotyping of *NAT2* and *SLCO1B1* genes, clinical biochemistry and hematology testing and determination of HIV status are also carried out. Random glucose > 200mg/dl will be considered as diabetes. Sputum at baseline and at end of ATT are collected and processed for smear and culture. Patients are followed up to determine treatment outcome.

We have recruited 902 patients, of which 86 have completed treatment. The complete pharmaco-kinetic study has been completed in 39 patients.

HIV LAB

STUDIES COMPLETED:

(i)   Enhancing immunogenicity of HIV-1 epitopes by rational modification

Principal Investigator	:	Dr. Luke Elizabeth Hanna
		(email: hanna@nirt.res.in)
Source of funding	:	Intramural
Study period	:	2012-2014

Background: More than 1000 HIV-1 epitopes have identified and demonstrated to induce immunogenic responses *in vitro*. Yet, we do not have an effective vaccine against HIV, suggesting that the best combination of epitopes is yet to be identified, or alternatively, even the best of these epitopes are not sufficiently immunogenic so as to induce a robust protective response in humans.

Aim: To identify a set of promiscuous, high affinity binding epitopes of HIV-1 subtype C isolates in our population, and to evaluate a strategy to enhance the immunogenicity of the epitopes by rational modification of the amino acid composition

Methods: Based on bioinformatics analysis and available literature, we identified a set of 12 epitopes of HIV-1C as promising candidate epitopes. These peptides were commercially synthesized with 95% purity and tested for immunogenicity using IFN-γ ELISPOT assay in PBMC obtained from 14 HIV-positive individuals who were ART naïve and had CD4 count >350 cells/ml of blood. Two of these epitopes that were widely recognized in the test subjects were subjected to *in silico* modification by substitution with amino acids other than the one present in the wild type form at position 2 (P2). All the analogs as well as the wild epitopes were modeled onto HLA-A*02:01 using MODPROPEP. Four analogs each that showed stronger binding affinity to HLA than the native epitope were selected and commercially synthesized. The two wild type peptides as well as the 8 analogs were tested *in* vitro for immunogenicity on PBMC obtained from HIV-infected individuals, by measurement of cytokines in culture supernatants using the Bio-Plex cytokine assay.

Results: Of the 12 promiscuous high affinity binding epitopes tested using the IFN-γ ELISpot assay, two epitopes (E2 and E9) were widely recognized among the

study subjects, with a positive response seen in 10 and 9 of the total 14 subjects included for the study. Analogs of E2 and E9 containing amino acids L, F, I, W and Y at P2 formed stronger complexes with HLA-A*02:01. Since I and L were similar, and L was found to score better than I, L alone was selected for further study. Thus, four variants of each peptide with amino acids F, L, W and Y were tested *in vitro* for immunogenicity. We found that in general all analogs of both E2 and E9 induced production of higher levels of Th1 cytokines, particularly IFN-γ, TNF-α and IL-1β. Further, analogs with L and W stimulated >100-fold higher levels of IFN-γ than the corresponding wild type epitope in at least one-third of the individuals (Fig. 7).

Fig. 7: IFN-γ production by PBMC upon *in vitro* stimulation with Gag-1w9 and Nef-RM9 and their respective analogs containing F, L, W and Y at position 2
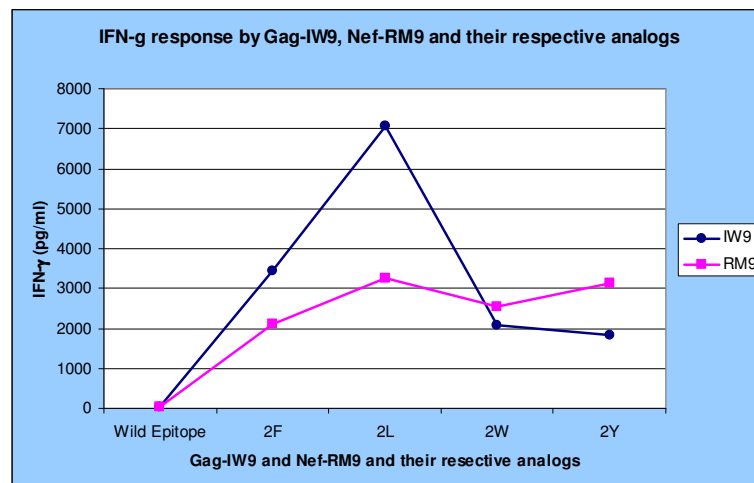
Conclusion: This study has demonstrated that immunogenicity could be improved by rational modifications of amino acid residues present at the anchor site in the epitopes. This finding needs to be validated in a large number of epitopes and tested in a large number of individuals.

STUDIES IN PROGRESS:

HIVL-1: Identification of virological factors that contribute to the varying pathogenicity of HIV-1 and HIV-2

Principal Investigator	:	Dr. Luke Elizabeth Hanna (email: hanna@nirt.res.in)
Source of funding	:	Intramural
Study period	:	2013-2015

Background: HIV-1 and HIV-2 are closely related retroviruses with varying degrees of pathogenicity and distribution. Compared to HIV-1, HIV-2 infection is associated with slower disease progression and transmission, longer latency period, low or undetectable plasmatic viral levels and reduced likelihood of progression to AIDS. Studies on the underlying mechanistic differences between HIV-1 and HIV-2 infections are limited and broader issues of differences in retroviral pathogenesis remain incompletely understood. A detailed comparison of the two closely related viruses and the infections they cause, from an epidemiological, virological and immunological viewpoint may provide a basis for hypothesis generation and testing for varied viral pathogenesis.

Aim: To investigate whether there exist differences in the codon usage patterns between HIV-1 and HIV-2, and to correlate these differences with the diminished virulence/pathogenesis of HIV-2 as compared to HIV-1

Methods: Sequence files of 35 full length HIV-2 genomes of various subtypes deposited in the Los Alamos National Laboratory database during the period 1986-2008 were downloaded. Ten HIV-1 full genome sequences of various subtypes deposited in the database during the same period were also downloaded. The effective number of codons, GC content, GC3s and Relative Synonymous Codon Usage data corresponding to each HIV gene were calculated for HIV-1 and HIV-2 using CodonW software. Correspondence analysis was also performed. Cumulative relative synonymous codon usage of all nine genes was analyzed using the GCUA software.

Results: Analysis of genome composition of HIV-1 and HIV-2 showed that more than 50% of the HIV-1 & 2 genomes was composed of AT bases (AT-rich). Comparative analysis of the effective number of codons (Nc) for each of the nine genes of the two viruses revealed that the tat

gene of HIV-2 had a comparatively higher Nc value (Nc=55.29) than that of HIV-1 tat gene, implying lower levels of expression of the HIV-2 tat protein (Table 10, Fig. 8). Tat protein of HIV is an important regulator of transcription and is one of the early expressed proteins. Lower levels of expression of HIV-2 tat in the early stages of infection could result in a lower viral set point leading to a lower viral load and delayed progression of disease in HIV-2-infected individuals as compared to HIV-1-infected subjects. Further, the GC composition of the regulatory genes of HIV-2 was >50%, unlike that of the structural genes which are AT-rich, indicating a firm effort by these viruses to adapt themselves to evolutionary survival.

Conclusion: Differential codon usage might be one of the possible factors responsible for the diminished immunopathogenicity of HIV- 2 in the host as compared to HIV-1.

Table 10:   Nucleotide composition at third synonymous position and NC value of HIV-2 and HIV-1 genes

| HIV-2 | | | | | | | | HIV-1 | | | | | | |
A3	T3	G3	C3	GC	GC3	ENC	Genes	A3	T3	G3	C3	GC	GC3	ENC
0.44	0.28	0.26	0.28	0.44	0.42	52.62	**Env**	0.49	0.33	0.23	0.21	0.40	0.33	46.83
0.48	0.20	0.28	0.25	0.48	.42	49.27	**Gag**	0.54	0.23	0.24	0.22	0.44	0.35	43.99
0.58	0.22	0.20	0.24	0.42	0.34	43.90	**Pol**	0.56	0.30	0.22	0.20	0.40	0.31	43.00
0.33	0.25	0.35	0.32	0.49	0.52	55.29	**Tat**	0.36	0.35	0.32	0.28	0.49	0.45	49.32
0.38	0.32	0.27	0.23	0.52	0.41	46.88	**Rev**	0.33	0.30	0.32	0.28	0.48	0.46	47.51
0.48	0.24	0.34	0.21	0.49	0.42	47.9	**Nef**	0.49	0.27	0.26	0.26	0.48	0.40	49.92
0.42	0.25	0.23	0.29	0.51	0.42	54.75	**Vpr**	0.48	0.34	0.22	0.20	0.44	0.31	43.65
0.45	0.24	0.27	0.27	0.47	0.43	50.79	**Vif**	0.54	0.31	0.23	0.18	0.41	0.33	41.68
0.44	0.23	0.28	0.27	0.51	0.44	53.28	**Vpx**	0.57	0.30	0.26	0.11	0.38	0.27	38.20

Fig. 8: Principal component analysis of HIV-1 and HIV-2 genomes
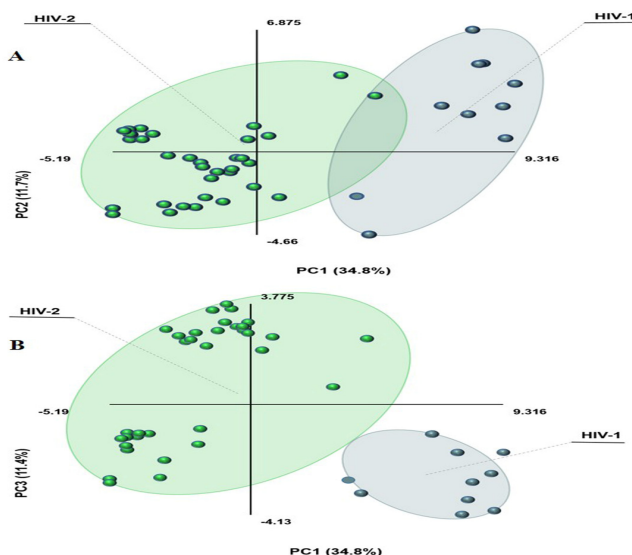
(A) First two principal component axis showing maximum variation for HIV-1 and HIV-2 genomes. (B) Principal component plot of axis 3 showing a variation closer to axis1.

HIVL-2: Structure-based design and synthesis of inhibitors of various enzymes of HIV

Emergence of drug-resistant viral variants in HIV-1-infected patients is a primary cause of treatment failure. Treatment with combinations of potent antiviral agents targeting the viral enzymes reverse transcriptase and protease, termed HAART, has been more successful than mono-therapeutic regimens. There are, however, problems with drug toxicity and multidrug resistance after prolonged therapy. NNRTIs, as components of HAART, have the advantage that they are minimally toxic; however, there are significant problems with the development of NNRTI resistance. Recently, inhibitors of the integrase enzyme

have emerged as a new promising class of therapeutics for the treatment of AIDS.

The present study involves:

- Design of newer leads employing structure-based drug design of inhibitors for HIV enzymes
- Synthesis and characterization of the designed inhibitors
- Enzyme inhibition studies
- Refinement of inhibitor molecules
- based on preliminary screening results and further design, synthesis, characterization and enzyme inhibition studies
- Anti-HIV activity in cell culture and cytotoxicity studies
- Pharmacokinetic and metabolism studies of potential molecules.

The design and synthesis of inhibitors is carried out at Dr. Sriram's lab and *in vitro* activity testing is being undertaken at NIRT. The study is on going.

Department of Immunology

STUDIES COMPLETED:

(i)   Use of alternative biomarkers other than IFN-γ

Principal Investigator	:	Dr. Alamelu Raja (email: alamelur@nirt.res.in)
Research Scholar	:	Ms. Maddineni Prabhavathi
Collaborator	:	Superintendent, Otteri Hospital
Source of funding	:	ICMR – Intramural; CSIR –Fellowship
Study period	:	2011 – 2014, Completed

Background: Early and accurate diagnosis and treatment of TB are the key strategies to control the global burden of TB. The suboptimal sensitivity of IFN-γ Release Assays (IGRAs) emphasizes the need for alternative markers for diagnosing active TB. Previously we had estimated the diagnostic ability of interferon-inducible protein (IP)-10, monocyte chemotactic protein (MCP)-2 and interleukin (IL)-2 in active TB diagnosis (NIRT Annual Report 2011-2012). We are now reporting on six other cytokines.

Aim: To evaluate whether *in vitro* Quantiferon TB Gold assay (QFT-GIT) antigens specific IL-1β, IL-6 IL-8, IL-2, IL-12 (p40) and TNF-α production is associated with active TB

Methods: In a cohort of 77 PTB patients, 67 healthy household contacts (HHC) and 83 healthy control subjects (HCS), the antigen specific cytokine levels were determined in supernatants generated from QFT- GIT tubes. Kruskal-Wallis statistical test was used to compare the groups. Receiver-operating-characteristic (ROC) curves were used to determine the cut-off points and discriminative ability was evaluated by the area under the ROC curve (AUC).

Results: Antigen specific IL-1β levels were significantly higher in PTB than HHC and HCS. At a fixed cut- off point (1108pg/ml), IL-1β showed positivity of 62.33% in PTB, 22.38% in HHC and 22.89% in HCS respectively (Fig. 9A). Moreover, antigen specific IL-1β assay could differentiate PTB and HHC (believed to be latently infected) ($p<0.0001$). Similar to IL-1β, significantly high level of antigen specific TNF-α was associated with PTB and displayed 43.63% positivity in PTB (Fig. 9B). The antigen

specific IL-2 level was associated both with PTB (54.54%) and HHC (48.14%) (Fig. 9C). Other cytokine levels did not differ among the groups.

Conclusion: Our results indicate that the existing biomarker of QFT-GIT i.e. IFN-γ can diagnose both active TB disease as well as latent TB infection (LTBI), which leads to its reduced utility for active TB diagnosis. However, as single biomarker IL-1β can effectively identify PTB alone, the elevated levels of IL-1β are mainly associated with PTB but not with LTBI.

Figs. 9 (A, B, C):

Fig A: Comparison of IL-1β levels   **Fig B: Comparison of TNF-α levels**   **Fig C: Comparison of IL-2 levels**
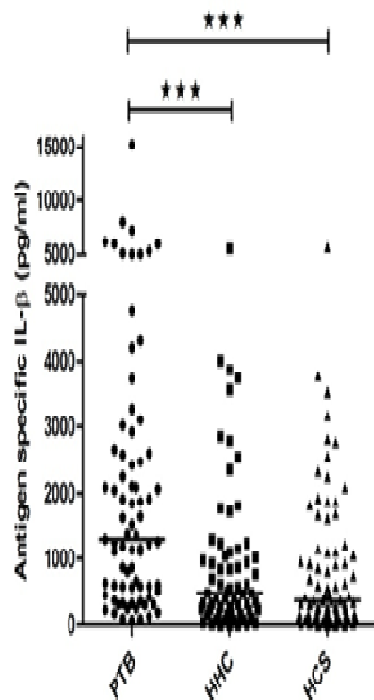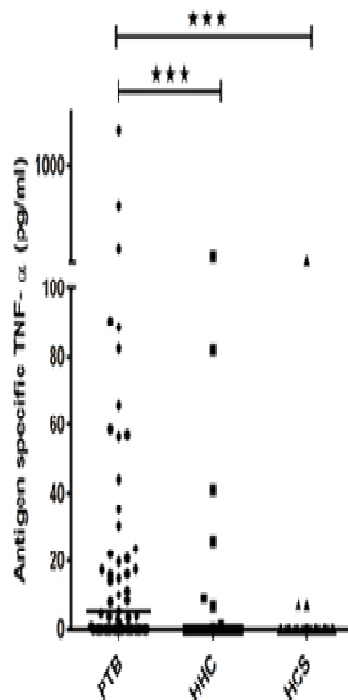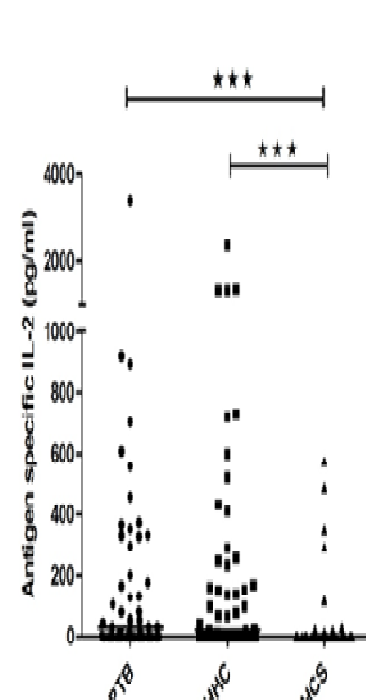
(ii) PknE, a serine/threonine protein kinase of _M. tuberculosis_ initiates survival cross-talk that also impacts HIV coinfection

Principal Investigator   :   Dr. Sujatha Narayanan
                                (email: sujathan@nirt.res.in)
Research Scholar   :   Dr.P. Dinesh Kumar
Source of funding   :   ICMR – Intramural; ICMR – Fellowship
Study period   :   2009-2014

Background: Serine/threonine protein kinases (STPK) play a major role in the pathogenesis of _M. tuberculosis_. Here, we examined the role of STPK pknE, using a deletion mutant ΔpknE in the modulation of intracellular signaling events that favour _M. tuberculosis_ survival. TB predominates in HIV-infected individuals due to weakened immune functions that lead to reactivation of latent bacilli. There are reports that disease progression in HIV/TB co-infected individuals is accelerated by both _M. tuberculosis_ and HIV. Cellular components of _M. tuberculosis_ are known to regulate co-receptors CXCR4 and CCR5 involved in HIV entry, but the molecular mechanisms underlying this phenomenon are not well-understood. Previously, we reported that pknE expressed under nitric oxide stress, suppresses multiple apoptotic pathways thereby supporting intracellular survival of _M. tuberculosis_ and that purified PknE cross-reacts with SAPK/JNK antibody.

Objectives:

(i)   to study the influence of pknE on intracellular signaling and to study its impact on the outcome of HIV/TB coinfection

(ii)   to study whether pknE of _M. tuberculosis_ influences the crosstalk between the MAPK pathways to regulate inflammation and HIV/TB coinfection

Methods: (i) Cell culture, infection, inhibitors and nitrate stress experiments: THP-1 cells were maintained, differentiated and infected as reported earlier. Cells were pretreated for 1 h with inhibitors of Akt (Wortmannin, 100 nM), arginase (Nv-Hydroxy-nor-Larginine diacetate, 100 mM), caspase-8 (Z-IETD-FMK, 25 mmol/L), caspase-9 (Z-LEHD-FMK, 25 mmol/L), ErkK (PD98059, 20 mM), p38 (SB203580, 10 mM), SAPK/JNK (SP600125, 10 mM) and TP53 (pifithrin-a, 5 mmol/L) and infected with _M. tuberculosis_ strains. For nitrate stress experiments, post-infection with _M. tuberculosis_, the cells were treated with 10 mM sodium nitroprusside.

(ii) HIV/TB co-infection: 24 hr post-infection with *M. tuberculosis*, THP-1 cells were infected with 500TCID50 of CCR5 (92UG005) and CXCR4 (92UG024)-tropic HIV-1 virus for 2 hr at 37°C. Post infection extracellular virus was removed by wash using serum-free RPMI and replenished with RPMI containing 10% FBS. The supernatant was harvested on day 4 to estimate the viral p24 levels by sandwich ELISA. Similarly, monocyte derived macrophages were isolated from the blood received from healthy volunteers.

ΔpknE infected macrophages modulate the expression of co-receptors CCR5 and CXCR4

We found that THP-1 macrophages infected with ΔpknE suppressed CCR5 but increased CXCR4 expression as compared to the wild-type strain. This finding was further confirmed by coinfection studies with *M. tuberculosis* and HIV-1 tropic strains. We examined various intracellular pathways that could influence this modulation. MAPK and arginase signaling were found to play an important role in the expression of CCR5 and CXCR4 in macrophages infected with ΔpknE. For the first time, we showed that ΔpknE induces apoptosis and down modulates intracellular events that suppress CCR5 expression. This concurs with a previous study where CCR5 was shown to induce anti-apoptotic signals via Akt and ErkK. The modulation of co-receptor expression was further investigated in MDM derived from normal healthy individuals. In contrast to THP-1 model of coinfection, ΔpknE increased the levels of p24 antigen upon co-infection with either R5 or X4 tropic HIV-1 strains. Among the MAPK and arginase signaling, SAPK/JNK was chosen for further validation. Inhibition of the SAPK/JNK signaling and co-infection with either R5 or X4 tropic HIV-1 strains increased the p24 antigen levels in ΔpknE co-infected macrophages. However, inhibition of SAPK/JNK signaling markedly reduced p24 levels in macrophages co-infected with either R5 or X4 tropic HIV-1 strains and Rv (Figs. 10A – D). These data suggests SAPK/JNK signaling as one among the cascade that regulates CCR5/CXCR4 expression.

Figs. 10 (A-D): ΔpknE coinfected with CCR5 and CXCR4 tropic HIV-1 increases p24 levels in MDMs
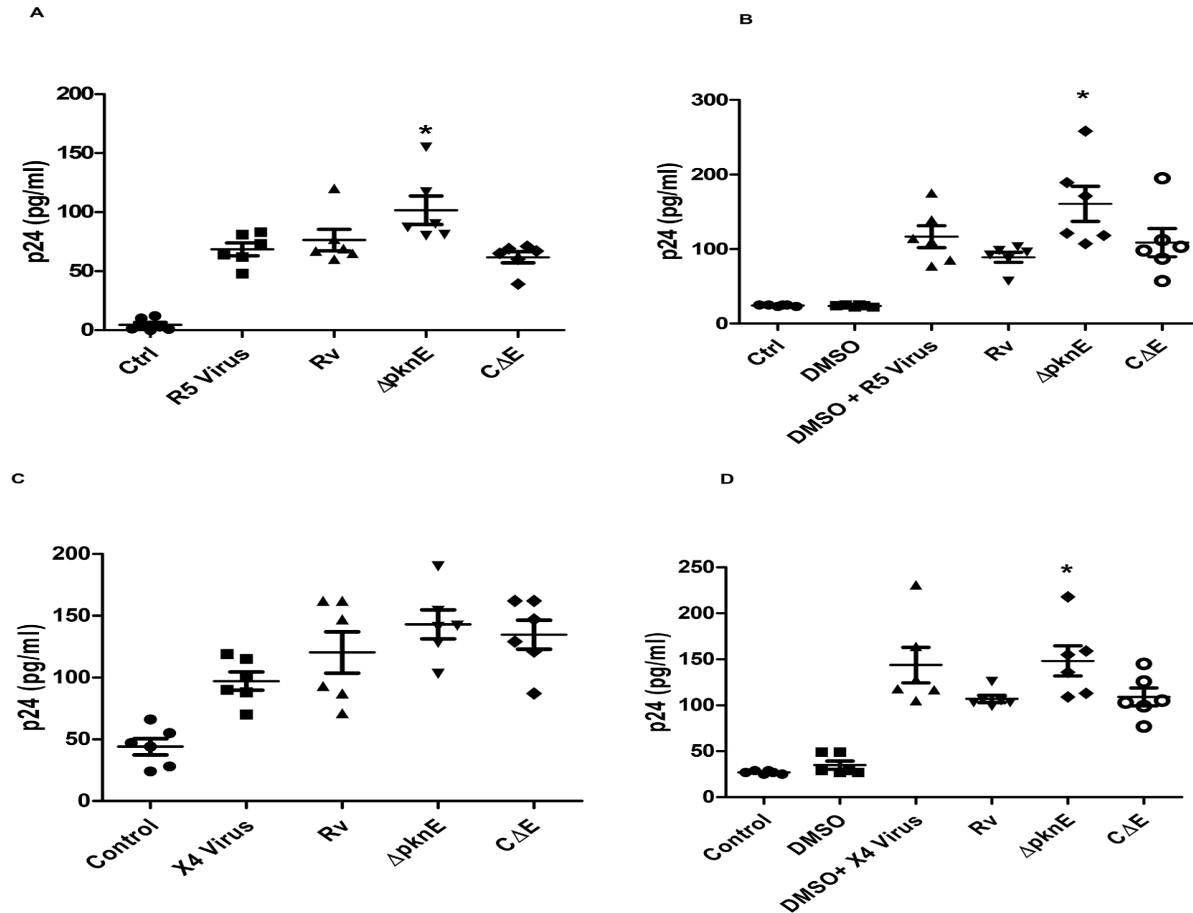
Legend for Figs.10A-D: Human monocyte derived macrophages (n = 6), were infected with *M. tuberculosis* strains followed by coinfection with a CCR5 tropic virus in the presence (A), and absence of SAPK/JNK inhibitor (B). Similarly, coinfection was performed using CXCR4 tropic virus in the presence (C) and absence of SAPK/JNK inhibitor (D). p24 antigen levels were estimated using ELISA on day 4. * denotes $p<0.05$ (one way – Anova) when ΔpknE was compared to Rv infected macrophages.

The expression kinetics of CCR5 and CXCR4 were examined on days 1 and 2 post-infection. Expression of CCR5 was reduced in ΔpknE-infected macrophages as compared to Rv-infected macrophages on both days ($p<0.05$; Fig.11A). Conversely, expression of CXCR4 was increased in macrophages infected with ΔpknE compared to Rv-infected macrophages ($p<0.05$; Fig.11B). CDE-infected macrophages had comparable levels of co-receptor expression to that of Rv-infected macrophages. Reduction in CCR5 by ΔpknE-infected

macrophages is influenced by intracellular signaling cascades.

Our previous and present findings persuaded us to examine the modulation of HIV receptors by MAPK, survival and apoptosis family of inhibitors. While MAPK inhibitors reduced the expression of CCR5 in ΔpknE-infected macrophages (p<0.05), SAPK/JNK inhibitors increased the expression of CCR5 in comparison with Rv-infected cells (Fig.11F). Akt inhibition did not have any effect on CCR5 expression (Fig.11C). ΔpknE infected macrophages had increased expression of CCR5 in the presence of arginase inhibitor as compared to Rv-infected macrophages (p<0.001). In the presence of TP53 inhibitor, both Rv and ΔpknE-infected macrophages had greater reduction in the expression of CCR5 (Fig.11D). CDE-infected macrophages were able to restore the expression levels similar to Rv-infected macrophages Increase in CXCR4 by ΔpknE-infected macrophages is influenced by intracellular signaling cascades Modulation of CXCR4 expression was also assessed in the presence of MAPK, survival and apoptosis inhibitors. In general, MAPK and Akt inhibitors increased expression of CXCR4 in Rv-infected macrophages. In contrast, ΔpknE-infected macrophages had significantly reduced CXCR4 expression in the presence of ErkK inhibitor (p<0.0001, Fig.118E) and moderate reduction in the presence of p38MAPK and SAPK/JNK inhibitors, compared to Rv infected macrophages (Fig.11F). Akt inhibitor did not affect expression of CXCR4 (Fig.11G), but arginase inhibitor reduced the expression of CXCR4 in ΔpknE-infected macrophages (p<0.05) when compared to Rv-infected cells (Fig.11G). TP53 inhibitor reduced the expression of CXCR4 in Rv, ΔpknE and CDE-infected macrophages (Fig.11H). CDE-infected macrophages reversed the changes observed in ΔpknE-infected macrophages.

MAPK inhibitors instead of reducing the phosphorylation in ΔpknE infected macrophages, revealed cross-talks with ErkK signaling influenced bySAPK/JNK and p38 pathways independently. Modulations in intracellular signaling altered the expression of co-receptors CCR5 and CXCR4 in ΔpknE infected macrophages. In conclusion, pknE plays a role in MAPK cross-talks that enables intracellular survival of *M. tuberculosis*.

This survival strategy also impacts HIV/TB co-infection.

Conclusion: Our previous and the current findings show that pknE contributes to the intracellular survival of MTB by initiating cross-talks within the intracellular signaling of the host.

Figs.11 (A-H): ∆pknE infected macrophages modulate the expression of coreceptors CCR5 and CXCR4 by intracellular cascades
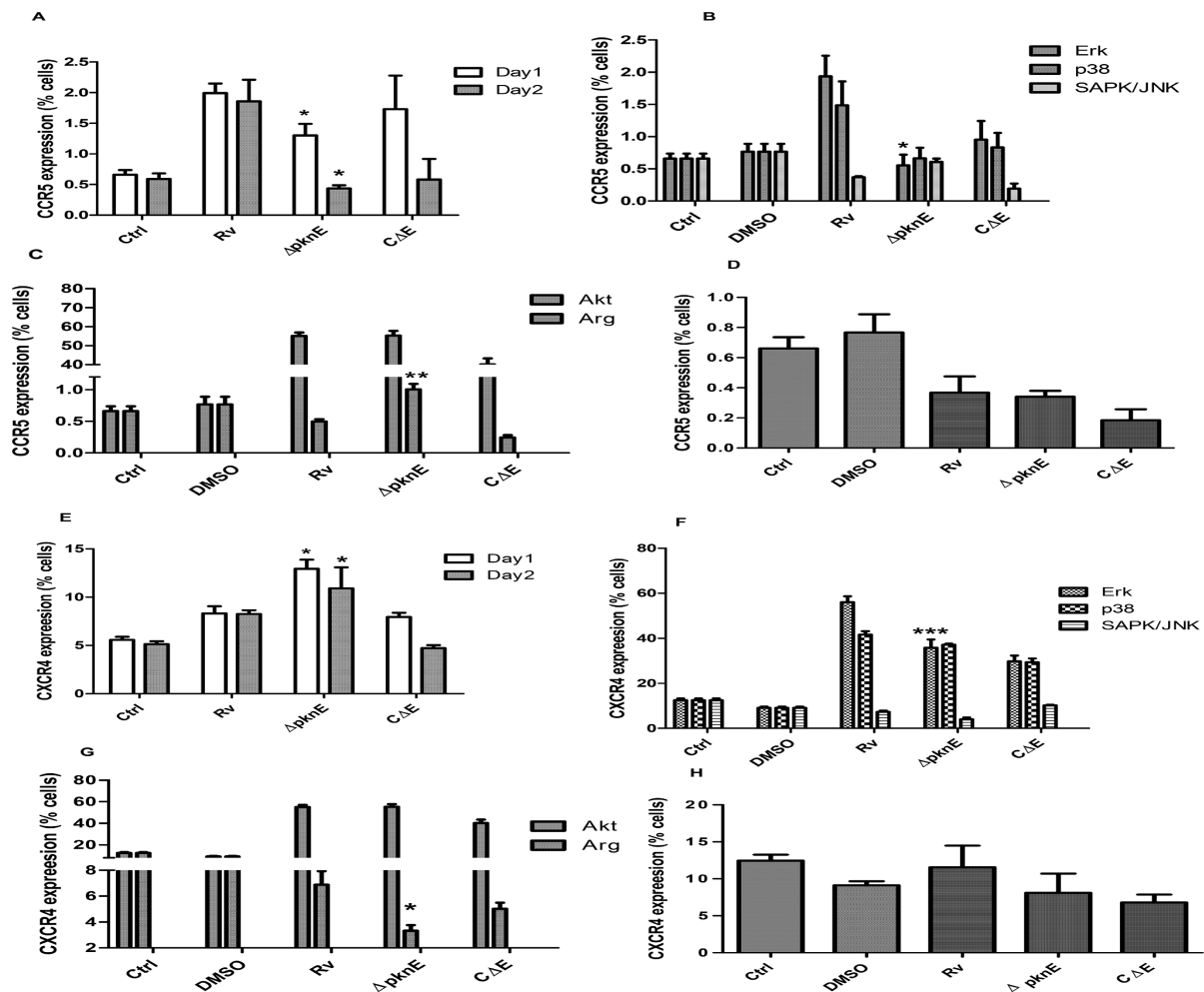
Legend for Figs.11 (A-H): Cells post infection were stained with CCR5 (A) and CXCR4 (E) antibody and the expression was analyzed in a time dependent manner using FACS, * denotes p,0.05 (Twoway – Anova) when ∆pknE was compared to Rv infected macrophages. Cells post infection in the presence of inhibitors CCR5 [B) MAPK family, C) survival family and D) TP53] and CXCR4 [F) MAPK family, G) survival family, and H) caspase family] expression was analyzed on day1 post infection using FACS. The symbols *, **, ***denotes p,0.05, p,0.001 and p,0.0001 respectively (one way – Anova) when ∆pknE was compared to Rv infected macrophages.

(iii)   Role of Chemokines, DC-SIGN and Toll-like receptor gene variants on immunity to TB

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Collaborators	:	1. Dr.N. Meenakshi, Institute for
		Thoracic Medicine, Chennai-31.
		2. Dr.R.T. Parthasarathy, Govt.
		Thiruvotteeswarar Hospital of Thoracic Medicine,
		Chennai – 12
Source of funding	:	Intramural
Study period	:	April 2009 to June 2013

Background: Invasion of the host by microbial pathogens causes activation of the innate immune response (first line defense) and triggers the secretion of various chemokines and cytokines and initiation of adaptive immunity. Chemokines, DC-SIGN and Toll-like receptor (TLR) gene polymorphisms have been studied and shown to be associated with susceptibility or resistance to various infectious diseases.

Aims: (i) to find out whether Chemokine, DC-SIGN and TLR gene polymorphisms are associated with susceptibility or resistance to TB

(ii) to understand the regulatory role played by the chemokine gene variants on chemokine expression in PTB and

(iii) to understand the immuno-modulatory effect of vitamin D_3 on innate and adaptive immunity to PTB

Under this main study, the following sub-studies were carried out.

(a) Regulatory role of chemokine gene polymorphisms on chemokine expression in PTB

Background: Polymorphisms in the chemokine genes have been shown to regulate the production of chemokines. In the present study, the regulatory role of various chemokine gene polymorphic variants on chemokine expression was studied in newly recruited PTB and healthy volunteers.

Aim: To understand the regulatory role of various chemokine gene variants on chemokine expression

Methods: The study was carried out with newly recruited PTB patients (n=60) and healthy volunteers (n=60). The chemokine levels of CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, CXCL10/IP-10 and CXCL12/SDF-1 were estimated

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in the 72hrs culture supernatants of peripheral blood mononuclear cells (PBMCs) stimulated with *M. tuberculosis* antigen and the cells were used for determination of intracellular chemokine expression. Chemokine gene polymorphisms were determined using DNA extracted from the white cells of the patients and healthy controls. Chemokine gene polymorphisms were correlated with the level of chemokines as well as intracellular chemokine positive cells.

Results: Regulatory role of various polymorphic variants of chemokine genes on culture filtrate antigen (CFA) of *M. tuberculosis* induced chemokine production was studied in PBMCs culture supernatant of HCs and PTB patients. CCL2 level was significantly lower in HCs with CCL2 -2518GG genotype compared to CCL2 -2518AA genotype in spontaneous (p=0.007) and CFA induced (p=0.035) cultures (Fig.12). In CCL4 -5725A/C polymorphism, a significantly decreased expression of CCL4 was observed in HCs with AA genotype compared to AC genotypes in CFA induced culture (p=0.047). When we

looked at the CCL5 expression in subjects with different genotypes of CCL5 -403G/A polymorphism, a significantly decreased expression of CCL5 was observed in PTB patients with -403AA genotype compared to -403GG genotype under different culture conditions (p<0.05). Moreover, CXCL10 level was significantly lower in CXCL10 -1447GG genotype compared to CXCL10 -1447AA genotype under unstimulated (p=0.047) and CFA stimulated (p=0.046) cultures. A similar regulatory role of various chemokine gene polymorphisms was observed with various intracellular chemokine positive cells.

Conclusion and Implication: The results suggest that decreased CCL2 expression in -2518GG genotype may be associated with protection against PTB through enhanced Th1 immunity. Moreover, -403AA genotype of CCL5 and -1447GG genotype of CXCL10 genes are associated with decreased CCL5 and CXCL10 expression respectively which might have a defective role in the recruitment of mononuclear cells to the inflammatory site and granuloma formation and may contribute to progression of TB.

Fig. 12: **CCL2 -2518A/G variants and CCL2 level in HCs and PTB patients**
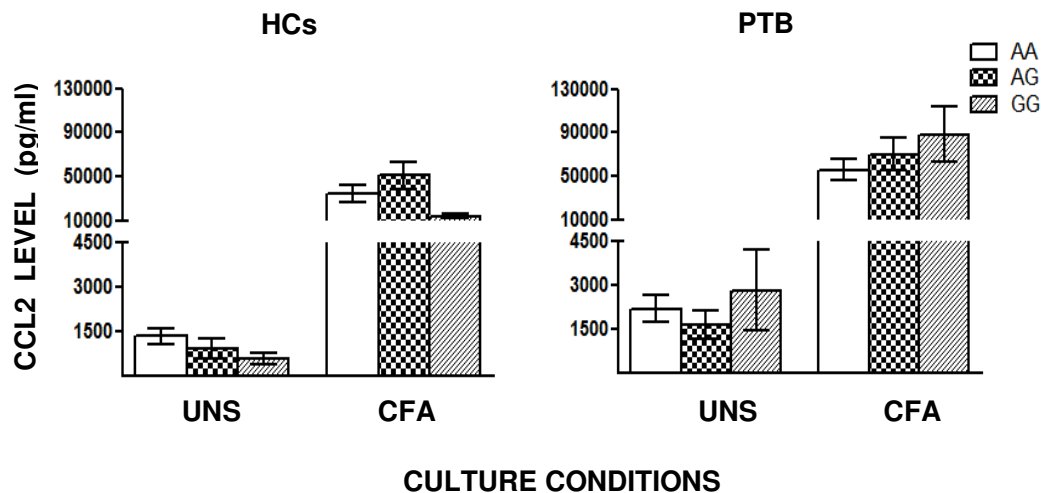
(b) Effect of 1,25 dihydroxy vitamin D$_3$ (1,25(OH)$_2$D$_3$) on CD14, CD206, CD209, Beclin and ATG-5 expression in macrophages infected with live *M. tuberculosis*

Background: Immune responses after mycobacterial infection are initiated by recognition of mycobacterial components through various host receptors. Phagocytosis and subsequent induction of autophagy plays key roles to eliminate the intracellular pathogen. Vitamin D$_3$ is known to play various immunomodulatory roles on innate immunity; it enhances macrophage phagocytosis by up regulating the expression of pattern recognition receptors and induces the expression of antimicrobial peptides.

Aim: To understand the effect of vitamin D$_3$ on CD14, CD206, CD209, Beclin and ATG-5 expression in monocyte/ macrophages in PTB

Methods: The study was carried out in 20 PTB patients and 20 healthy control subjects. PBMCs were cultured for 72 hrs with live *M. tuberculosis* and its CFA in the presence and absence of 1,25 dihydroxy vitamin D$_3$. The non-adherent cells were aspirated gently and the adherent 72 hrs old monocytes/macrophages were used for RNA

extraction and cDNA synthesis. The relative quantification of target genes such as CD14, CD206, Beclin, ATG-5 and the housekeeping gene, GAPDH was done using real-time PCR with TaqMan assay primers and probes.

Results: 1,25 dihydroxy vitamin D_3 significantly increased the expression of CD14, Beclin1 and ATG5 genes in peripheral blood monocyte derived macrophages and soluble CD14 levels in the PBMC culture supernatants as compared to ethanol treated control cultures in both the study groups (p<0.05). Similarly, $1,25(OH)_2D_3$ significantly up regulated CD14, Beclin1 and ATG5 gene expression in CFA stimulated and Mtb infected macrophages.

Addition of 1, $25(OH)_2D_3$ significantly increased the soluble CD14 levels in CFA and Mtb stimulated PBMCs culture supernatants of both study groups (p<0.05) (Fig.13). $1,25(OH)_2D_3$ also enhanced CD206 gene expression, whereas it suppressed the CD209 expression in CFA with $1,25(OH)_2D_3$ stimulated macrophages of HCs (p=0.0318).

Conclusions and Implications: 1,25 dihydroxy vitamin D_3 significantly upregulate CD14, Beclin1 and ATG5 gene expression that may enhance macrophage phagocytosis and induce autophagy of infected cells and elimination of intracellular mycobacteria.

Fig.13: Effect of 1,25(OH)₂D₃ on soluble CD14 levels in PBMCs culture supernatants
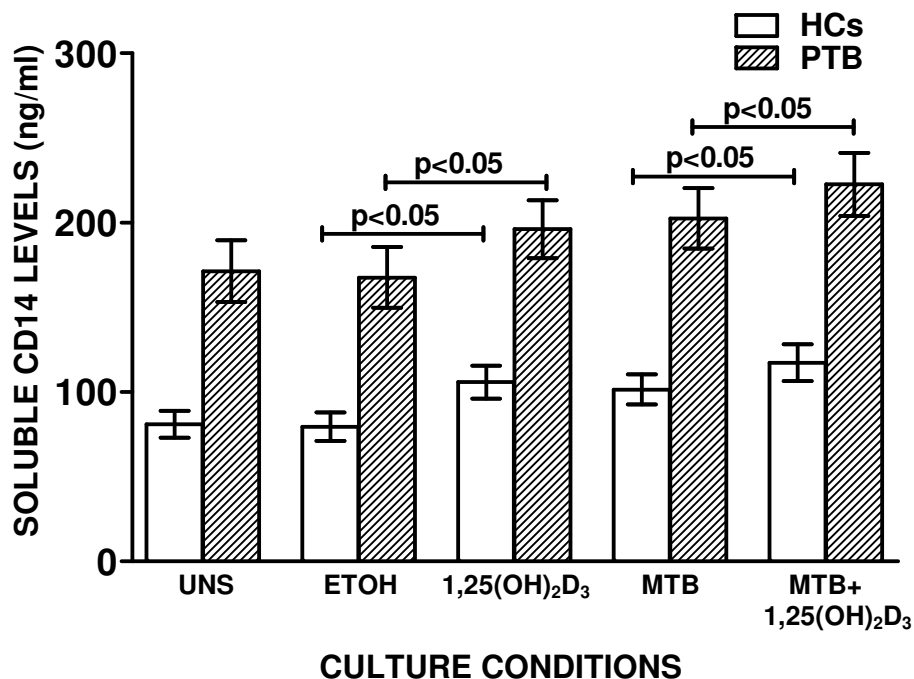
Uns: Unstimulated cells; ETOH: Ethanol control; $1,25(OH)_2D_3$: 1,25-dihydroxy vitamin $D_3 1X10^{-7}M$ Concentration; MTB: *M. tuberculosis*; HCs: Healthy controls; PTB: Pulmonary tuberculosis patients.

(c) Regulatory role of vitamin D receptor gene variants on 1,25 dihydroxy vitamin D_3 modulated intracellular chemokines in PTB

Background: Our earlier studies revealed the regulatory role of vitamin D receptor (VDR) gene variants on various immune functions in PTB. We have reported that vitamin D_3 significantly alter the various extracellular chemokine levels in the cell culture supernatants of peripheral blood mononuclear cells stimulated with *M.* *tuberculosis* antigens. In the present study, the regulatory role of VDR gene variants on vitamin D_3 modulated intracellular chemokine expression is explored in PTB.

Aim: To find out the regulatory role of VDR gene promoter region polymorphism *Cdx-2* and 3' untranslated region polymorphisms *TaqI* and *BsmI* on vitamin D_3 modulated

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intracellular chemokine expression in PTB

Methods: The VDR gene polymorphisms were determined in 50 HCs and 50 PTB patients. Cdx-2 polymorphism was studied by polymerase chain reaction (PCR) with allele-specific primers. TaqI and BsmI polymorphisms were studied by PCR based restriction fragment length polymorphism (RFLP) method. Whole blood cells were cultured for 72 hrs with live *M. tuberculosis* and its antigen with or without vitamin D_3 at a concentration $1X10^{-7}M$. After 72 hrs, the cells were processed for immunostaining using specific monoclonal antibodies against CD3, CD4 and CD8 surface markers and intracellular chemokines which included MCP-1, MIP-1α, MIP-1β, RANTES and IP-10, and analysed by flow cytometry.

Results: The regulatory role of VDR Cdx-2 and 3'UTR *Bsm*I and *Taq*I gene polymorphisms on intracellular chemokine expression was analysed in CD3+ T-cells of HCs and PTB patients. In HCs, a significantly decreased CCL4 /MIP-1β and CCL5/RANTES expression was observed in *Cdx*-2 AA genotype compared to GG

genotype in culture filtrate antigen (CFA) of *M. tuberculosis* with $1,25(OH)_2D_3$ treated cells (p=0.027) (Fig.14). A similar decreased expression was observed in *Taq*I tt genotype compared to TT genotype in MIP-1β expression in HCs (p=0.042) and RANTES expression in PTB patients (p=0.037). No such regulatory role of VDR gene variants was observed on CCL2/MCP-1, CCL3 /MIP-1α and CXCL10/IP-10 levels in HCs and PTB patients.

Conclusions and Implications: The present study suggests that *Cdx*-2 AA and *Taq*I tt genotypes are associated with decreased intracellular expression of CCL4/MIP-1β and CCL5/RANTES. Since these chemokines are involved in activation and proliferation of T-cells and granuloma formation, the decreased expression observed in *Cdx*-2 AA and *Taq*I tt genotypes may be associated with impaired T-cell responses and recruitment of immune cells at the site of infection which may lead to susceptibility to TB.

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Fig. 14: Cdx-2 gene polymorphism and intracellular CCL5/RANTES expression in CD3+ T-cell in HCs and PTB patients
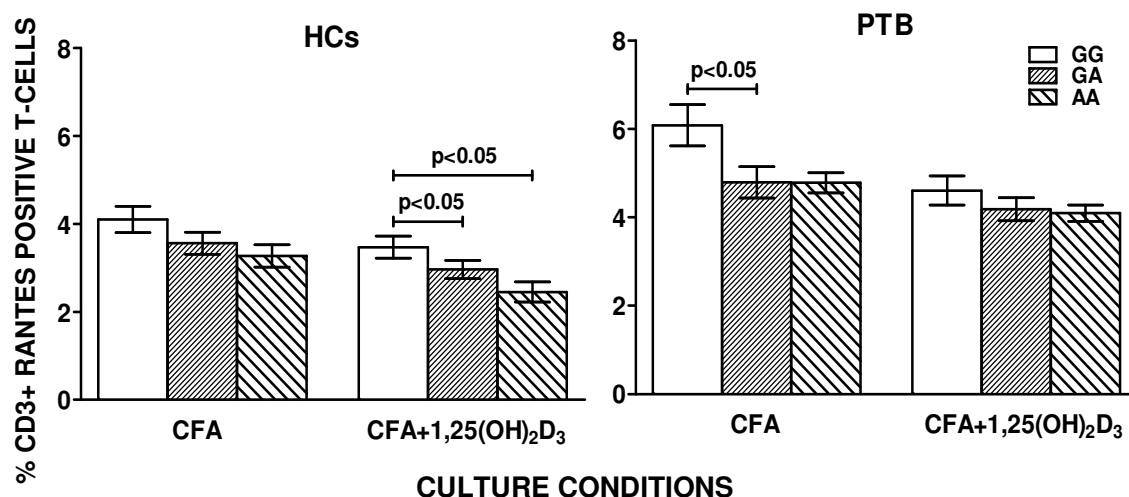
HCs, GG (n=19); GA (n=25) and AA (n=06).PTB, GG (n=15); GA (n=24) and AA (n=11).
n= number of individuals studied.
HCs-healthy controls; PTB- pulmonary tuberculosis patients; CFA-Culture filtrate antigen.

STUDIES IN PROGRESS:

I-1: **Identification of serologically reactive antigens in culture filtrate of *M. tuberculosis***

Principal Investigator	:	Dr. Alamelu Raja (email: alamelur@nirt.res.in)
Research Scholar	:	Mr.P. Pukazhvandhen
Source of funding	:	ICMR – Intramural; ICMR - Fellowship
Collaborators	:	Superintendent, Otteri Hospital
Study period	:	2011 – 2015

Background: Detection of *M. tuberculosis* (*M. tb*)-specific human antibodies has been an important diagnostic aid in the diagnosis of TB. Proteins secreted into the extracellular environment by *M. tb* are recognized by the immune system in the infected host, constituting an important source of antigens that induce immune responses with diagnostic value.

Aim: To identify serologically reactive fractions in culture filtrate antigens of *M. tb*

Methods: CFA of *M. tb* was subjected to preparative 2-Dimensional electro-phoresis which separated CFA into 600 fractions. To

identify serologically reactive fractions, each fraction was tested with pooled sera of 50 TB patients and 50 healthy control subjects by indirect ELISA.

In the initial standardization experiments, it was observed that 16 fractions showed ≥3 fold difference and 94 fractions exhibited >2 fold but <3 fold difference in terms of OD value between TB and healthy controls. The 94 fractions were further assessed for their serodiagnostic potential with 110 sera from HCS, 110 sera from HHC, 88 sera from PTB patients and 88 sera from HIV-infected TB patients (HIV-TB). Cut off value for each fraction was ascertained by mean +2SD. Mass spectrometry analysis was carried out to identify antigens in these sero reactive fractions.

Results: On assessing individual fractions, sensitivity ranged from 18% to 56% in PTB, 14.5% to 34.5% in HHC and 15.5% to 45%

in HIV-TB with the specificity ranging from 93% to 100% in HCS. Combining seven fractions (3_30 + 5_27 + 6_01 + 7_04 + 7_26 + 11_06 and 13_23) increased sensitivity to 93% in PTB, 72.7% in HHC and 83% in HIV-TB with specificity to 93% in HCS (Table 11).

Exploration of these serologically reactive fractions (3_30, 5_27, 6_01,7_04, 7_26, 11_06 and 13_23) by LC-MS/MS method identified 42 antigens.

Most of these sero reactive fractions contained already reported immuno-dominant antigens such as phoS1, adk, bfrB, dnaK, fbpA, Frr, GlcB, glnA1, groEL2, grpE, hspX, icd-2, katG and lppZ. But they also contain seven novel B cell antigens such as Rv0020c, Rv0815c, Rv1630, Rv2185c, Rv2204c, Rv2721c, Rv3036c which are not reported elsewhere (Table 12).

Table 11: Representative sensitivity and specificity of individual and combination of fractions among 94 fractions

S.no	IEF_WGE Fractions	% Specificity	% Sensitivity		
		HCS	HHC	PTB	HIV-TB
1	5_16	100	18.1	46.5	23.8
2	7_01	100	21.8	39.7	21.5
3	2_06	99	34.5	52.2	37.5
4	2_19	99	27.2	46.5	32.9
5	6_10	99	15.4	43.1	28.4
6	7_04	99	12.7	38.6	19.3
7	10_12	99	24.5	40.9	34.0
8	2_30	98	21.8	22.7	15.9
9	4_24	98	22.7	38.6	21.5
10	5_11	98	16.3	30.6	15.9
11	6_18	98	19.0	23.8	17.0
12	8_10	98	14.5	45.4	27.2
13	9_09	98	23.6	50.0	27.2
14	10_09	98	20.0	46.5	27.2
15	11_06	98	25.4	44.3	28.4
16	13_23	98	22.7	35.2	31.8
17	14_12	98	21.8	50.0	42.0
18	6_01	97	15.4	34.0	26.1
19	5_27	97	32.7	52.2	37.5
20	3_30	97	25.4	38.6	31.8
21	7_12	97	19.0	42.0	26.1
22	6_04	97	19.0	44.3	37.5
23	7_10	97	16.3	53.4	36.3
24	5_21	97	27.2	47.7	38.6
25	5_26	97	25.4	44.3	37.5
26	5_27	97	32.7	52.2	37.5
27	6_01	97	15.4	34.0	26.1
28	6_16	97	24.5	43.1	31.8
29	8_01	97	24.5	50.0	29.5
30	8_06	97	24.5	51.1	29.5
31	8_08	97	27.2	48.8	29.5
32	9_28	97	24.5	36.3	25.0
33	10_05	97	15.4	44.3	28.4
34	10_07	97	20.0	42.0	30.6
35	13_08	97	27.2	39.7	32.9
36	7_26	96	28.1	56.8	45.4
	3_30 + 5_27 + 6_01 + 7_04 + 7_26 + 11_06 + 13_23	88	77.3	93.2	83

Table 12: Antigens identified in sero reactive fractions (3_30, 5_27, 6_01,7_04,7_26,11_06, and 13_23)

S.No	Protein name	Gene number	Molecular weight	S.No	Protein name	Gene number	Molecular weight
1	acpM	Rv2244	12473.8	22	phoS1	Rv0934	38211.0
2	adk	Rv0733	20075.6	23	pks13	Rv3800c	186428
3	bfrB	Rv3841	20423.6	24	ppa	Rv3628	18278.3
4	cysA2	Rv0815c	30997.2	25	ProA	Rv2427c	43774.6
5	dnaK	Rv0350	66812.9	26	rpsA	Rv1630	53183.5
6	fadA3	Rv1074c	42638.1	27	sucB	Rv2215	57070
7	fbpA	Rv3804c	35668.3	28	wag31	Rv2145c	28259.6
8	frr	Rv2882c	20809.9	29	hypothetical protein	Rv1926c	16495.8
9	GlcB	Rv1837c	80402.0	30	hypothetical protein	Rv1174c	10863
10	glnA1	Rv2220	53522	31	hypothetical protein	Rv1827	17200.5
11	groEL2	Rv0440	56709.3	32	hypothetical protein	Rv2376c	16617
12	grpE	Rv3418c	10754.1	33	hypothetical protein	Rv3036c	24388.7
13	hspX	Rv2031c	16209.6	34	hypothetical protein	Rv2204c	12526.2
14	Icd-2	Rv0066c	82552.2	35	hypothetical protein	Rv2721c	72321.5
15	katG	Rv1908c	80555.9	36	hypothetical protein	Rv3881c	47575.7
16	lppZ	Rv3006	38734.2	37	hypothetical protein	Rv0020c	56862.2
17	lpqH	Rv3763	15096.2	38	hypothetical protein	Rv1827	17200.5
18	LprA	Rv1270c	24843.7	39	hypothetical protein	Rv2204c	12526.2
19	modD	Rv1860	32702.8	40	hypothetical protein	Rv1314c	20676.9
20	mpt53	Rv2878c	18364.9	41	hypothetical protein	Rv2140c	18616
21	mpt64	Rv1980c	24805.3	42	hypothetical protein	Rv2185c	16275.7

Conclusion: Eight fractions were identified as serologically reactive fractions and the antigens present in these fractions would be promising candidates for serodiagnosis of TB.

I-2: Dormancy associated antigens of *M. tuberculosis*

Principal Investigator	:	Dr. Alamelu Raja
		(email: alamelur@nirt.res.in)
Research Scholar	:	Mr.D. Santhi
Collaborators	:	Superintendent, Otteri Hospital
Source of funding	:	ICMR Task Force – Extramural
Study period	:	2011 – 2014

Background: Analysis of mycobacterial antigens associated with the slowly replicating, post-logarithmic phase growth of *M. tuberculosis*, the so-called "dormant" phase, is our interest. Three different strains of *M. tuberculosis* were used in this study: the laboratory strain H37Rv and two of the clinical strains, most prevalent in South India, S7 and S10. Clinical strains were used since no literature reported dormancy associated antigens from clinical strains.

Aim: To identify differentially regulated genes under hypoxia from laboratory strain H37Rv and two clinical strains (S7 and S10) of *M. tuberculosis*

Methods: H37Rv, S7 and S10 strains were grown aerobically (MB7H9 media, 300rpm, 37°C) and anaerobically (0.5 ratio of headspace air volume to liquid volume, MB7H9, 120 rpm, 37°C). RNA was isolated from pellets collected by standard Trizol reagent method. 10-200ng of isolated RNA was used for cDNA synthesis by WT primer method and labeled with cy3 (aerobic cultures) and cy5 (anaerobic cultures) by two color labeling kit. Labeled RNAs were hybridized on a 60mer oligonucleotide based custom array chip. The image analysis was done using Feature extraction tool version 9.5.3.1.

Results: Total numbers of genes that were differentially regulated in H37Rv, S7 and S10 during hypoxia is given in Fig.15. Out of 3951 genes tested in custom array chip, 15.6% of genes in H37Rv were expressed under oxygen depletion, whereas in S7 11.5% genes were upregulated; In S10 the percentage of genes that responded to hypoxia was higher than other two strains (37%). Approximately 13% (12.7%) genes in H37Rv were expressed less and in S7 and S10, 8.2% and 29.8% of genes were suppressed under hypoxia. A total of 134 upregulated genes which were common among all three strains were of our interest (Fig.16) to target them for vaccine approaches and drug targets.

Conclusion: Genes, such as fad4 (Rv0214), Rv0719 (50S ribosomal protein L6) and Rv0347 are expressed almost 4 fold in all three strains. Highest level of upregulation (9.85 fold) was observed for Rv2293 in H37Rv, Rv1307 (atpH) 4.84 fold overexpressed in S7 and Rv2300c (hypothetical protein) was overexpressed in S10. These variations in gene expression highlight the need for studying the most prevalent strains of *M. tuberculosis* under various stimuli.

Fig.15: Number of genes regulated during oxygen depletion in *M. tuberculosis* lab strain H37Rv and south India prevalent strain S7 and S10

a. No of genes that are expressed more than 1.5 fold **during hypoxia**

b .No of genes that are suppressed more than **1.5 fold during hypoxia**
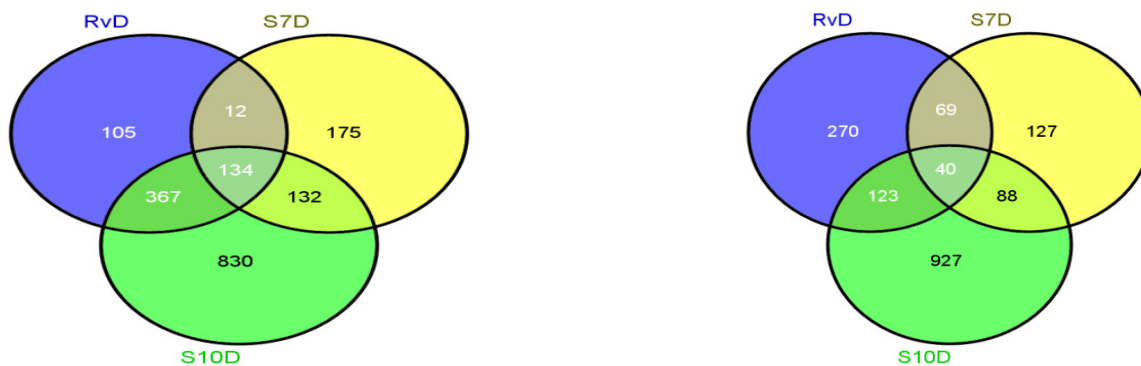
Fig.16: Heat map of upregulated genes that are found to be common among the strains of this study
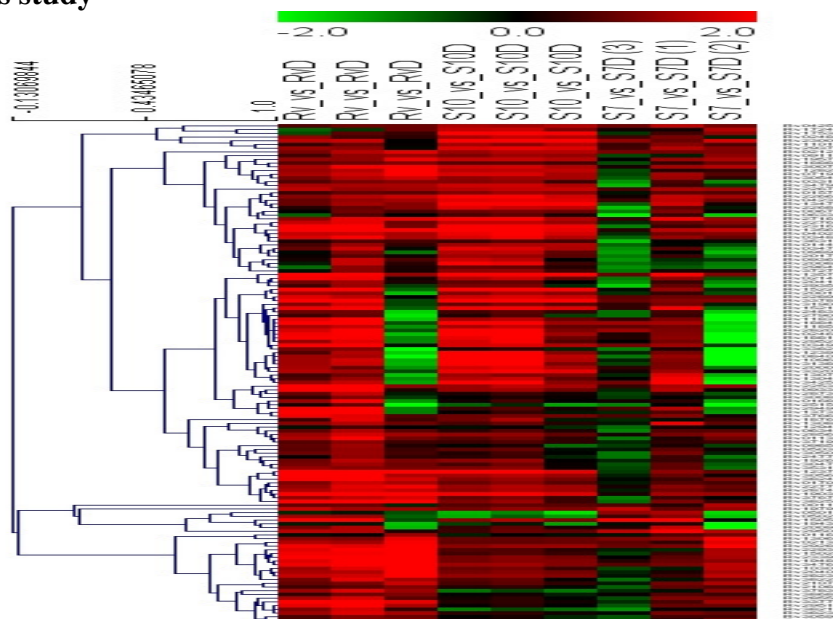
I-3: Immunoproteomically identified *M. tb* antigens for differential diagnosis of LTBI and active TB disease

Principal Investigator	:	Dr. Alamelu Raja (email: alamelur@nirt.res.in)
Research Scholar	:	Ms. Maddineni Prabhavathy
Collaborators	:	Superintendent, Otteri Hospital
Source of funding	:	CSIR Fellowshjip / ICMR Intramural
Study period	:	2011-2016

Background: An estimated one-third of the world's population who harbor *M. tb* remains asymptomatic and termed latently infected. Early diagnosis and treatment of individuals with LTB1 are crucial in effective TB control programmes. The existing TST and IGRAs are inefficient diagnostic tools for LTBI detection. Earlier we had carried out the separation of culture filtrate proteins (CFP) of *in vitro* grown *M. tb* by two dimensional-liquid phase electro-phoresis (2D-LPE) and tested for the ability to stimulate T-cells in human models of TB. Of those, 10 fractions were exclusively recognized only by the HHC and not by TB patients. A total of 16 proteins were identified from the 10 contact specific (CS) fractions by using LC-MS/MS method.

Aims: (i) to analyze the T-cell mediated *in vitro* cytokine (IFN-γ) response to differentiate LTBI and active TB, by using 16 antigens

(ii) to undertake cloning, over expression and purification of all 16 antigens and comparison of IFN-γ levels in response to these antigens by using whole blood from the study subjects

Methods: All the recombinant proteins were over expressed by using BL21 (DE3)/ BL21 (DE3) pLysS *E. coli* system. We prospectively enrolled, 40 PTB and 35 HHC subjects. Blood from the study subjects were stimulated with individual antigens (5µg/ml) for six days (1:10 diluted whole blood). IFN-γ secretion was measured by ELISA in plasma harvested at day 6 post-culture. Mann-Whitney test was used to compare the groups. ROC curves were used to determine the cut-off points and discriminative ability was evaluated by the area under the ROC curve (AUC).

Results: As described in the previous annual report (NIRT Annual Report 2012-2013), all the 16 proteins were purified successfully by

affinity column chromatography. All the purified antigens were used to characterize the T-cell mediated *in vitro* cytokine response (IFN-γ) to differentiate LTBI and active TB. All the 16 antigens induced significantly higher level of IFN-γ in HHC compared to TB subjects; Table 10 indicates individual antigen sensitivity and specificity. Among the 16 antigens, Rv2626c followed by TrxC and Rv3716c showed optimal differentiation between the two groups (Fig. 17).

Conclusion: Rv2626c antigen can be used in conjunction with the existing QFT-GIT. The QFT-GIT positives can be either LTBI or TB, whereas QFT-GIT and "Rv2626c antigen" positivity will rule out LTBI in QFT-GIT positives**.**

Table 13: Specificity and sensitivity of individual antigens

S. No	Name of the Antigen	AUC	Specificity	Sensitivity	*p* value
1	ESAT-6	0.6264	71.43	53.85	0.06
2	CFP-10	0.7575	88.57	56.41	0.0001
3	Ag85B	0.863	88.57	71.79	< 0.0001
4	GroES	0.6692	88.57	41.03	0.01
5	ADK	0.7458	88.57	43.59	0.0002
6	Rv2626c Hypothetical protein	0.9487	88.57	92.31	< 0.0001
7	Trx C	0.9341	88.57	87.18	< 0.0001
8	Dna K	0.9509	88.57	84.62	< 0.0001
9	Gro EL	0.8103	88.57	43.59	< 0.0001
10	HspX	0.8623	88.57	69.23	< 0.0001
11	Pst S1	0.848	88.57	71.79	< 0.0001
12	AcpM	0.7355	82.86	53.85	0.0005
13	Psts2	0.8872	88.57	74.36	< 0.0001
14	Rv3716c Hypothetical protein	0.8971	88.57	87.18	< 0.0001
15	TB8.4	0.8788	88.57	84.62	< 0.0001
16	KatG	0.9033	88.57	82.05	< 0.0001

Fig. 17: IFN-γ secretion against the best antigens among the 16 antigens
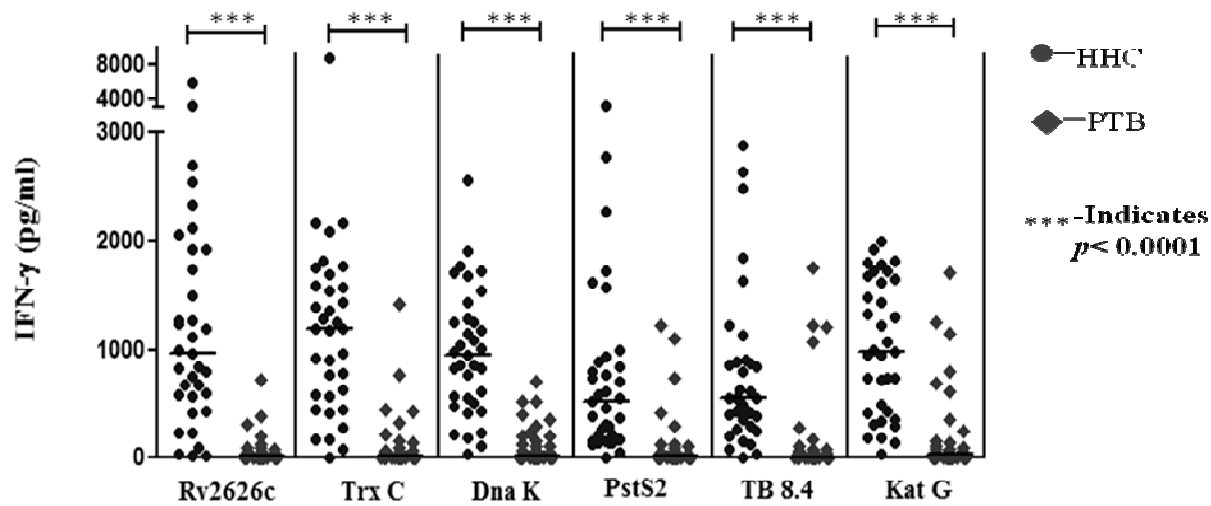
I-4: **Immunological characterization of novel T- cell antigens of *M. tuberculosis***

Principal Investigator	:	Dr. Alamelu Raja (email: alamelur@nirt.res.in)
Research Scholar	:	Mr. Pathakamuri Balaji
Collaborators	:	Superintendent, Otteri Hospital
Source of funding	:	ICMR Fellowship / ICMR-Intramural
Study period	:	2011-2016

Background: In our earlier study we identified Rv2204c, (hypothetical protein), Rv0753c (mmsA) and Rv0009 (PpiA) as novel T-cell antigens in our endemic setting. Purification of these recombinant proteins and IFN-γ response against these antigens in 15 household contacts and 19 active TB patients (PTB) has been reported in NIRT Annual Report 2012-2013. In continuation of this study, we further assessed IFN-γ response in more study subjects.

Aim: To analyze whether the antigen specific IFN-γ response against Rv2204c, Rv0753c, Rv0009 can be used for the differentiation of LTBI and active TB disease (PTB)

Methods: During the period under review, 20 HHC and 20 PTB patients were recruited. Along with standard antigens (ESAT-6, CFP-10), Rv2204c, Rv0753c, Rv0009 whole antigens and Rv0009 peptide specific IFN-γ level was measured in the supernatant of diluted whole blood, following six days

stimulation. Rv0009 whole protein and its 18 peptides were performed in totally 35 HHC and 27 PTB cases. Unpaired t test was used for statistical analysis. Cut-off values were calculated by ROC curve analysis using graph pad prism.

Results: Rv2204c, Rv0753c, Rv0009 and standard antigens induced significantly higher IFN-γ production in HHC compared to active TB. The test antigens showed higher sensitivity when compared to standard antigens. Rv2204c, Rv0753c,

Rv0009 had shown 74%, 68.5%, 74% sensitivity respectively with 90% specificity (Table 14). Compared to the peptides, whole Rv0009 antigen showed a higher sensitivity of 77% and a specificity of 89%. Among the peptides, P11 (54%), P2 (51%), P3 (48%), P4 (48%) had shown maximum sensitivity and 90% specificity (Table 15).

Table 14: IFN-γ response to Rv2204c, Rv0753c and Rv0009 antigens

S.NO	Protein name	% of Sensitivity	% of Specificity
1	ESAT-6	20(7/35)	90(4/39)
2	CFP-10	29.5(10/35)	90(4/39)
3	Rv2204c	74.29(26/35)	94.87(2/39)
4	Rv0753c	68.5(24/35)	90(4/39)
5	Rv0009	74.29(26/35)	90(4/39)

Table 15: IFN-γ responses to Rv0009 antigen and its peptides

S.NO	Protein name	% of Sensitivity	% of Specificity
1	P1	45.71(16/35)	92.59(2/27)
2	P2	51.43(18/35)	92.59(2/27)
3	P3	48.57(17/35)	92.59(2/27)
4	P4	48.57(17/35)	92.59(2/27)
5	P5	40(14/35)	92.59(2/27)
6	P6	37.14(13/35)	92.59(2/27)
7	P7	51.43(18/35)	92.59(2/27)
8	P8	40(14/35)	92.59(2/27)
9	P9	37.14(13/35)	92.59(2/27)
10	P10	22.86(8/35)	92.59(2/27)
11	P11	54.29(19/35)	92.59(2/27)
12	P12	28.57(10/35)	92.59(2/27)
13	P13	34.29(12/35)	96.3(1/27)
14	P14	45.71(16/35)	92.59(3/39)
15	P15	37.14(13/35)	92.59(3/39)
16	P16	31.43(11/35)	100(0/39)
17	P17	45.71(16/35)	92.59(2/27)
18	P18	40(14/35)	96.3(1/27)
19	P19	31.43(11/35)	96.3(1/27)
20	PpiA	77.14(27/35)	89(3/27)

Conclusion: This preliminary study shows that the three antigens namely Rv2204c, Rv0753c, Rv0009 differentiated LTBI from active TB disease in terms of IFN-γ levels. However, the number of study subjects has to be increased, which may help to confirm whether these antigens can be used as biomarker for differential diagnosis of LTBI and PTB.

I-5: Protein Interaction studies

Principal investigator : Dr. Sujatha Narayanan
(email: sujathan@nirt.res.in)
Co-investigator : Mr.V. Arunkumar
Source of funding : ICMR Fellowship / ICMR-Intramural
Study period : 2012-2015

Background: Studies addressing protein-protein interactions continue to be our research priority. Inside the host, *M. tuberculosis* resists the oxidative and nitrogen stress by employing its antioxidant defence mechanism. Rv2159c is one of the

hypothetical proteins of *M. tuberculosis.* The closely related orthologs suggest that Rv2159c belongs to the family of alkylhydroperoxidases, AhpD family core containing domain. The protein has CMD domain in its structure, and appears to contain two cysteine residues Cys-X-X-Cys, responsible for peroxidase activity. Previous studies from our lab suggest that, PknI, a serine/threonine protein kinase of *M. tuberculosis* could interact with two novel proteins Rv2159c and Rv0148.

Objectives:

(i) to clone and over-express Rv2159c from *M. tuberculosis*

(ii) to confirm the PknI-Rv2159c protein interactions

Methods:

Cloning of Rv2159c:

The Rv2159c gene from *M. tuberculosis* was amplified using the following primers, FP: 5'CCC GGA TCC CCA GGA GGG AGT CGA ATC ATG AA3' and RP: 5'CCC GAA TTC GGC CAG CGA TGA CAC CCT ACC3', and cloned into *BamH1* and *EcoR1* sites of pGEX 4T-1 vector harbouring tac promoter and N-terminal glutathione-S-transferase (GST) tag.

Expression & purification of rRv2159c:

The *Rv2159c* cloned in pGEX 4T-1 vector was transformed into *E. coli* BL21 cells. The construct was then over-expressed in LB broth with 1µg/ml Carbenicillin (50mg/ml) and 100mM of IPTG was added as an inducer. The cells were grown at 37oC and pelleted down by centrifugation at 8,000 rpm for 10 min at 4°C.

The over-expressed Rv2159c cells were sonicated using Buffer A (50mM Tris chloride pH:8, 300mM Nacl, 20% Glycerol and 0.5mM PMSF) to extract the cytoplasmic and crude membrane proteins. The resultant crude cytoplasmic proteins were purified using GST affinity chromatography and the GST-tagged recombinant Rv2159c protein was eluted using buffer A containing 20mM reduced glutathione.

Protein-protein Interaction assays:

(i) Far-western blotting:

To investigate the interaction between PknI and Rv2159c using Far-western blotting, the PknI was separated by SDS-PAGE as prey protein and transferred to the PVDF membrane. After denaturation and renaturation of the PVDF membrane with AC buffer (5M Nacl, 1M Tris, pH:7.5, 0.5M EDTA, 10% Tween-20, 8M Guanidine-HCL, Skim milk powder, Glycerol and 1M DTT), the Rv2159c was incubated onto the antibody and developed using enhanced chemiluminescence.

PknI-Rv2159c complex was eluted using buffer A with 200mM imidazole. Eluted proteins were separated by SDS-PAGE and immunoblotted using anti-His and anti-GST antibody respectively.

Results:

The Rv2159c gene was successfully cloned and expressed in pGEX 4T-1 vector (Fig.18). Rv2159c protein was purified using affinity chromatography and the resolution was obtained in SDS-PAGE and confirmed with western blotting. Rv2159c protein was purified at 65kDa and correlates with its molecular weight (Figs.19a & b).

PknI interacts with Rv2159c:

To verify whether PknI and Rv2159c can interact directly *in vitro*, a far-western blot assay was used with purified full-length His-PknI and GST-Rv2159c fusion proteins. The results showed that PknI could bind to Rv2159c, but the control could not.

Moreover, absence of the non-specific protein around ~54kDa in the Far-western blot showed the specificity of the interaction (Fig.20).

The pull-down technique detects physical interactions between proteins directly; as a result, it is a useful tool in the confirmation of protein-protein interactions predicted by other techniques. Here, pull-down assay with equimolar concentration of the two proteins were used to confirm interactions between PknI and Rv2159c. Immunoblotting with anti-His and anti-GST antibodies showed that the His-tagged PknI could pull down GST tagged Rv2159c (Figs.21a&b). Moreover, reaction with Rv2159c protein alone could not be pulled down with Ni-NTA resin, thereby confirming that the interaction was specific.

Conclusion: The interaction between PknI and Rv2159c has been confirmed.

Fig.18: Rv2159c gene cloning

Agarose gel electrophoresis of digested clone of Rv2159c. Lane 1 and 4: 100bp and 1kb ladder respectively.
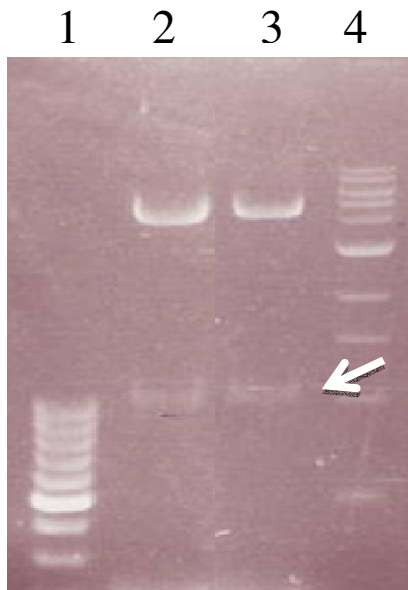
Figs.19a & b: Expression & purification of rRv2159c

(a)

(b)
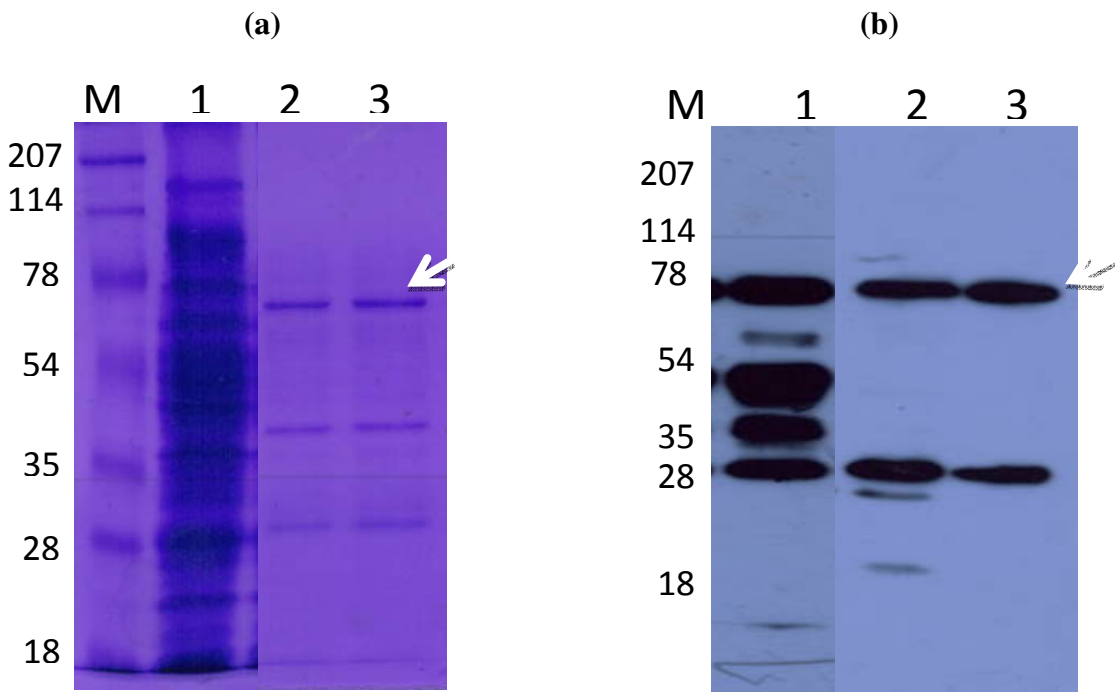
Lane M Marker; Lane1: Rv2159c crude lysate; Lane 2 &3 Rv2159c protein elution.

Fig. 20: PknI-Rv2159c protein interaction using far-western blotting
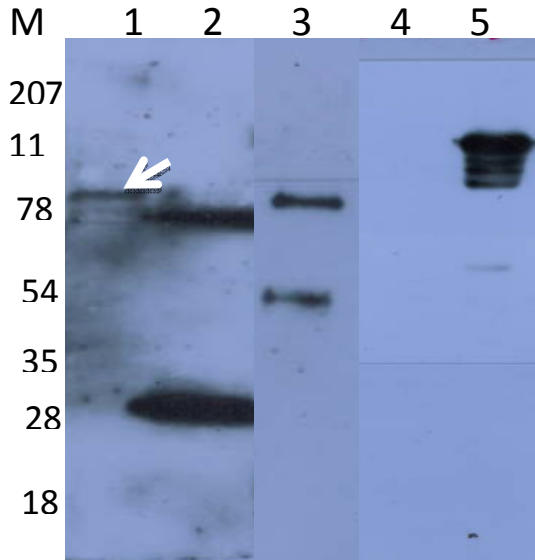
Lane 1 PknI prey protein vs Rv2159c bait – anti-GST antibody (FWB); Lane 2 Rv2159c protein western blotting with anti-GST antibody; Lane 3 PknI protein western blotting with anti-His antibody; Lane 4 Control protein vs Rv2159c bait – anti-GST antibody (FWB); Lane 5 Control protein western blotting with anti-His antibody; lane M Molecular weight marker.

Figs.21a & b: PknI-Rv2159c protein interaction using pull down assay
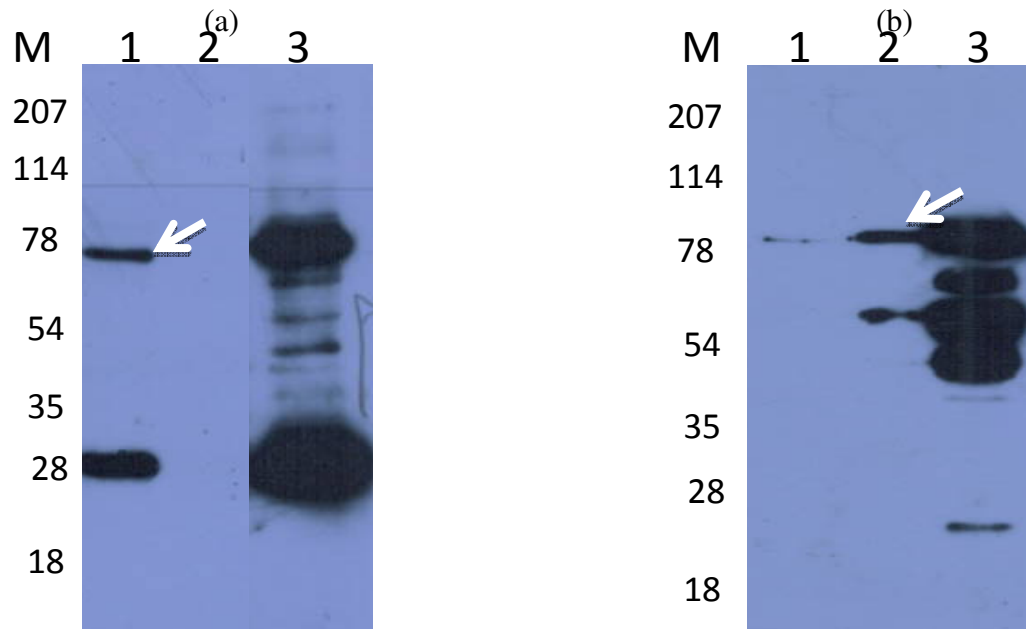
Immunoblots of Rv2159c pulled down by PknI. (a) Lane 1 Rv2159c vs PknI elution with anti-GST antibody; Lane 2 Control reaction, Rv2159c bounded with Ni-NTA resin; Lane 3 Purified Rv2159c; (b) Lane 1 Control reaction, Rv2159c bounded with Ni-NTA resin; Lane 2 PknI vs Rv2159c elution with anti-His antibody; Lane 3 Purified PknI.

I-6: Construction of *M. tuberculosis* double knockout strain lacking pknI and dacB2

Principal Investigator	:	Dr. Sujatha Narayanan
		(email: sujathan@nirt.res.in)
Source of funding	:	ICMR Fellowship / ICMR-Intramural
Study Period	:	2010- 2014

Background: The physiological roles of several mycobacterial STPKs and penicillin binding proteins (PBPs) are connected to cell division/shape and cell envelope biosynthesis but the exact mechanisms remain unexplored. It has been reported that, the pknI (STPK) and dacB2 (PBP) located in the same cluster is predicted to play a role in cell division and peptidoglycan biosynthesis. In our lab we have extensively characterized the PknI, a STPK and DacB2, a PBP independently. Both the PknI and DacB2 overexpressed strains caused similar phenotypes like growth retardation and altered colony morphological changes. With respect to virulence also both the PknI and DacB2 mutants exhibited hypervirulent phenotype. The genomic proximity and similarity of function of proteins DacB2 with PknI prompted us to construct the double knockout (DKO) strain of PknI and DacB2; this DKO will help us to understand the functional relationship between STPK and PBP of *M. tuberculosis*.

Aims: (i) to construct DKO strain of PknI and DacB2 (ΔPknI/ΔDacB2) using specialized transduction protocol

(ii) to construct DKO complement strain

Methods:

Construction of ΔPknIΔDacB2 double knockout:

The available single mutants of ΔPknI and ΔDacB2 were used for the construction of DKO strain. The DKO was constructed by single step homologous recombination using a specialized transducing phage system (Fig.22).

Fig.22: Construction of DKO by single step homologous recombination
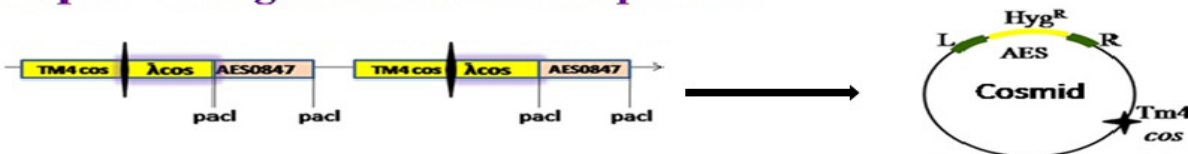
Hygromycin cassette was removed from ΔPknI using resolvase phage. High titer phage for DacB2 gene was prepared as follows: The DacB2 containing Allelic exchange substrate phasmid was electroporated into *M. smegmatis*. The electroporated phasmid gave plaques after 3 days incubation at 30°C. The plaques were purified and propagated into *M. smegmatis* for high titre phages. All the phage isolation and propagation were done at permissive temperature (30°C). Phage titre of 10^{10} or 10^{11} was used for transduction. The unmarked PknI (ΔIUM) H37Rv strain were grown to an OD_{600} of 0.8-1.0. 10^9 cells of ΔIUM were mixed with 10^{10} phages and transduction was performed at 37°C. The double knockout colonies were selected on Hygromycin containing 7H10 agar plate. The desired DKO phenotype was confirmed by PCR and southern Blot.

DKO complement strain construction:

The cloning experiments were done in *E.coli* XL-10 gold and DH5α strains. Initially, the PknI coding region along with 400 bp upstream sequences was PCR amplified using primers mentioned in Table 13. The PCR product was cloned into the ECoRI and HindIII site of PMV306 vector and named as pRG3. Similarly, the DacB2 (~900bp) coding region was PCR amplified using one pair of primers which had EcoRI overhang on 5' end and BamHI overhang on 3' end. The amplified DacB2 ligated into the downstream region of hsp60 promoter of PMV261 vector and this construct as pKS1. Ten individual colonies were randomly picked out and clones were confirmed by PCR amplification and restriction enzyme digestion.

All the PCR products were sequenced to make sure that it did not have any mutations.

Furthermore, the DacB2 along with hsp60 promoter region was removed from pKS1 and sub-cloned into the downstream region of pRG3. The clone was confirmed by restriction enzyme digestion and named as pPKS2 (Table 16). This final construct pPKS2 was electroporated into the *M. tuberculosis* DKO strain and named as DKO complemented strain.

Table 16: List of plasmids and primers for double knock-out construction

Plasmid/ Strain/ Primers	Description	Source
pMV261	*E. coli - Mycobacterium* shuttle vector carrying *hsp60* promoter; Kan^r	Dr.William.R. Jacobs (AECOM)
pMV306	*E. coli - Mycobacterium* integrative promoterless vector carrying Kan^r	Dr.William.R. Jacobs (AECOM)
pKS1	pMV261 harbouring 900bp DacB2 coding region with *hsp60* promoter; Kan^r	This study
pRG3	pMV306 harbouring 1758 bp PknI coding region with its 400 bp upstream region harbouuring the homologous promoter, Kan^r.	This study
pPKS2	pMV306 harbouring DacB2 with hsp60 promoter and PknI gene with homologus promoter	This study
Primers		
2911 BamH1 Fwd 261	CGGATCCG– ATG CGA AAG CTC ATG ACC GC	This study
2911 EcoRI Rev 261	CCG GAA TTC CGG - CTA GAG CGA GCC GAC GCT GG	This study
Rv2914c 306 Fwd	CCGGAATTCCGG- ACATGCGCGACCTGTATGCC	This study
Rv2914c 306 Rev	CCCAAGCTTGGG- TCGTTGAGTTGCCGCCAAGC	This study

Results:

a) **Construction of DKO:** The hygromycin cassette was removed from ΔPknI strain by using high titer resolvase phage. The unmarking of hygromycin cassette from ΔIUM was confirmed by PCR (Fig. 23) and sequencing analysis. The DacB2 gene was deleted in ΔIUM by transducting DacB2 high titer phage. The DacB2 deletion in ΔIUM was confirmed by PCR (Fig. 24) and southern blotting (Fig.25) and it was named as DKO.

b) **Construction of DKO complemented strain:** The PknI coding region was PCR amplified and cloned into pMV306 vector. The clone was confirmed by restriction enzyme digestion. Similarly, the DacB2 (~900bp) gene was PCR amplified and cloned into EcoRI and BamHI site of PMV261 vector. The clone was confirmed by restriction enzyme digestion. Furthermore, the DacB2 gene with hsp60 promoter region was removed from pKS1 and sub-cloned into the downstream of PknI gene. This construct was named as pKS2 and the clone was confirmed by restriction enzyme digestion. The pKS2 was electroporated into DKO strain and plated on antibiotic containing 7H10 agar plate. The kanamycin and hygromycin resistant colonies were picked and confirmed by PCR analysis (Fig.26).

Fig.23: PknI unmarking confirmation by PCR
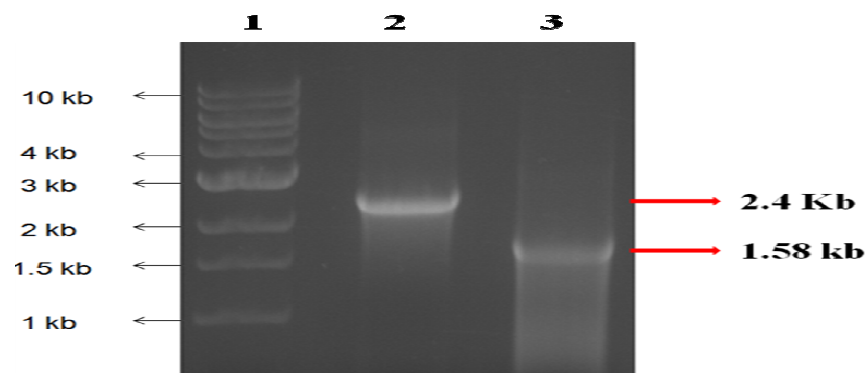
Lane 1: 1 Kb Ladder
Lane 2: 2.4 Kb PCR product amplified from wild type PknI genomic DNA
Lane 3: 1.58 Kb PCR product amplified from ΔIUM

Fig.24: DKO was confirmed by PCR
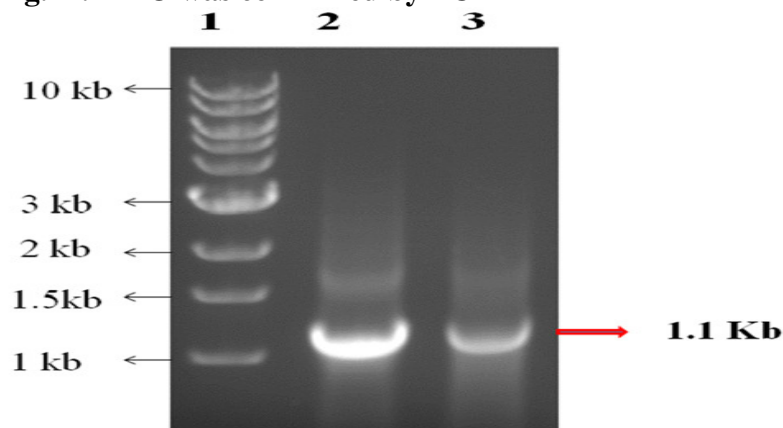
Lane 1: 1 kb ladder; Lane 2 & 3: DKO colony 1 and 2

Fig.25: DKO was confirmed by Southern blotting
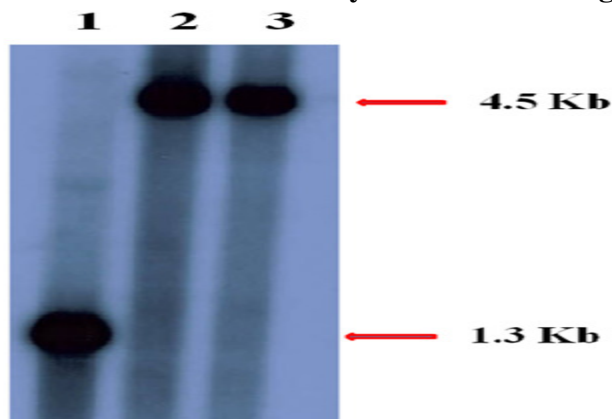
Lane 1: *M. tuberculosis* H37Rv wild type control DNA; Lane 2 & 3: DKO colony 1 & 2

Fig.26: DKO complement confirmation
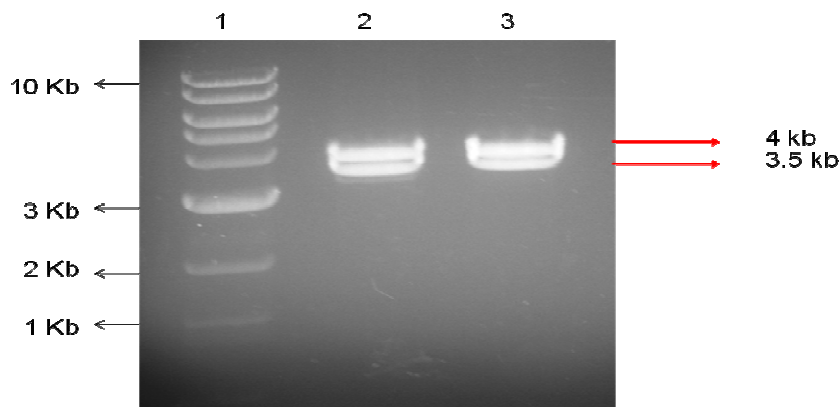
Lane 1: 1 Kb ladder; Lane 2 & 3: pKS2 RED showed that PknI and DacB2 insert (3.5 Kb) released from pMV306 vector (4 Kb).

Conclusion: For the first time in India, we successfully created a *M. tuberculosis* double knockout strain which lacks PknI and DacB2 genes. We also constructed a DKO complemented strain; it was included in all the experiments as to confirm whether the changes are due to the double deletion. Further, we are planning to characterize the phenotypic characteristics as well as virulence of DKO strain in comparison with wild type *M. tuberculosis* H37Rv strain.

I-7: Characterization of *M. tuberculosis* double knockout strain lacking PknI and DacB2

Principal Investigator : Dr. Sujatha Narayanan
                            (email: sujathan@nirt.res.in)
Source of funding : Intramural
Study Period : 2010- 2014

Background: In the earlier project, we created *M. tuberculosis* DKO strain in our lab which is lacking PknI and DacB2 genes. In this project, we characterized DKO phenotypic characteristics by different methods. Initially, we carried out *in vitro* growth kinetics of DKO strains in enriched growth (7H9) and/or nutrient minimal (Sauton's) media under different stress conditions such as acidic pH low oxygen availability conditions. It has been predicted that both PknI and DacB2 has a role in maintenance of colony morphology, hence we analysed colony morphology by spotting early log-phase culture on 7H10 agar plate. We also tested the PknI and DacB2 role in colony morphology by analyzing biofilm formation capacity.

Aims:

(i) to analyze the *in vitro* growth kinetics of DKO strain

(ii) to analyze the colony morphology, clump and biofilm formation

Methods:

(i) *In vitro* growth kinetics:

To determine the *in-vitro* growth characteristics, log-phase cultures grown in 7H9-ADS-tween 80 medium were washed thrice and diluted in different growth media to be tested. The growth of wild type and knockout strains were analyzed in two different media such as normal 7H9 and Sauton's medium which is a nutrient depletion media supplemented with 0.05% Tween 80 at pH 7.0 as well as pH 5.5. All the experiments were performed in duplicate at olling and standing culture (low oxygen availability) conditions. Aliquots of 1mL were taken from the cultures for OD600 nm readings at specified time points as indicated.

(ii) Colony morphology analysis:

All bacterial strains were grown to mid log phase and adjusted to an optical density of 0.05. An aliquot of culture was dropped in to 7H10 and 7H11 agar plates. The plates were incubated for 3-4 weeks.

(iii) Biofilm analysis:

M. tuberculosis biofilms were generated by modifying published protocols in either 12-well polystyrene plates or petri plates. Cells

from a 7H9 aerated culture (OD_{600} ~1) were diluted 1:100 (v:v) in Sauton's medium excluding detergent and added to each well as well as petri plates. The plates were wrapped twice in parafilm and incubated at 37° C for 5 weeks without shaking.

Crystal violet staining was carried out according to methods previously described. The medium was removed from wells by pipetting underneath the biofilm at the interface. Biofilms were dried in a biosafety cabinet and incubated with 500ul of 1% crystal violet for 10 minutes. Wells were washed three times with water and dried again. One milliliter of 95% ethanol was added to each well for 10 minutes. Then, 2-fold serial dilutions were made and read at an absorbance at 600nm on a spectro-photometer.

Results:

(i)   Growth kinetics:

In the standard 7H9 growth medium, the growth of DKO was not altered at normal pH both at shaking and standing conditions. But, DKO showed growth retardation at pH 5.5 in both conditions. The PknI single mutant growth was high at acidic and standing conditions but there was no change in other conditions. Similarly, the DacB2 mutant did not show any differences in growth in this media.

In the Sauton's nutrient minimal media, the growth of DKO was reduced in both normal and acidic pH and also in shaking and standing conditions. The PknI single mutant growth was high at Ph 5.5 and standing conditions as like standard media, it did not show any difference in other conditions. But the growth of DacB2 single mutant was reduced at pH 5.5 under standing conditions alone and it did not show any difference in other conditions (Fig.27).

(ii)   DKO morphology on solid agar:

The morphology of DKO strain was analyzed by spotting 5 µl of culture on 7H10 +OADC containing plate. The DKO showed smoother morphology when compared to the wild type strain. The single mutants of PknI and DacB2 did not show any difference (Fig.28).

(iii)   Biofilm analysis:

The wild type *M. tuberculosis* H37Rv strain showed thick biofilms and it covered entire plate but the DKO strain was defective in biofilm formation.

Conclusion: Both PknI and DacB2 play a role in maintenance of cell division under stress conditions and also in normal colony morphology.
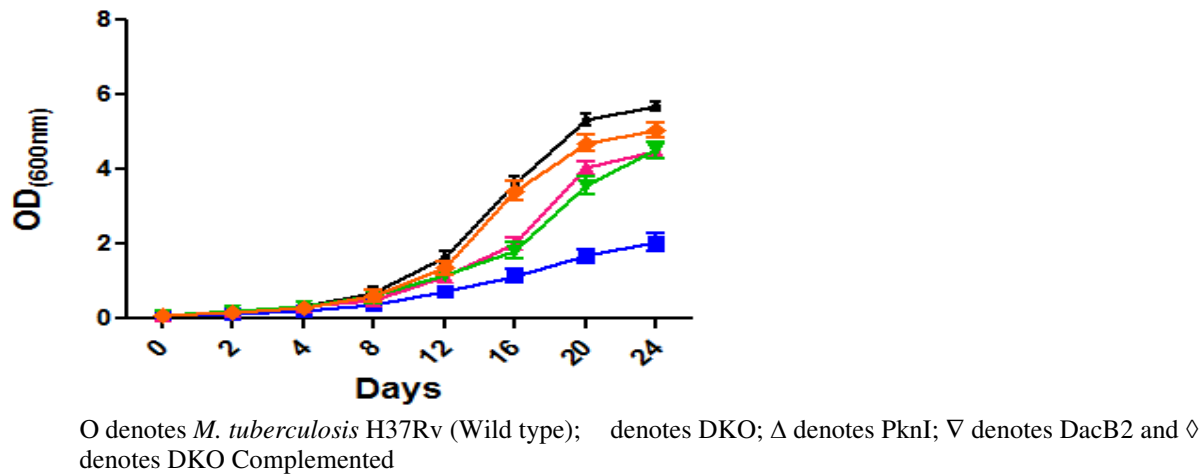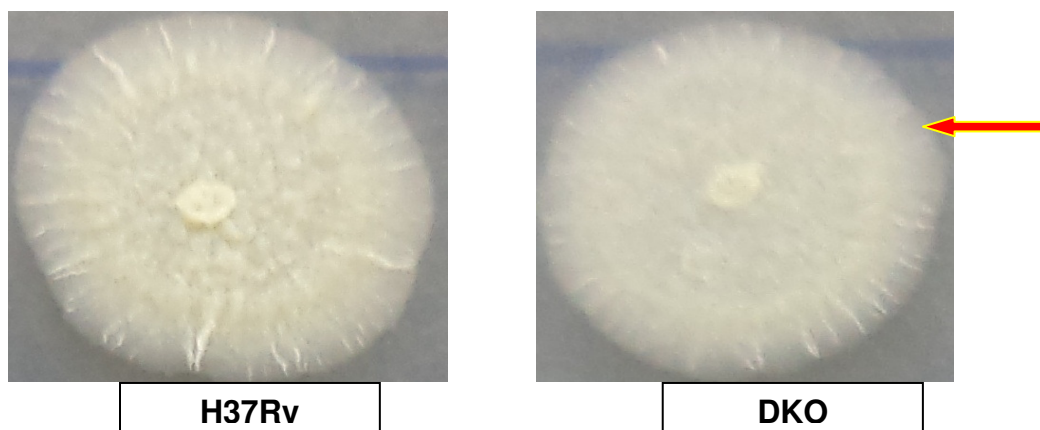
Fig.27: *In vitro* growth kinetics

O denotes *M. tuberculosis* H37Rv (Wild type);   denotes DKO; Δ denotes PknI; ∇ denotes DacB2 and ◊ denotes DKO Complemented

Fig.28: Colony morphology analysis on 7H10 agar plate

I-8: **Vitamin D receptor gene polymorphisms and sputum conversion during anti-TB treatment**

Background: Vitamin D_3, a potential immunomodulator, is known to influence innate and adaptive immunity. Vitamin D_3 exerts its activity through VDR, a nuclear hormone receptor. VDR gene variants have been

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associated with altered VDR expression as well as with susceptibility or resistance to TB. Polymorphic variants of VDR gene have been shown to be associated with faster sputum conversion during anti-TB treatment.

The present study was planned to understand the role of various VDR gene polymorphisms on sputum mycobacterial culture conversions during anti-TB treatment in south Indian PTB patients.

Aim: To find out whether vitamin D receptor gene polymorphisms are associated with sputum mycobacterial smear / culture conversion during anti-TB treatment and treatment outcome

Experimental Design: Two milliliter blood sample in anti-coagulant is collected from sputum positive PTB patients receiving standard anti–TB treatment ($2EHRZ_3/ 4HR_3$). DNA is extracted from the white cells and used for assessing Vitamin D receptor gene polymorphisms. VDR gene polymorphisms in the 5′ regulatory region (Cdx2 and A-1012G), coding region (FokI), and 3′ untranslated region (UTR) BsmI, ApaI, and TaqI) is carried out. Data available on sputum mycobacterial culture conversion during anti-TB treatment will be correlated with the data on allele and genotype frequencies of VDR gene polymorphisms to find out the role on sputum conversion during anti-TB treatment.

Two hundred patients who received anti-TB treatment with the standard TB treatment regimen ($2EHRZ_3$ / $4HR_3$) from the clinical trials in NIRT whose sputum status (smear and culture status) for TB are available for the time points - pretreatment, 15 days, 30, 45, 60, 90, 120, 150, and 180 days of treatment are included for this study. So far 51 patients have been studied. The study is in progress.

I-9: Cytokine gene polymorphisms in HIV and HIV-TB

Principal Investigator	:	Dr.P. Selvaraj (email: selvarajp@nirt.res.in)
Source of funding	:	Intramural
Study Period	:	2014-2017

Background: HIV -1 infection has increased the burden of TB, especially in populations where the prevalence of TB is high among young adults. Host genetic factors have been suggested to serve as genetic markers to find out susceptibility or resistance of the host to various infectious and non-infectious diseases. Numerous studies have emphasized the role of host genetic factors (HLA and non-HLA genes) on susceptibility or resistance to HIV and HIV-TB.

Studies on cytokine gene polymorphisms in HIV infected individuals with TB are meagre in Indian population. In the present study, various cytokine gene polymorphisms are carried out in HIV and HIV-TB co-infection in south Indian population. This study is carried out in DNA samples collected earlier for the ICMR Task Force project.

Aim: To find out whether cytokine gene polymorphic variants are associated with susceptibility or resistance to HIV and HIV-TB in south Indian population

Methodology:

Study subjects: The study population consists of:

1. HIV-1 seropositive patients without TB (HIV+TB-), (n= 150)

2. HIV-1 seropositive patients with TB (HIV+TB+), (n=115)

3. HIV-1 seronegative patients with PTB (HIV-PTB+) (n=150)

4. Healthy controls (n=150)

The following cytokine gene polymorphisms will be studied using the stored DNA samples extracted from the patients and controls.

1) Tumour Necrosis Factor (TNF)-α (TNF-α -308 G/A) and TNF-β (TNF-β intron2/exon3).

2) Interleukin (IL)-10, (IL-10 -1082 A/G and -819 C/T and -592 C/A).

3) IL-12, (IL-12A 3' UTR G/A and 5' UTR T/G, IL-12B 3' UTR+1188 A/C)

4) IL-2, (IL-2 -330 T/G and +166G/T)

5) IL-4, (IL-4 5'UTR -33C/T, promoter region of IL-4 -589 C/T)

6) Interferon (IFN)-γ (IFN-γ +874A/T and 3' UTR 5644A/G)

7) IFN- γ inducible protein-10 (IP-10), (IP-10 -1596 C/T, -1447 A/G and -135 G/A)

8) IL-2, (IL-2 -330 T/G and +166G/T)

The study has been initiated.

DEPARTMENT OF STATISTICS

STUDIES COMPLETED:

(i)   Tuberculosis disease classification using genetic-neuro hybrid system

Principal Investigator   :   Dr. P. Venkatesan
                                           (email: venkatesanp@nirt.res.in)
Study period   :   2012-2014

Introduction: Machine learning algorithms offer a principled approach for developing sophisticated, automatic and objective algorithms for analysis of high dimensional multi-modal biomedical data. Machine learning, a branch of artificial intelligence is about the construction and study of models that derive information from data. A machine learning model could be trained on the data set to learn to distinguish the different patterns of the data sets which then can be used to classify a similar data. This study investigated the application of the hybrid technique of Genetic-Neuro approach for TB disease classification.

Methodology: Evolutionary algorithms are proved to be the efficient methods for optimization problems and their primary component namely genetic algorithm was used to select the significant features for disease classification. Artificial neural network was used for classification and training was done by methods like Levenberg Marquardt algorithm. The database had 539 patients each with 9 features –age, gender, marital status, place of living, literacy level, family income, history of tuberculosis in the family, habits such as smoking and drinking and food habits and tuberculosis (yes/no). In this study a multi-layer neural network structure with one hidden layer was developed for classification purpose, considering all the 9 features.

Results: The results are given in Table 17. The results reveal that the hybrid technique of Genetic-Neural system outperforms the conventional technique of Artificial Neural Network for disease classification.

Table 17: Classification accuracy

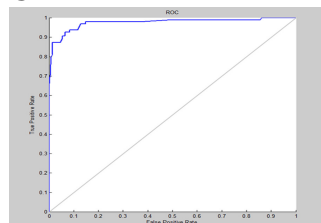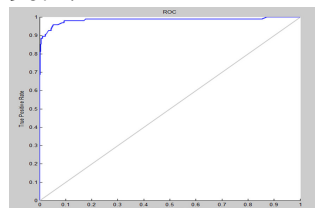| Approach	Training	Validation	Testing
ANN	93.9%	86.4%	86.4%
GA	94.2%	90.1%	96.3%

Fig.1. ROC for ANN

Fig.2. ROC for GA-ANN

(ii)   Bayesian random effects model for disease mapping of relative risks

Principal Investigator   :   Dr. P. Venkatesan
(email: venkatesanp@nirt.res.in)
Study period   :   2011-2013

Introduction: The applications of Bayesian methods for disease mapping, risk assessment and prediction within spatial research are numerous. There are two dominant approaches- empirical and fully Bayesian method. In the Empirical Bayesian (EB) method, parameters of prior distributions are estimated using observed marginal distributions, but in the fully Bayesian approach, the prior and posterior distributions are obtained via Markov Chain Monte Carlo (MCMC) computations.

Disease mapping is useful to find the geographical distribution of disease burden and diseases incidence.

Methodology: Bayesian methods for disease mapping of HIV in Chennai ward using Poisson-gamma model and log-normal model was explored. The aim was to compare whether Bayesian estimates of random effects model of log normal model are more stable than the Poisson gamma model estimates.

Results: The result of the study reveals that the random effects model, gives better smoother values of relative risk than the Poisson gamma model. The results are given in Figs. 29-31.

Conclusion: Spatial analysis is proved to be more useful for studying spread of HIV analysis.

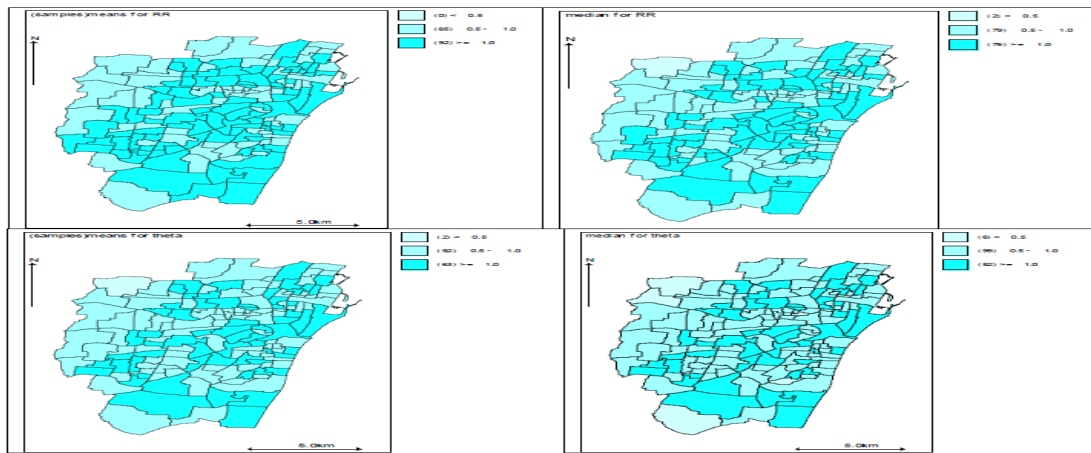**Fig. 29: Posterior expected values under Poisson gamma model**

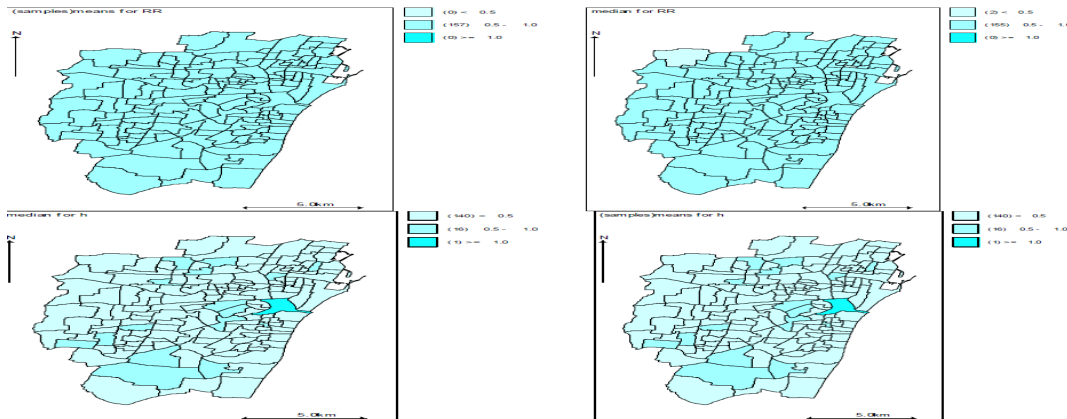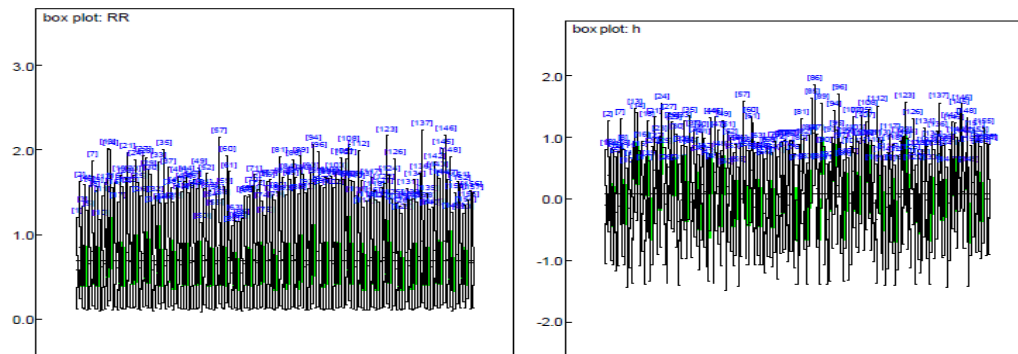**Fig. 30: Posterior expected values under lognormal model**

Fig. 31: Box plot for RR and residuals using lognormal model

STUDIES IN PROGRESS:

S-1:   Genetic algorithm based approach for comparative docking analysis

Principal Investigator   :   Dr. P. Venkatesan
                                    (email: venkatesanp@nirt.res.in)
Study period   :   2012-2016

Introduction: In recent years, the demands on drug discovery process have increased dramatically, partly because of the necessity to recognize novel targets that are both pertinent to disease and chemically tractable. The emergence of bioinformatics gives room to investigate diseases at the molecular level using computational techniques. The key protein breast cancer type 1 susceptibility (BRCA1), which upon mutation, plays a major role in cyst formation and carriers have a 4-fold increased risk of colon cancer and increased chance of bilateral cancers.

Materials and Methods: Curcumin, is one of the biologically active photochemical compounds which acts as a target, for treatment of number of diseases including cancer. In this study, docking scores and protein-ligand interactions are obtained using genetic algorithm, local search algorithm and simulated annealing algorithm for molecular docking with curcumin.

Results: Among the docking scores and interactions obtained using genetic algorithm, curcumin is indicated as a potential and natural therapeutic agent to combat breast cancer than the other algorithms. The efficiency of genetic algorithm relative to simulated annealing algorithm and local search algorithm are given in Figs. 32 and 33 and Table 18. The

genetic algorithm shows the better results compared to other two algorithms. Also the efficacy of genetic algorithm is authenticated in molecular docking by an attempt to dock aspirin and ibuprofen to cyclooxygenase 2. Further studies are needed to confirm these findings.

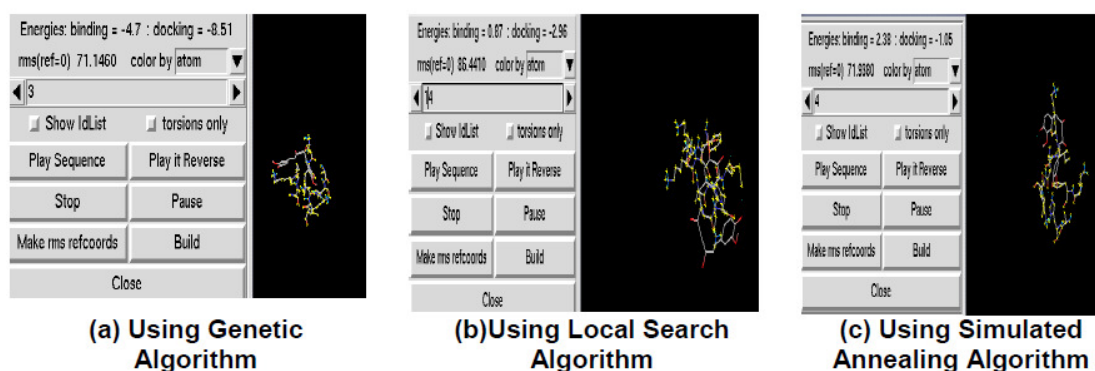**Fig. 32: Docking score for BRCA1 against Curcumin**

(a) Using Genetic Algorithm

(b) Using Local Search Algorithm

(c) Using Simulated Annealing Algorithm

Table 18: Comparison of docked interactions

Algorithms	BRCA1		Curcumin	Distance (A^0)	H-BONDS	Docked energy (kcal/mol)
	Residue	Atom				
Genetic algorithms	HIS1672	HE2	O	2.43594	2	-8.51
	LYS1667	HZ1	O	2.49698		
Local search algorithm	LEU1676	HN	O	1.91277	1	-2.96
Simulated annealing algorithm	-	-	-	-	0	-

Fig. 33: Docked conformation using (a) Genetic Algorithm, (b) Simulated Annealing and (c) Local Search Algorithm

(a) H-Bond distance

(b) H-Bond distance

(c) H-Bond distance
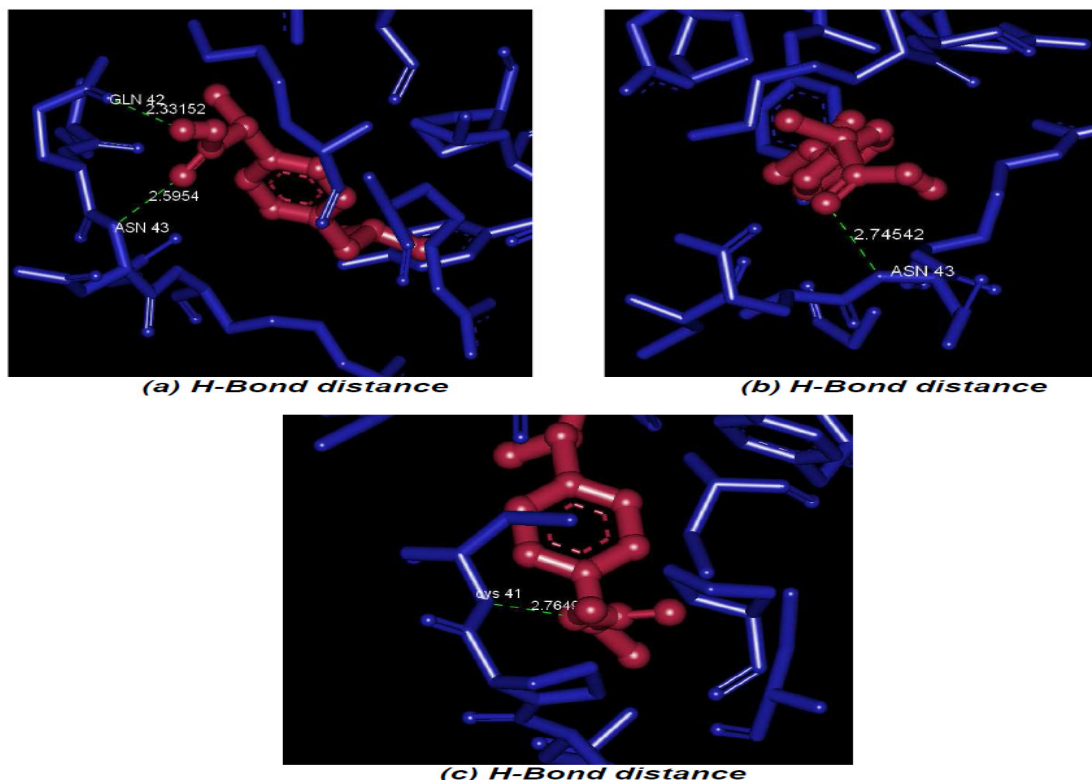
S-2: **SOM component planes in visualization of high dimensional databases**

Principal Investigator : Dr. P. Venkatesan
(email: venkatesanp@nirt.res.in)
Study period : 2012-2016

Introduction: Artificial Neural Network is capable of handling data exploration efficiently and effectively, and hence enhances the decision making in the medical field when used for classification, clustering, pattern recognition and prediction. Data mining is 'non-trivial extraction of implicit, unknown and potentially useful information. Its aim is to search through the data for its hidden features and other relations. Scatter

plot is one of the traditional techniques to identify the dependencies or the relations between the variables. But this technique does not help for high dimensional data exploration. Self Organizing Maps (SOM) based visualization technique such as component planes helps to find the correlating attributes. A self-organizing map consists of components called nodes or neurons. SOM describes mapping from a higher dimensional input space to a lower dimensional map space, usually two dimensional. SOM combines the features of vector quantization and vector projection.

Materials and Methods: SOM is trained using unsupervised learning to produce a low-dimensional (typically two-dimensional), discretized representation of the input space of the training samples, and called a Map. SOM is different from other artificial neural networks in the sense that they use neighbourhood function to preserve the topological properties of the input space. SOM is organized so that similar data are mapped on to the same node or to the neighbouring nodes and thereby similar input patterns are spatially clustered and get organized themselves. Breast cancer data obtained from UCI -Machine Learning Repository is used to illustrate the methodology. Data consists of details of 699 individuals tested for breast cancer based on 32 real valued features contributing the disease attributes and nine important features, *viz,* radius, texture, perimeter, area, smoothness, compactness, concavity, concave points and symmetry. The component planes for the attributes are obtained for both actual and normalized data with Gaussian neighborhood and bubble kernel. SOM_PAK (version 3.1) has been used in Matlab (version 7.9.0.529-R2009b) for the purpose. Correlation co-efficient values are obtained with the help of discovery SOMine 5 software. Map is trained using batch algorithm.

Results: The results are given in Figs. 34, 35, 36.

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Fig.34: Component planes (Actual Data) with gaussian neighbourhood
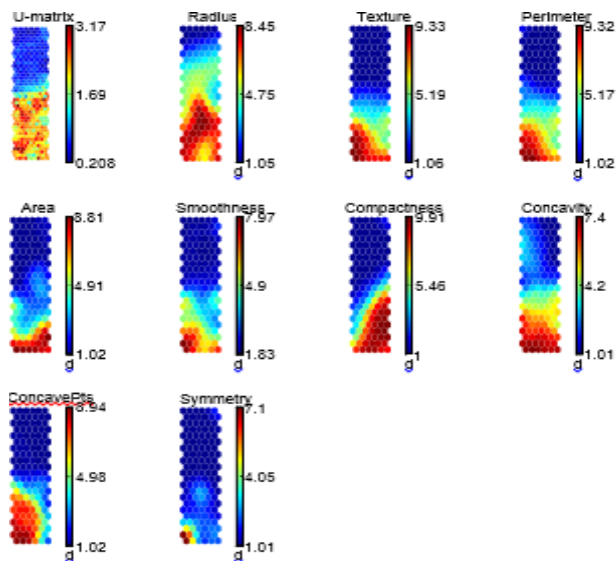

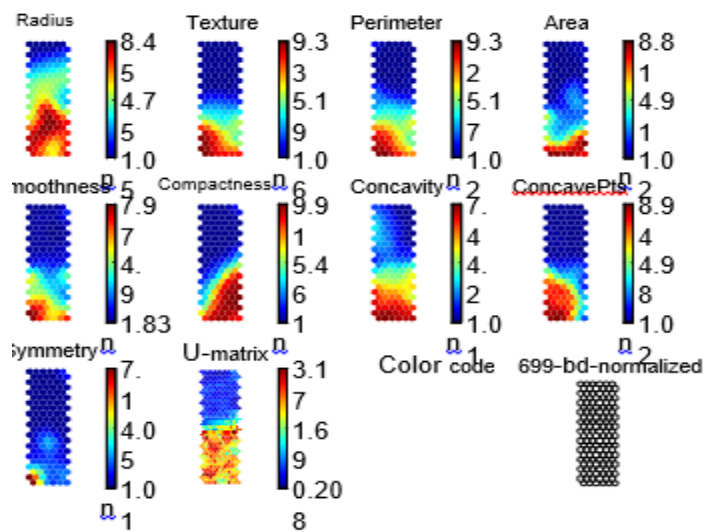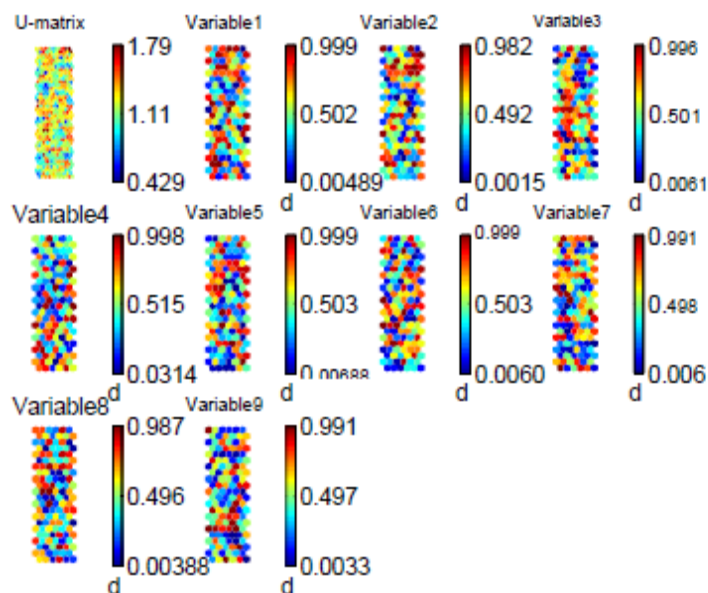**Fig. 35: Component planes (normalized data) with Gaussian neighbourhood**

Fig. 36: Component planes (normalized data) with Bubble neighbourhood final quantization error

Conclusion: The component plane presentation of integrated SOM is a powerful tool for exploring, large, complex, biological databases. This approach allows the display of multi-dimensional SOM outputs disease databases in multiple sample specific presentations providing distinct advantages in visual inspection of biological significance of features clustered in each unit for further analysis.

S-3: **Rough set theory based feature extraction for disease prediction**

Principal Investigator   :   Dr. P. Venkatesan
                                (email: venkatesanp@nirt.res.in)
Study period   :   2012-2016

Introduction: One of the fundamental steps in classifier design is reduction of pattern dimensionality through feature extraction and feature selection. Feature selection is often isolated as a separate step in the processing of pattern sets. This work presents rough sets methods for feature selection in high dimensional databases in the context of feature selection in pattern classification.

Materials and Methods: An approach based on the rough set theory and induction of decision rules is applied to analyses relationships between condition attributes describing breast cancer and their treatment results. The data set contains breast cancer described by four attributes and is divided into two classes: the 1st class – who had not experienced cancer recurrence; the 2nd class - who had cancer recurrence. In the first phase of analysis, the rough sets based approach was applied to determine attribute importance for the classification. The set of selected attributes, which ensured high quality of the classification, was obtained. Then, the decision rules were generated by means of the algorithm inducting the minimal cover of the learning examples. The data base consisted of 228 breast cancer patients (all women after mastectomy) consisting of 162 patients who had no recurrence of cancer (class 1) and 66 patients who had cancer recurrence (class 2)

(Wielkopolska Oncology Center). This situation of unbalanced classes often occurs in medical data and requires particular attention during the analysis. The conditional attributes were age, number of parturition, breast cancer in the 1st or 2nd generation and tumor size. The breast cancer information system was analysed using the rough set theory-creating classes of indiscernibility relations and building approximations of the objects' classification, evaluating the ability of attributes to approximate the objects' classification; the measure of the quality of approximation of the classification, defined as the ratio of the number of objects in the lower approximations to the total number of objects, examining the significance of attributes by observing changes in the quality of approximation of the classification caused by removing or adding given attributes.

Results: The number of classes of indiscernible objects was equal to 204. The majority of them were single element attributes. The approximations of decision classes were created and their accuracies were calculated. The minimum set of rules were induced using the algorithm LEM2

(considering all 16 attributes) and obtained set contained 60 rules. A total of 72 decision rules were generated including 3 nondeterministic ones (i.e. 35 rules for class 1 and 34 rules for class 2). Ten-fold cross-validation evaluation was performed for the reduced and non reduced information

system. The mean accuracy of classification is determined. The classification accuracies in both information systems are similar. The results are presented in the Table 19 and Figs. 37, 38.

Table 19: Rough approximation of Cancer data

Rough Approxi.	Class I (Non Recurrence)	Class 2 (Recurrence)	All
No. of patients	162	66	228
Lower approxi.	160	65	225
Upper approxi.	163	68	231
Accuracy (%)	98.8	98.4	98.7

Fig. 37: Reduced system
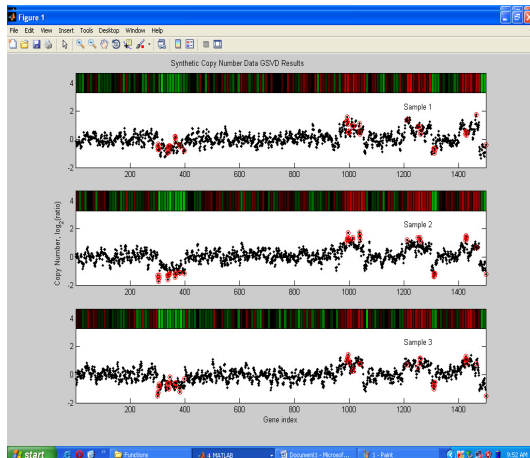
Fig. 38: Non-reduced system
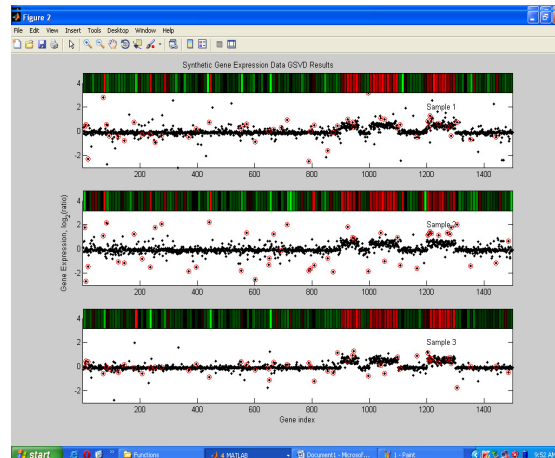
Conclusion: This kind of knowledge representation is easy for human inspection, interaction and clinical interpretation. The classification performance of induced rules (both for all and selected attributes) is comparable with results of using other learning systems.

DEPARTMENT OF EPIDEMIOLOGY

STUDIES COMPLETED:

(i)   Prevalence of pulmonary disease in homeless people of Chennai

Principal Investigator	:	Dr.C.K. Dolla
		(email: chandrakumar.d@nirt.res.in)
Source of funding	:	Intramural
Study period	:	2013

A pilot study was initiated to determine the prevalence of chest symptomatics or occurrence of TB among the homeless people in Chennai. Among these people, those above 15 years of age were X-rayed and queried about the occurrence of chest symptoms such as, cough for >2 weeks, chest pain for more than a month, fever for more than a month and haemoptysis. From those found having abnormal x-rays and with any of the above chest symptoms, two sputum samples were collected and were processed for TB bacilli through smear and culture examination.

Results: A total of 301 homeless individuals living on the streets were screened. Blood glucose and blood pressure tests were done for persons above 35 years.

The prevalence of TB among the homeless was 17 per 1000. A well designed larger study would be necessary to estimate the prevalence of TB among the homeless people in Chennai.

(ii)   Screening of inmates and prison guards for TB (May 2013 to Dec.2013) Central Jail, Puzhal, Chennai

Principal Investigator	:	Dr.C.K. Dolla
		(email: chandrakumar.d@nirt.res.in)
Source of funding	:	Intramural
Study period	:	2013

Introduction: In consultation and with approval of IG Prisons, Chennai, it was decided to screen all the inmates and staff of Puzal central prison. The exercise was intiated in May 2013. *M. tuberculosis*, the organism that causes TB, is transmitted

through airborne respiratory droplets when an individual with active pulmonary TB coughs, sneezes, speaks, or sings. Transmission of *M. tuberculosis* depends on the length of time and frequency of the exposure, the degree of contagiousness of the infected person, the environment and airflow in which the exposure occurred, and intensity of the contact with the TB organism itself. Infection with *M. tuberculosis* usually requires prolonged contact with an infectious case in an enclosed space. Correctional facilities have often been cited as reservoirs for TB, presenting a potential threat to the general population, and to public health.

Studies from other countries have, shown that the prevalence of TB in prisons is higher, than in the general population. Mortality rates for TB among prisoners are high. Released prisoners, as well as prison staff and visitors are also at higher risk for TB.

Screening: The inmates of the Puzhal prison, in-house staff and their family members were screened by X –ray and for TB symptoms. An informed written consent was obtained before screening from each inmate and staff. Symptomatic persons and those with abnormal radiography and those who had history of previous TB treatment were asked to provide sputum samples, which was processed and examined for *M. tuberculosis* by smear and culture by standard methods. In addition, hematology, liver and kidney function tests and blood sugar tests were performed for individuals $\geq$ 35 years.

Results: The study findings are shown in Table 20. A total of 2292 individuals (Convicts- 23%, Remand prisoners-58% and In-house staff and their family-19%) were recruited. More than 90 percent were males.

Table 20: Details of demography, clinical status and symptoms of TB and its positivity among the persons examined in the Puzhal prison, Chennai, Tamil Nadu.

Characteristics	Convicts (n=529)		Remand Prisoners (n=1325)		In-house Staff and family (n=438)	
	Number	%	Number	%	Number	%
Gender						
Females	33	6	92	7	65	15
Males	496	94	1233	93	373	85
Age (in years)						
15-25	16	3	329	25	151	34
26-35	158	30	548	41	123	28
36-45	221	42	260	20	70	16
45-55	79	15	133	10	80	18
>=56	55	10	55	4	14	3
Smoking						
Number responded	515	97	1192	90	433	99
Smoking	331	64	805	68	107	25
Alcohol						
Number responded	514	97	1191	90	431	98
Alcohol	276	54	811	68	157	36
Symptomatics						
Cough >2 weeks	87	16	227	17	25	6
Chest pain	47	9	204	15	7	2
Fever > 2 weeks	1	0	0	0	0	0
Haemoptysis	34	6	55	4	4	1
History of previous TB treatment						
Number who have taken TB treatment in past	30	5.7	31	2	11	2.5
Blood pressure						
Total assessed	527	100	1323	100	435	99
Hypertension (Systolic - >=140 and Diastolic - >=90)	26	5	56	4	52	12
Sputum						
Positive for smear and or culture	5		10		2	

Out of 17 cases of TB, 5 were among convicts, 10 were among remand prisoners and 2 among staff. DST results revealed one case to be drug resistant among the convicts. The rate was 10 per 1000 inmates among convicts, 8 per 1000 inmates among remand prisoners and over all rates for both groups combined was 8 per 1000. This rate is more than that observed in the general population, which is 3.41 per 1000 in Chennai.

Of the 17 patients, three were already on treatment. We followed those who had started on treatment and found them to be regular. One person who went on bail from the remand prison could not be traced.

Based on these findings, the following recommendations were submitted to the prison authorities.

1. Need of TB screening at entry:

Symptomatic screening and X-ray (preferred) should be done for all inmates at entry. If abnormal, it can be referred to Medical Officer.

2. Medical Officer in each Prison:

Medical officer is mandatory to identify the suspected cases, referring them for further investigation and to give proper guidance about treatment.

3. Diagnostic facilities:

A binocular microscope with a well-trained Lab Technician is needed. They should be trained in smear microscopy.

4. Isolation ward:

Suspected and infectious persons must be isolated in a separate ward till the disease is confirmed to avoid transmission.

5. Volunteers from each block:

Suitable inmates can be identified and trained by the RNTCP Staff or NIRT. They can play the following vital roles in the prevention and treatment of TB.

- DOT provider
- Counsellor
- Intensive search for symptomatics
- Recognise and report about the side effects of the drug.

6. Health Education:

Health Education can be done once in week for each group, by standard education materials.

7. Follow up:

The infected inmates are to be motivated to follow and complete the treatment. If they are going to be released on bail or on completion of sentence, they must be counselled to continue TB treatment. Treatment compliance must be ensured.

8. Hypertension:

Medical treatment along with yoga and counselling can be provided for the staff.

9. Diabetes and Pre-diabetes:

Appropriate treatment should be given for the control of blood sugar including advice on diet control and regular monitoring.

Salient findings:

1. A high rate of TB was found in Puzhal, compared to the general population Chennai city.

2. Remand prison has higher rate of TB than general population of Chennai city, which is a mobile group compared to convicts.

3. A significant number of people in inmates had elevated blood pressure and diabetes mellitus.

4. Prison staff working also had higher rate of hypertension and TB.

STUDIES IN PROGRESS:

E-1: Survival rate due to TB disease: a study among TB patients treated under Revised National TB Control Programme in Tiruvallur district of Tamilnadu

Principal Investigator	:	Dr. V. Chandrasekaran
		(email: chandrasekaranv@nirt.res.in)
Source of fundnig	:	USAID (MDP)
Study Period	:	March 2014 - September 2015

TB remains an important cause of death from an infectious agent, second only to HIV. TB control is high on the international public health agenda, not only because of the enormous burden of disease, but also because short-course chemotherapy (SCC) is recognized as one of the most cost-effective health interventions. TB mortality has decreased from over 42/100,000 population in 1990 to 24/100,000 population in 2008 as per the WHO Global TB Control-updated 2009 Report. However, there is a need to understand the longevity of these patients after their TB is cured.

Aims:
(i) to compare the survival rate of successfully treated TB patients with Non-TB controls

(ii) to estimate the survival rate of treated TB patients during 2000 – 2004 under DOTS programme

(iii) to compare the Quality of Life of treated TB patients with non-TB controls

Methodology:

Sample size: According to a cross-sectional study carried out in one Tuberculosis Unit (TU) of Tiruvallur district in Tamil Nadu, south India, around 4000 TB patients were registered for treatment under Government health facilities with the age between 15 to 64 years during 2000-2004. A control group (not affected by TB disease) of 12000 persons was selected at the ratio of 3 per 1 TB case.

Survey method: Semi-structured and a pre-coded interview schedule was used for data collection after pilot testing. The interview schedule could capture the demographic details (age, sex) general health status and quality of life assessed using the WHO-BREF questionnaire. In case of patients who are not alive at the time of interview, details such as year of death and cause of death were collected from the patient's closest family member.

Analysis plan: The primary outcome of interest is time from TB diagnosis to death, which will be calculated by subtracting the date of the death from the date on which the patient was diagnosed with TB, in the case of TB patients. Similarly, among the controls, the time from survey date to death will be calculated. Appropriate survival analysis will be done to compare the survival rates based on the primary outcome between the TB patients and control individuals.

Progress: With the pilot study experience and by considering the Indian life expectancy, the age of the study participants (retrospectively) was restricted to be within the range of 15 to 64 years.

Table 21 provides the details on the number of individuals (TB and non-TB controls) covered upto March 2014. It also provides the split up of the cases (and controls) those surviving, those died and those migrated/not traceable etc among those surveyed till now. Based on this data, preliminary findings show that overall mortality rate among TB cases and the controls was 37% and 19% respectively. However, confirmed death due to TB was 52% and 4% among TB cases and controls respectively.

Data collection is still in progress and is expected to complete by March 2015.

Table 21: Preliminary findings based on the data collected upto March 2014

	Control n(%)	TB cases n (%)
Sample size visited (till March 2014)	12245 1166 (10%)	4064 640 (16%)
Alive	803 (69%)	287 (45%)
Died	223 (19%)	235 (37%)
Migrated	140 (12%)	118 (18%)
Of those who died, due to TB	8 (4%)	121 (52%)
Other diseases (Cardiovascular, Cancer, etc.)	146 (65%)	41 (17%)
Other causes (suicides, accidents, etc.)	69 (31%)	73 (31%)

Expected outcome:

This study is expected to provide a better understanding on the survival rate of TB patients treated under DOTS strategy of RNTCP and also the difference in survival rate of treated TB persons as compared to non-TB persons. This information will be useful for policy makers and programme managers of the TB control Programme.

E-2: **Community volunteers, solidarity and case management of TB**

Principal Investigator	:	Dr. Soumya Swaminathan (email: soumyas@nirt.res.in)
Collaborators	:	Dr. Nancy Luke, Dr.K. Munshi, Pennsylvania State Unviersity, USA & Dr. Shanthidani Minz, CMC, Vellore
Source of funding	:	Pennsylvania State University, USA
Study period	:	2012 – 2018

The unsatisfactory performance of public health systems, particularly in poor rural areas, has led to calls for decentralization and greater community participation throughout the developing world.

Aims: (i) to assess the feasibility of using DOT providers from the patient's

community for the case management of TB

(ii) to establish where and why these community volunteers will be effective

Methods: There are three groups of DOT providers, such as

(i) Community DOT provider within the patient's kin-group

(ii) Community DOT provider outside the patient's kin-group

(iii) Government DOT provider (control arm)

Village census:

Data collection: The census of all households in the study area, which based on the 2000 Population Census of India and consisting of approximately 280,000 households residing in 420 villages is being conducted.

Household survey:

Sampling frame and sample size: Once the census has been completed, a stratified random sample of 12,000 households will be selected from the list of 280,000 households in the study area. From this, 25 households will be sampled in each of the 420 villages. So far, 2, 65, 120 households have been completed (Table 22). The study is in progress.

Table 22: Household survey details

Name of TU	No. of households
PUNNAI	94,216
PUTHUPADI	93,076
NATRAMPALLI	77,828
Total	2, 65,120

BIOMEDICAL INFORMATICS

STUDIES COMPLETED:

(i)   Homology modeling, docking, pharmacophore and site directed mutagenesis analysis to identify the critical amino acid residue of PknI from *M. tuberculosis*

Principal Investigator	:	Dr. Sujatha Narayanan
		(email: sujathan@nirt.res.in)
Source of funding	:	ICMR Biomedical Informatics Project
Study period	:	2013

Background: PknI is one of the 11 functional Serine/Threonine protein kinases predicted to regulate cell division in *M. tuberculosis*. In order to find newer drugs for TB, a deeper understanding on the pathogenesis of the disease is essential.

Aim: To identify the functionally active residues of PknI using bioinformatics tools and validate the results using *in vitro* experiments

Methods: We created a homology model for PknI and performed comparative structural analysis of PknI with other kinases and molecular docking studies were done with a library of kinase inhibitors.

Results: T95 and B31 were found to be the most potent inhibitors for PknI. Based on structure-based pharmacophore analysis of kinase substrate complexes, Lys 41 along with Asp90, Val92 and Asp96 were identified as functionally important residues (Fig. 39).

Conclusion: Using site directed mutagenesis technique we mutated Lys 41 to Met and observed a defect in cell division in *M. smegmatis* mc^{2}155, thus confirming our *in silico* prediction that Lys41 is critical for the function of PknI.

Fig.39: PknI interaction with B31 and T95
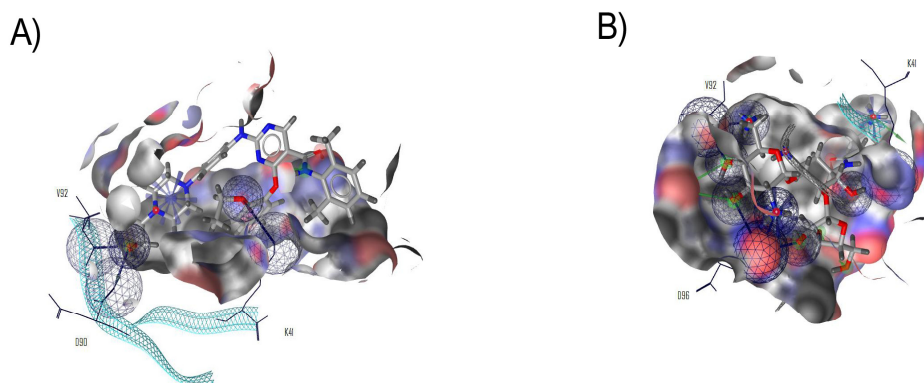
A) The 3D image depicts B31 interaction with active site of PknI. B) 3D view of T95 interaction with active site of PknI. Both the compounds interacted with Lys 41 & Val 92 residues of PknI.

(ii)   Phylogenetic analysis of HIV-1C isolates circulating in India

Background: Genetic diversity is one of the major challenges for vaccine development against HIV. Almost 97% of HIV-1 infections in India is reported to be caused by subtype C. Further, clade C HIV-1 isolates circulating in India are reported be more closely related among themselves than with clade C viruses from other countries. Majority of the phylogenetic studies on HIV-1C viruses from India have based on individual HIV proteins like Gag, Env, Vpr.

Aim: To perform phylogenetic analysis for whole genomes as well as individual proteins of HIV-1 subtype C sequences from India, in order to clearly understand the phylogenetic dynamics of Indian HIV-1C strains

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Methods: Sixty five whole HIV-1 subtype C genome sequences sampled from 19 different countries during the years 2000-2010 and used as reference sequences in the HIV Sequence Compendium were downloaded from the HIV Sequence Database. Twenty of these sequences were from India, 12 were from Botswana, 8 from South Africa, 5 from Tanzania, 3 from Brazil, and the rest were from 14 other countries. Phylogenetic analysis was performed for the 65 full length sequences as well as individual proteins of HIV-1C using MEGA (Molecular Evolutionary Genetics Analysis, version 4.0 and version 5.0). Neighbor-joining algorithm with Tamura-Nei substitution model was employed to construct the phylogenetic tree. Bootstrap replications were set at 100.

Results: Of the 20 Indian isolates, 19 (95%) were placed in a single cluster and one isolate (Accession ID: AF286223) was an outlier (Fig. 40). It was noted that a lone HIV-1C isolate from China (Accession ID: AY967806) was part of the cluster of the Indian cluster. Phylogenetic analysis was also extended to individual proteins of HIV, and phylogenetic trees were constructed using MEGA. Majority of the Indian sequences were found to form a single in the phylogenetic tree. Of the 18 envelope sequences from India included for the construction of the phylogenetic tree, 17 formed a cluster into which a lone sequence from China was also included. One Indian sequence (AF286223) that was found to be an outlier in the whole-genome based phylogenetic tree was also found to be an outlier in the envelope sequence based tree.

Fig. 40: Phylogenetic tree of full-length HIV-1 subtype C genomes from India and other parts of the world
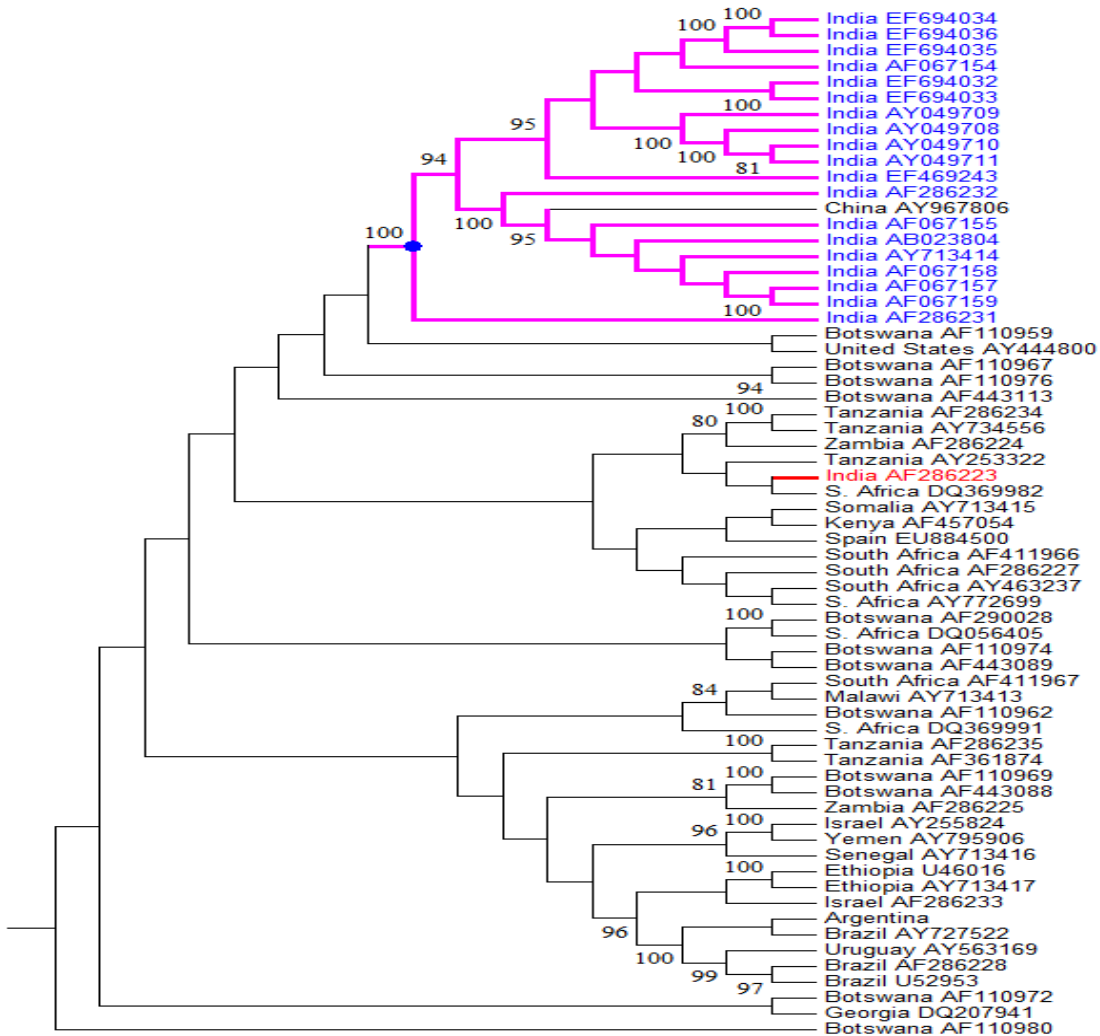
Bootstrap values ≥80 are shown. The Indian sequences are shown in branches with pink color. The outlier among the Indian isolates is highlighted in red color.

Conclusions: The present phylogenetic study confirms previous observations that HIV-1C strains from India are more closely related among themselves than with HIV-1C isolates circulating in other countries. Interestingly, the conservation has been demonstrated using sequence data generated over a period of 15 years, thereby suggesting that HIV-1C isolates from India continue to be conserved across time. These observations suggest for a possibility of designing a vaccine specifically targeting HIV-1C isolates circulating in India.

(iii) Identification of promiscuous epitopes in HIV-1C of Indian sequence using *in silico* tools

Principal Investigator	:	Dr. Luke Elizabeth Hanna (email: hanna@nirt.res.in)
Source of funding	:	ICMR Biomedical Informatics Project
Study period	:	2012-2014

Background: Efforts towards vaccine design for HIV is challenged by rapid changes in the organisms genetic components, latent infection of the HIV in the host and genetic diversity of HIV. Indian epidemic comprises predominantly of subtype C infection. The viruses circulating in India are reported to be more closely related among themselves than with clade C viruses from other countries. Thus, we hypothesized that a consensus sequence for HIV-1C isolates circulating in India would help to identify potential epitopes for vaccine design, specifically targeting the virus in India.

Aim: To identify promiscuous epitopes from consensus sequence of HIV-1C from India using multiple bioinformatics tools

Methods: Promiscuous CTL epitopes were predicted from Indian consensus HIV-1C protein sequences using three different bioinformatics tools, namely, NetCTL 1.2 server, IEDB Consensus method and ProPred1. Epitopes that were identified by all the three methods and could bind to 10 or more HLA alleles listed in ProPred1 were shortlisted as promiscuous epitopes. Promiscuous T_H cell epitopes were identified using MetaMHCII, NetMHCIIPan2.1 and ProPred. Consensus sequence for HIV-1C from India was established *in house* using the data obtained from HIV database.

Results: Totally forty nine potential promiscuous CTL epitopes were identified – about one third of these epitopes were from the envelope (9 from Gp120 and 6 from Gp41), nine epitopes were from RT, seven were from Gag and five from Nef. Out of the 49 epitopes, 43% (21/49) were found to be previously reported as immunogenic and listed in HIV Database. A total of 63 peptides with a length of 15 amino acids were identified as CD4-specific epitopes using NetMHCIIpan and MetaMHCII. Though the CD4+ epitopes 15-mers in length, the core peptide that fits into the ligand-binding groove of the HLA molecule is reported as only nine amino acids. Thus, core epitopes with the length of nine amino acids were identified using ProPred. Totally, 52 core epitopes (9-mers) were identified to be part of the 63 15-mers shortlisted in this

study. The majority of the promiscuous core 9-mer epitopes (28 of the 52) shortlisted in this study were from three proteins, Gag, Env and Vif (Gag (10), Env: Gp120 (7), Gp41 (13) and Vif (8).

Further, eight CTL epitopes (three from Gag, and one each from Gp120, G41, Integrase, RT and Rev) were found to be present in the set of 15-mer T_H epitopes (Table 23), indicating that these epitopes could potentially induce both CD4+ T-cell as well as CT8+ T-cell responses. Population coverage cumulatively for all of the eight promiscuous-cum-polyfunctional epitopes was calculated to be 60.89% for Class I HLA alleles, 88.64% for Class II HLA alleles, and 95.56% when HLA-I and HLA-II were taken together.

Conclusion: The shortlisted epitopes deserve further evaluation to be included in a vaccine formulation as they were identified from the consensus sequence of HIV-1 isolates circulating in India over a period of 15 years, and they are also potentially polyfunctional and promiscuous in nature.

Table 23: Promiscuous and polyfunctional (CD4+ and CD8+ T-cell specific) epitopes shortlisted from HIV-I consensus sequence

S.No.	Protein-Start position	End position	Th Epitope - 15mers	15-mer epitopes specific to HLA-DRB1	Core Epitopes (9mer-CD4+ specific)	Total number of class II HLAs that bind to the epitope	Core Epitopes (9mer-CD8+ specific)	Promiscuous to HLA (I) Supertypes	Total number of class I HLA alleles that bind to the epitope
1	Gag-71	85	GTEELRSLFNTVATL	0401, 0405	75: LRSLFNTVA	14	77:SLFNTVATL	A1,A2,A26, B39,B62	12
2	Gag-266	280	GLNKIVRMYSPVSIL	0802, 1501	271: VRMYSPVSI	47	272:RMYSPVSIL	A1,A2,A3, B7,B8,B27, B39,B58, B62	15
3	Gag-327	341	CKTILRALGPGASLE	0101	331: LRALGPGAS	37	332:RALGPGASL	B7,B8,B27, B58,B62	14
4	Gp120-162	176	KVYALFYRLDIVPLN	0401	168:YRLDIVPLN	16	162:KVYALFYRL	A2,A3,A24 B8,B27,B39 B58,B62	14
5	Gp41-232	246	DRSIRLVNGFLALAW	1501	235:IRLVNGFLA	23	236:RLVNGFLAL	A1,A2,A3, B7,B8,B27 B39,B62	13
6	Integrase-254	268	NSDIKVVPRRKAKII	0802	259:VVPRRKAKI	30	260:VPRRKAKII	B7,B8	12
7	RT-276	290	VRQLCKLLRGAKALT	0802, 0901, 1501	276::VRQLCKLLR	37	281:KLLRGAKAL	B8,B62	11
8	Rev_exon1-10	24	EALLKAVRIIKILYQ	0701	16:VRIIKILYQ	50	14:KAVRIIKIL	B7,B8,B58	17

STUDIES IN PROGRESS:

B-1: Database for Drug Resistant Tuberculosis – DDR-TB

Principal Investigator : Dr. Luke Elizabeth Hanna
(email: hanna@nirt.res.in)
Source of funding : ICMR Biomedical Informatics Project
Study period : 2013-2018

Background: Emergence of resistance to anti-TB drugs is one of the major challenges for the control of TB globally. Multi-drug resistant TB (MDR-TB) has now been reported in almost all parts of the world, and extensively drug resistant TB (XDR-TB)

cases have been confirmed in 58 countries. As per the WHO 2013 report, about 3.7% of new TB patients are reported to have MDR-TB, and 9% of them have XDR-TB. China and India carry approximately 50% of the global burden of drug resistant TB. Close follow-up and monitoring of the disease will help us understand and prevent the emergence and spread of TB drug resistance.

Aim: To develop an online resource called 'Database for Drug Resistant TB' (DDR-TB)

Method: Data on 170 unique variables about drug resistant TB patients, including demography, contact history, medical history (past illnesses and treatment history), present clinical profile (general and systemic examination reports, presenting complaints, co-morbidity status), treatment profile (regimen details, adverse drug reactions, treatment monitoring Index), laboratory reports (clinical, microbiology and biochemistry), etc. is captured from the patients' case files and entered electronically. MySQL and HTML has been used to develop the database. In the initial phase, the database will be local and capture available data on all MDR-TB patients registered at NIRT since February 2008, and all XDR-TB patients registered at NIRT since February 2013. Rights to access, edit and modify data in the database are restricted to persons authorized by the Director of NIRT.

Results: Currently the database contains clinical and laboratory data on 21 XDR-TB and 26 MDR-TB patients. Genotypic NIRT mutation data is available on some of the XDR-TB isolates.

Conclusion: Systematically captured clinical data of this nature will help clinical researchers to easily access and review data for evaluation and management of drug resistant TB cases. Further, large scale analysis of the data will help us to understand the changing pattern of the disease from drug sensitive to drug resistant form, and thus help clinicians in timely decision making. The database will also serve as an educational tool for medical students and researchers. The work is ongoing.

B-2: A user-friendly web portal for analyzing conformational changes in structures of *M. tuberculosis* proteins

Principal Investigator   :   Dr. Luke Elizabeth Hanna
                                 (email: hanna@nirt.res.in)
Source of funding   :   ICMR Biomedical Informatics Project
Study period   :   2014-2015

Background: With the initiation of the TB structural consortium, the protein structural space for *M. tuberculosis* (MTB) has been steadily increasing. This has led to furtherance of our understanding of *M. tuberculosis,* and has provided a base for structure-based drug designing. Currently, 969 experimental structures are available for 354 of the 4,018 proteins of MTB. This indicates that multiple structures exist for certain MTB proteins solved under different functional conditions such as bound forms with different ligands, mutant proteins, etc.

Aim: To develop a user friendly web portal for analyzing conformations changes in structures of MTB proteins

Methods: Detailed systematic analysis of the available multiple structures of each MTB protein has been carried out to determine the amount of conformational changes that the given protein structure can accommodate. Torsion angles were used to perform principal component analysis (PCA).

Results: Data on root means square deviation, sequence identity, presence of mutations, torsional angles, etc. for the multiple protein structures have been calculated and provided (Fig.41). We also present here a case study for three important MTB drug targets *viz.* inhA, alanine dehydrogenase and FabH proteins having multiple crystal structures.

Conclusion: This online resource is useful for selecting appropriate protein structures for molecular docking and structure-based drug designing studies.

Fig. 41: Conformational analysis page in MTBSD showing the RMSD, sequence identity, presence of mutations and active site residues

B-3: Updating of *M. tuberculosis* structural database

Principal Investigator	:	Dr. Luke Elizabeth Hanna (email: hanna@nirt.res.in)
Source of funding	:	ICMR Biomedical Informatics Project
Study period	:	2013-2014

Background: We developed a database of all experimentally solved protein structures of *M. tuberculosis* which is available online at www.bmi.icmr.org.in/mtbsd/MtbSD.php (*Tuberculosis* 2011;91(6):556-62). The database contains systematically analyzed and categorized structural data for each protein and serves as a very useful resource for structure based drug designing studies.

Aim: To update the database with the newly solved MTB protein structures

Results: The database has been updated with 130 newly solved MTB protein structures and their structural homologues. A functional annotation page has been provided for hypothetical proteins and a search option has been added for searching for structures based on PDB id, country, authors and journals. For example, the user can now search for MTB structures solved by different countries (Fig. 42). For the selected country, the different institutes and departments will be listed. Further selecting

the institute, the different MTB structures deposited by the institute will be listed in a tabular form. The database is now integrated with BLAST, ClustalW tools. BLAST tool can be used for searching *M. tuberculosis* homologues in MtbSD database. Further, the user can select any number of Blast hits that are similar to the submitted sequence and submit for multiple sequence alignment using ClustalW. Option for saving both BLAST as well ClustalW results are provided.

Fig. 42: Search page for list PDB IDS based on country, journal, and authors

ELECTRONIC DATA PROCESSING DIVISION

Electronic Data Processing

(Contact person: Mr.R. Subramani: email: subramanir@nirt.res.in)

The Electronic Data Processing division plays a key role in the conduct of Epidemiological surveys. The data collected during the epidemiological surveys forms the basis of subsequent data analysis which in turn will help in writing reports for publications. Once subject enrollment begins, the data is collected, computerized, validated, completed and checked for consistency. In addition, the division provides data entry/verification support for several studies conducted at the Institute.

The existing IT equipments are being maintained under comprehensive annual maintenance contract. This includes managing the IT related facilities and ensuring that the IT equipments are maintained and kept up to-date.

The management of functionality of LAN facility is carried out with the support given by NIH-ICER project. The video conferencing facilities are maintained by project staff attached to ICMR-HCL project and NIH-ICER project.

Highlights

- Created login facility on the network system for 30 new users and added with existing user account database.

- The NIRT campus has been upgraded the Wi-Fi access points with secured connection and dashboard monitoring.

- A new virtual server with more memory and hard disk drive space was installed. The users account with share folders was moved to the new virtual server.

- NIRT new domain was created as 'nirt.res.in' and made all e-mail access work with this domain.

- Provided additional network access points to NIRT Administration.

- Added a new Airtel high bandwidth internet line with existing network as a backup internet connection.

- Data analysis and a report on one-time prevalence survey were completed.

Computerization and data processing

The quantum of documents of epidemiological, clinical, laboratory and program based studies entered and verified from April, 2013 to March, 2014 is shown below.

No. of documents entered: 25,512

No. of documents verified: 25,419

A total of 41,773 records were processed for the One-time disease prevalence survey undertaken by the Epidemiology unit of NIRT. Data analysis and a report on comparison of findings of one-time survey (2008-2009) with that of 3rd repeat disease prevalence survey (2006-2008) were completed to study the effect of repeat surveys on decline of PTB in Tiruvallur district.

INTERNATIONAL CENTRE FOR EXCELLENCE IN RESEARCH

STUDIES COMPLETED:

(i)   Host immune responses in lymphatic filariasis: IL-1- and IL-23-mediated expansion of filarial - antigen specific Th17 and Th22 cells in filarial lymphedema

Principal Investigators	:	Dr. Subash Babu, Dr.P. Paul Kumaran (email:sbabu@nirt.res.in / ppkumaran@nirt.res.in)
Source of funding	:	ICER
Collaborators	:	Dr. Thomas Nutman (NIH); Dr. R. Nandini (GGH), Dr.V. Lakshmi (CDH)
Study period	:	2012-2013

Background: Lymphatic filarial disease is known to be associated with elevated Th1 and normal or diminished Th2 responses to parasite-specific antigens. The role of Th17 and the recently described Th22 cells has not been examined in detail in either filarial infection per se or in filarial disease (e.g. lymphedema and elephantiaisis).

Aim: To explore the role of Th17 and Th22 cells and their subsets, we examined the frequency of these cells in individuals with filarial lymphedema (CP), in clinically asymptomatic, infected (INF) and in uninfected (UN) individuals, ex vivo and in response to parasite and non-parasite antigens

Results: Those with disease (CP) had a significantly expanded frequency of Th17 and Th22 cells compared to either INF or UN individuals at baseline (ex vivo) and in response to parasite antigen. This antigen driven expansion of Th17 and Th22 cells was dependent on IL-1, IL-23 and to lesser on extent on TGFb as blockade of any of these cytokines resulted in significantly diminished frequencies of Th17 and Th22 cells.

Conclusion: Our findings, therefore, suggest that filarial – parasite driven expansion of Th17 and Th22 cells is associated with the pathogenesis of filarial infection and disease.

(ii)   Immunology of helminth-TB co-infections: Helminth infections coincident with active PTB inhibit mono - and multifunctional CD4$^+$ and CD8$^+$ T-cell responses in a process dependent on IL-10

Background: Tissue invasive helminth infections and TB are co-endemic in many parts of the world and can trigger immune responses that might antagonize each other. We have previously shown that helminth infections modulate Th1 and Th17 responses to mycobacterial - antigens in latent TB.

Aim: To determine whether helminth infections modulate antigen - specific and non - specific immune responses in active PTB

Methods: We examined CD4$^+$ and CD8$^+$ T-cell responses as well as the systemic (plasma) cytokine levels in individuals with pulmonary TB with or without two distinct helminth infections - *Wuchereria bancrofti* and *Strongyloides stercoralis* infection

Results: By analyzing the frequencies of Th1 and Th17 CD4$^+$ and CD8$^+$ T-cells and their component subsets (including multifunctional cells), we report a significant diminution in the mycobacterial – specific frequencies of mono - and multi – functional CD4$^+$ Th1 and (to a lesser extent) Th17 cells when concomitant filarial or Strongyloides infection occurs. The impairment in CD4$^+$ and CD8$^+$ T-cell cytokine responses was antigen - specific as polyclonal activated T-cell frequencies were equivalent irrespective of helminth infection status (Fig. 43). This diminution in T-cell responses was also reflected in diminished circulating levels of Th1 (IFN−γ, TNF-α and IL-2)- and Th17 (IL-17A and IL-17F)-associated cytokines. Finally, we demonstrate that for the filarial co-infections at least, this diminished frequency of multifunctional CD4$^+$ T-cell responses was partially dependent on IL-10 as IL-10 blockade significantly increased the frequencies of CD4$^+$ Th1 cells.

Conclusion: Thus, co-existent helminth infection is associated with an IL-10 mediated profound inhibition of antigen–specific CD4$^+$ T-cell responses as well as protective systemic cytokine responses in active PTB.

Fig. 43: Helminth infections are associated with decreased frequencies of mono - and multifunctional mycobacterial antigen - specific CD4⁺ Th1 cells in active TB
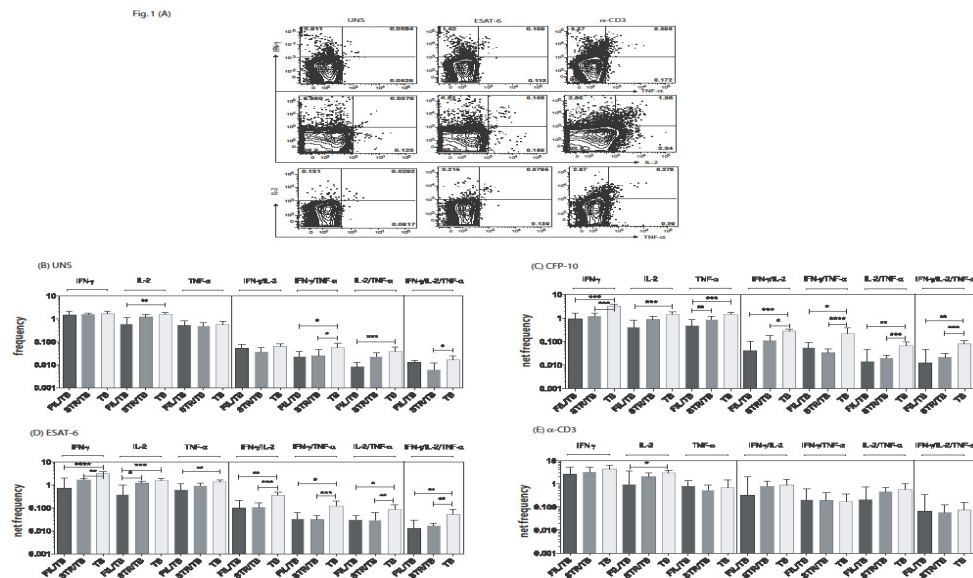
Whole blood was cultured with media alone, CFP-10, ESAT-6 or anti-CD3 for 6 h and the baseline or net frequencies of mono - and multifunctional Th1 cells determined. (A) Representative whole blood intracellular cytokine assay flow data from an active TB individual showing expression of Th1-associated cytokines. The plots shown are gated on CD3$^+$CD4$^+$ T cells. (B-E) The spontaneous frequencies (B) and the net frequencies of mono – and multifunctionalCD4$^+$ Th1 cells following CFP-10 (C) or ESAT-6 (D) or anti-CD3 (E) stimulation are shown as bar graphs. All individuals had pulmonary TB with concomitant filarial infection (FIL/TB, n=17) or concomitant Strongyloides infection (STR/TB, n=13) or no helminth infection (TB, n=20). The bars represent the geometric mean and 95% confidence intervals. Net frequencies were calculated by subtracting baseline frequency from the antigen – induced or anti-CD3 induced frequency for each individual. P values were calculated using the Kruskal-Wallis test with Dunn's multiple comparisons (* p < 0.05, ** p < 0.01, *** p < 0.001).

(iii)   Immunology of TB and its co-morbidities: IL-10-, CTLA-4- and PD-L1-dependent contraction of circulating CD4$^+$ T follicular helper cell subsets in active PTB

Background: Circulating T follicular helper (Tfh) cells represent a distinct subset of CD4$^+$ T-cells and are important in immunity to infections including TB.

Methods: In order to determine the distribution of circulating Tfh cell subsets in human TB, we measured the frequencies of Tfh subsets ex vivo and following TB - antigen or polyclonal stimulation in PTB (PTB; n=30) and latent TB (LTB; n=20) individuals

Results: We found that both ex vivo TB and antigen induced frequencies of Tfh cell subsets was significantly lower in PTB compared to LTB individuals. Similarly, antigen induced frequencies of Tfh cells expressing IL-21 was also significantly lower in PTB individuals and this was reflected in diminished circulating levels of IL-21 and IFNγ (Fig. 44). This was not accompanied by diminished frequencies of activated or memory B cell subsets, although those with PTB had diminished levels of TB antigen - specific IgG4. Finally, the diminution in frequency of Tfh cells in PTB individuals was mediated by IL-10, CTLA-4 and PD-L1 *in vitro.*

Conclusion: Thus, PTB is characterized by an IL-10, CTLA-4 and PDL-1 mediated diminution in the frequency of Tfh cell subsets, suggesting an association of these cells with the pathogenesis of PTB.

Fig. 44: Expansion of Tfh cells subsets in response to TB antigens and anti-CD3 in PTB
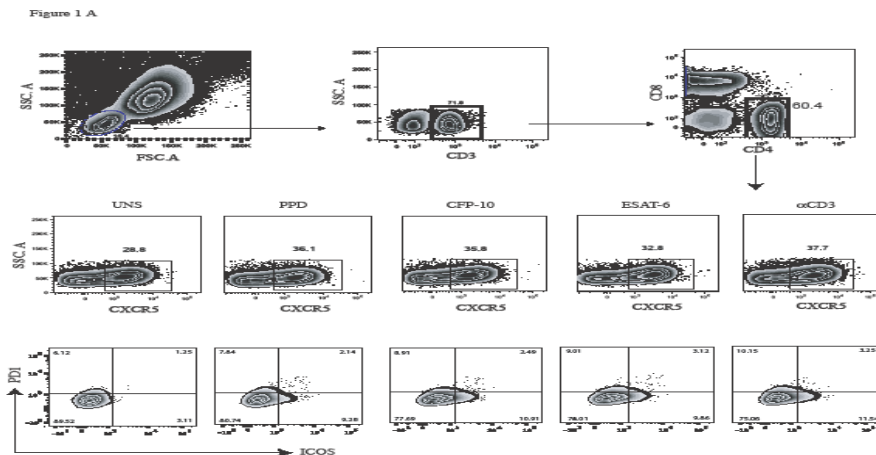
Whole blood from PTB individuals was stimulated with media alone (UNS), PPD, CFP-10, ESAT-6 or anti-CD3 for 6 h and the Gating strategy for Tfh cell subsets and a representative plot is shown.

STUDIES IN PROGRESS:

ICER-1: Characterization of immune responses in filarial-TB co-infection

Principal Investigators	:	Dr. Subash Babu and Dr.P. Paul Kumaran (email: sbabu@nirt.res.in/ppkumaran@nirt.res.in)
Source of funding	:	ICER
Collaborators	:	Dr. Thomas Nutman (NIH)
Study Period	:	2012-2017

We are studying the influence of filarial infection on the immunological responses to TB antigens in latent TB infected individuals. This study is being conducted as a prospective case-control study in Kanchipuram district, Tamil Nadu. We are screening individuals by tuberculin skin test and Quantiferon-in-Tube Gold assay to detect latent TB and ELISA to detect filarial infections. We will perform whole blood cell cultures and multi-parameter flow cytometry to determine the immunological consequences of co-infection. We have performed *ex vivo* phenotyping on a variety of T, B, NK, DC and monocyte markers including regulatory

T-cells, plasmacytoid and lymphoid dendritic cells, regulatory B cells and inflammatory monocytes on all our samples. We plan to recruit 60 patients and controls in this study and recruitment and follow up is ongoing.

<u>ICER-2:</u> Characterization of immune responses in treatment induced latency in PTB

Principal Investigators	:	Dr. Subash Babu, Dr.V.V. Banurekha and Dr. Dina Nair (email: sbabu@nirt.res.in / banurekha@nirt.res.in dinanair@nirt/res.in)
Source of funding	:	ICER
Collaborators	:	Dr. Thomas Nutman (NIH)
Study Period	:	2010-2015

The immune responses in latent TB are poorly understood. While it is difficult to define the onset of latency during natural infection, patients undergoing treatment for TB are driven into a state of latency or cure. The present study on the effect of 3 and 4 month regimens containing MFX in sputum smear and culture positive PTB offers us the opportunity to study definitive immune responses pre and post treatment. We are evaluating a variety of innate and adaptive immune responses in patients before and after treatment and our study is comparing the differences in immunophenotype (eg. Markers of T, B and NK cell activation, proliferation and regulatory phenotype) and function (eg.

production of cytokines, proliferative responses to TB antigens) at different time points following treatment. The kinetics of immune responses in patients who relapse are used to assess immunological predictors of relapse in TB. In addition, we are also trying to determine immunological differences between PTB, extrapulmonary TB, latent TB and uninfected individuals. We have performed *ex vivo* phenotyping on a variety of T, B, NK, DC and monocyte markers including regulatory T-cells, plasmacytoid and lymphoid dendritic cells, regulatory B cells and inflammatory monocytes on all our samples. Recruitment is over and follow up is in progress.

ICER-3: Host immune responses in lymphatic filariasis

Principal Investigators	:	Dr. Subash Babu and Dr.P. Paul Kumaran
		(email: sbabu@nirt.res.in/ ppkumaran@nirt.res.in)
Source of funding	:	ICER
Collaborators	:	Dr. Thomas Nutman (NIH);
		Dr. R. Nandini (GGH); Dr.V. Lakshmi (CDH)
Study Period	:	2012-2017

This study has been initiated to determine the presence of and the immune response to filarial infections in an area endemic for lymphatic filariasis in south India. This study aims to examine the presence of filarial infection at a community level as well as in hospital settings. After routine clinical evaluation and screening, individuals enrolled in this study are studied in depth immunologically, and their blood cells and/or serum are collected to address the broader questions of immunodiagnosis, immunoregulation and immunopathology. Careful observations of the individual's clinical and immunologic responses to therapy are made, as well as long-term follow-up of these changes. In addition to infected individuals, this study is also used to study individuals with filarial pathology and endemic normal individuals. This will enable us to characterize the immunological profiles of infected, uninfected and diseased individuals in an endemic area and provide greater insight into the pathogenesis of lymphatic filarial disease. Patient recruitment is ongoing.

ICER-4: Host immune responses in tuberculous lymphadenitis

Principal Investigators	:	Dr. Subash Babu, Dr.D. Bhaskaran
		(email: sbabu@nirt.res.in / baskar.d@nirt.res.in)
Source of funding	:	ICER
Collaborators	:	Dr. R. Sridhar (Stanley Hospital),
		Dr. Meenakshi (GGH)
Study period	:	2012-2017

TB lymphadenitis is the most common presentation of extra-pulmonary TB, accounting for 30–40% of cases. It constitutes a significant disease burden and differs from other forms of TB in that patients have large tuberculin reactions and

there is a strong female preponderance. The immune responses in TB lymphadenitis are poorly understood. In addition, no study till date has evaluated the immune responses pre-and post treatment in TB lymphadenitis. The NIRT study on the effect of a 4 month regimen containing OFX in superficial lymph node TB offers us the opportunity to study definitive immune responses pre and post treatment. We are evaluating a variety of innate and adaptive immune responses in patients before and after treatment and our study compares the differences in immuno-phenotype (eg. markers of T, B and NK and dendritic cell activation, proliferation and regulatory phenotype) and function (eg. production of cytokines and proliferative responses to TB antigens). Patient recruitment is ongoing.

CONTRIBUTION TO THE NATIONAL PROGRAMMES

(I)   <u>Clinical:</u>

Treatment outcome of Category V and individually tailored regimens in the management of extensively drug resistant, Pre-XDR and chronic poly-resistant PTB patients

(Contact person: Dr. Soumya Swaminathan: email: soumyas@nirt.res.in)

Introduction: Extensively drug-resistant TB (XDR-TB) is defined as resistance to any fluoroquinolone and to at least one of three second line injectable drugs (capreomycin, kanamycin and amikacin), in addition to multidrug- resistant TB (MDR-TB). According to the World Health Organisation (WHO) report, at least one case of XDR-TB has been reported by 92 countries by the end of 2012. On average, an estimated 9.6% of MDR-TB cases have XDR-TB. XDR-TB has been reported in India by isolated studies with non-representative and highly selected clinical samples. The magnitude of the problem remains to be determined due to the absence of laboratories capable of conducting quality assured second line DST.

RNTCP introduced the Programmatic management of drug resistant TB (PMDT) services since 2007 to address the needs of MDR/XDR-TB patients and is now rapidly scaling up services across the country while also expanding services towards universal access. The treatment for XDR-TB in PMDT is the Category V regimen which comprises of seven drugs - Inj. Capreomycin, Para-amino salicylic acid, MFX, High dose-INH, Clofazimine, Linezolid, and Amoxyclav during 6-12 months of the Intensive Phase and six drugs- Para-amino salicylic acid, MFX, High dose-INH, Clofazimine, Linezolid, and Amoxyclav during the 18 months of the Continuation Phase. A meta-analysis documented higher proportion of favourable treatment outcomes in studies with a higher proportion of XDR-TB patients treated with a later-generation FQ.

Pre – XDR TB is resistance to any fluoroquinolone or to at least one of three second line injectable drugs (capreomycin, kanamycin and amikacin), in addition to MDR-TB. Poly-resistance is defined as resistance to more than one first line anti-TB drug (other than both INH and RMP). WHO recommends individually designed treatments based on drug susceptibility

profile for poly-resistance.

NIRT is a WHO-recognised supranational reference laboratory for mycobacteriology where DST for First line drugs (FLD) and Second Line drugs (SLD) (Kanamycin, OFX and ethionamide) is being performed. The information on treatment outcomes of XDR-TB, pre XDR-TB and chronic poly-resistant PTB patients treated with category V regimen of PMDT or individualized regimen is important for effective management of these patients in the TB Control programme.

Objectives: (i) to document the treatment outcomes in XDR-TB, Pre-XDR-TB and chronic poly-resistant PTB patients treated with Category V regimen of PMDT or individually tailored regimens

(ii) to document adverse drug reactions to category V regimen of PMDT and individually tailored regimens

Study sites: NIRT, Chennai and Madurai.

The treatment outcomes of the category V and individually tailored regimen are studied in terms of the proportion of patients who had

- Sputum culture conversion
- Favourable response
  Unfavourable response to treatment
- Adverse drug reactions.

The study was started in January 2013; so far 26 patients have been initiated on treatment. The study is ongoing.

(II) HIV Laboratory services:

(Contact person: Dr. Luke Elizabeth Hanna – email: hanna@nirt.res.in)

Viral load testing:

The laboratory continues to serve as a Regional Reference Laboratory for viral load testing for NACO's Second Line ART program. For this program, the Molecular HIV-1 Viral load facility has processed over 1800 samples to confirm clinical and/or immunological failure in patients on first line antiretroviral therapy.

Diagnostic testing:

The laboratory also continues to serve as a Regional Reference Laboratory for NACO for testing of samples from infants received from different districts across the states of Tamil Nadu, Kerala and Pondicherry for early diagnosis of HIV. Detection of HIV-1 DNA in dried blood spots (DBS) and whole blood samples is done using Roche AMPLICOR HIV-1 DNA PCR Test Kit, v1.5 which detects HIV-1 proviral DNA integrated into human genome by amplification of the HIV-1 gag gene. Between April 2013 and March 2014, a total of 1761 HIV-1 DNA PCR tests were performed on DBS samples. Among all the DBS samples tested, 1168 samples were from newly enrolled infants/children, and 593 were follow-up cases. The proportion of positives among newly infected and follow-up cases is shown in Fig. 45A. The age-wise distribution of positive cases is shown in Fig. 45B.

Fig. 45A
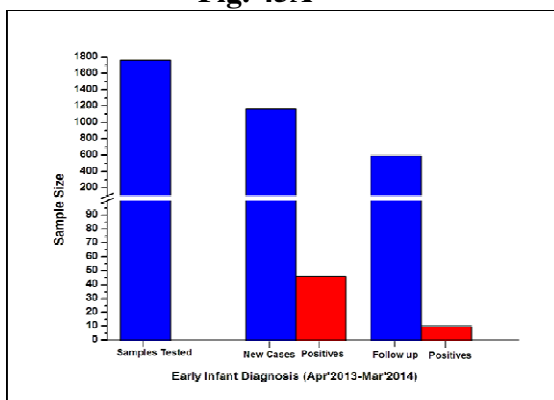
Fig. 45B
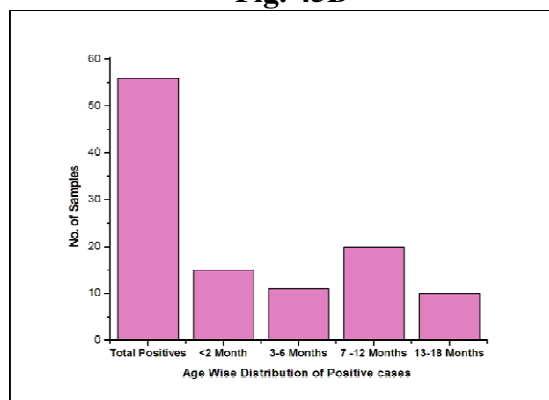
Participation in external quality assessment programs:

During the year 2013-14, the laboratory participated successfully in various external quality assurance programs such as NIH-VQA and CDC-GAP for HIV-1DNA PCR, NIH-VQA and RCPA-QAP for HIV-1 viral load testing, NIH-VQA and WHO for HIV-1 drug resistance genotyping, and QASI-NARI for CD4 Count testing. As part of continuous quality improvement, the laboratory's endeavors in the flow cytometry, HIV molecular and sequencing divisions have received good quality scoring and accreditation from the international agencies like NIH and WHO.

III. Bacteriology Lab services:

(i) RNTCP activities in National Reference Laboratory, NIRT, Chennai (2013-14)

RNTCP-External quality assurance on drug resistant survey (EQA-DRS)

Contact person	:	Dr.K.R. Uma Devi (email: umadevi.r@nirt.res.in)
Source of Funding	:	Ministry of Health and Family Welfare,
		Central TB Division, New Delhi.

Certification for culture and DST: Pre accreditation assessment was completed for three laboratories and the process is on going for 16 labs including IRLs, Medical Colleges and Private Laboratories. Certification process was completed for one laboratory for first line DST (FLD) by solid culture and two labs by liquid culture. Certification was completed for one laboratory for second line DST (SLD) by solid culture and two labs by liquid culture. Renewal of Certification for FLD by solid culture has been completed for nine laboratories and by liquid culture for two laboratories. Renewal of Certification for SLD by solid culture has been completed for one laboratory and by liquid culture for two laboratories. Certification for DST by LPA has been completed for eight laboratories.

Proficiency Testing: The fifth round of proficiency testing for mycobacterial culture and DST for 2013-2014 has been completed for labs under NIRT. In addition, as part of Supra National Reference Laboratory activity, proficiency testing is in progress for National Tuberculosis Program of DPR Korea.

XDR diagnosis: A total of 924 cultures from XDR suspects were received from different states of India and processed for second line DST by solid culture method.

EQA of smear microscopy: Five states were visited for on site evaluation of sputum smear microscopy and 500 panel slides were used to assess 100 laboratory personnel of RNTCP.

Training: Onsite training on EQA smear microscopy was given for 75 senior TB laboratory supervisors from the state of Punjab and for 30 IDSP Microbiologists of Tamil Nadu.

Line Probe Assay Services for rapid detection of MDR-TB among patients attending RNTCP from NIRT

Contact person   :   Dr.N.S. Gomathi

(email: gomathisharma@nirt.res.in)

Source of Funding   :   FIND

Diagnosis of patients with MDR-TB by Line Probe Assay was done for 5897 patients during the period between 1st April, 2013 and 31st March 2014 as part of service to the Tamil Nadu PMDT activities of RNTCP. The districts covered were Chennai and Kanchipuram. Of them, 257 were true MDR, 99 were resistant to RMP only, 400 were resistant to H only and 3431 were sensitive to both HR (Table 22). In addition, 405 follow-up samples were processed by MGIT960 system.

Table 22: Line probe assay results during 2013-14

	H Resistant	R Resistant	HR Resistant	HR Sensitive	Neg for MTB	Cult. Neg (of smear negatives)
2nd Q/2013	91	28	77	797	95	261
3rd Q/2013	107	31	81	830	92	322
4th Q/2013	108	20	57	899	58	411
1st Q/2014	94	20	42	905	13	458
Total	**400**	**99**	**257**	**3431**	**258**	**1452**

WORLD TB DAY CELEBRATIONS ON 24th MARCH, 2014

A collage of few paintings done by the students

Community programme in the corporation centre

Among school children

Quiz competition   Prize Distribution

LIBRARY AND INFORMATION CENTRE

Library and Information Centre

(Contact person: Dr.R. Rathinasabapati: email: rrathinasabapati@nirt.res.in)

Library plays a key role in furthering the research mission of NIRT with the space of around 1800Sqft. Besides holding an excellent print and online collection of over 17000 Volumes of books and journals on health science, it also holds CD-ROMs, Gratis materials, photographs, reprints, slides, theses, video cassettes, and WHO Publications. During the last fourteen years the Library makes significant investments in acquiring e-journals. It strives to keep pace with a dynamic and technology-enabled information environment to meet the expectations of its users. For providing access to the e-journal literature, a customized Digital Library portal has been established in 2001. It provides web-based access to the full-text journals and databases being subscribed by the library. It also provides gateway to ICMR consortium journals, ICMR Resource sharing portal JGate@ICMR, and open access resources. Further to, this portal provides an integrated gateway to access more than 41000 titles. It enhances a customized integrated (24hrs) access (browse, search, locate & download) facility to our patrons; all it needs few mouse click.

Services

The library offers a range of value added services including:

- Access to electronic resources through **digital library portal (24hrs)**
- Electronic Check- in and Check-out services
- Current Awareness Service
- Document Delivery Service (Print & Electronic)
- e-Mail co-ordination
- Internet Lab
- Publication (TB Alert)
- Reference assistance
- Resource Sharing
- Facilitating digital medial resources and web based services.

Institutional Repository

The NIRT created phenomenal amount of scholarly knowledge on tuberculosis as part our research activities. To maximize the visibility of these literatures and to promote research on tuberculosis, an **Institutional cum Specific Subject Repository on**

tuberculosis is being constructed. Through this repository the institute's intellectual output can be preserved, searched, and shared. We expect the service to facilitate long-term preservation of our research output and provide easy access to our publications.

Publication

As part of our Value Added Services, a monthly publication **TB Alert** is being published and circulated to all ICMR institutes and all the major tuberculosis institutes in India and abroad.

APPENDICES

LIST OF PUBLICATIONS

Publications in Journals   :   **82**

Published   i) International   :   66

                ii) National   :   16

Books   :   1

Accepted   i) International   :   18

International:

1.   Anbarasu D, Ponnuraja C, Raja A. Multiplex analysis of cytokines/chemokines as biomarkers that differentiate healthy contacts from tuberculosis patients in high endemic settings. *Cytokine*.2013;61(3):747-754.

2.   Anbalagan S, Kanthikeel SP, Bhavani PK, Hanna LE, Pahwa S, Swaminathan S. Defective dendritic cell response to toll like receptor 7/8 agonists in perinatally HIV infected children. *Pathog Dis.*2013;69:184-193.

3.   Andrade BB, Pavan Kumar N, Mayer-Barber KD, Barber DL, Sridhar R, Rekha VV, Jawahar MS, Nutman TB, Sher A, Babu S. Plasma heme oxygenase-1 levels distinguish latent or successfully treated human tuberculosis from active disease. *PLoS One.*2013;8:e62618.

4.   Anuradha R, George PJ, Hanna LE, Chandrasekaran V, Kumaran P, Nutman TB, Babu S. IL-4-, TGF-β-, and IL-1-dependent expansion of parasite antigen-specific Th9 cells is associated with clinical pathology in human lymphatic filariasis. *J Immunol.* 2013;191:2466-2473.

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Chapters:

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Accepted:

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3. Anuradha R, George PJ, Hanna LE, Kumaran P, Chandrasekaran V, Nutman TB, Babu S. Expansion of parasite-specific CD4+ and CD8+ T cells expressing IL-10 superfamily cytokine members and their regulation in human lymphatic filariasis. *PLoS Negl Trop Dis.*

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6.	Balaji S, Kannan P, Arunkumar V, Narayanan S. Expression, purification and functional characterization of AmiA of acetamidase operon of *Mycobacterium smegmatis*. *Microbiol Res.*

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10.	Jawahar MS, Swaminathan S. Rifamycin drugs as alternatives to standard isoniazid treatment to prevent active tuberculosis (TB) in HIV-negative people at risk of developing active TB. *Clin Epid Global Health.*

11.	Kumar RS, Kumar AMV, Claassens M, Banurekha VV, Gomathy S, Venkatesan P, Swaminathan S. Are we justified in treating for multidrug-resistant tuberculosis based on positive follow-up smear results? *Int J Tuberc Lung Dis.*

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13.	Makeshkumar V, Madhavan R, Narayanan S. Prevalence of drug resistance in *Mycobacterium tuberculosis* in a teaching hospital of Kanchipuram District, Tamilnadu, south India. *American J Microbiol Res.*

14.	Narendran G, Andrade BB, Swaminathan S. Tuberculosis-immune reconstitution inflammatory syndrome in HIV: from pathogenesis to prediction. *Expert Rev Clin Immunol.*

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17. Ramana Rao PV, Anbalagan S, Sikhamani R, Raja A. Interleukins 15 and 12 in combination expand the selective loss of natural killer T cells in HIV infection *in vitro*. *Clin Exp Med.*

18. Singh B, Chitra J, Selvaraj P. CCL2, CCL3 and CCL4 gene polymorphisms in pulmonary tuberculosis patients of south India. *Int J Immunogenet.*

Awards/Honours

Dr.P. Selvaraj, Scientist 'F', for being awarded with "ICMR Chaturvedi Ghansyham Das Jaigopal Memorial Award" for the year 2009. The award distribution ceremony was held on 24th eptember, 2013 at New Delhi.

Ms. Jayalakshmi Vadivel, DNS, is being honoured with "National Florence Nightingale" award for Nursing 2013, given in public recognition of valuable services to the community as Nurse by Ministry of Health & Family Welfare, Government of India, New Delhi on 12th May 2013.

Special Assignments

Membership in Committees

Dr. Soumya Swaminathan

<u>International:</u>

- Chair of the HIV section of the International Union Against TB and Lung Diseases
- Member of the TB steering committee of the IMPAACT network (NIH)
- Member, Childhood TB subgroup, DOTS Expansion Working Group, Stop TB Partnership
- Member, International Conference Organizing Committee, International AIDS Society Conference, Kuala Lumpur
- Founder member, Sentinel Project on Pediatric Drug-Resistance TB

<u>National:</u>

- Member, Country Coordination Mechanism (CCM) for the Global Fund – to fight against AIDS, Tuberculosis and Malaria
- Chair, Indo-Brazil working group on Biotechnology
- Member, Indo-Russian working group on Medical Research, DST
- Member, Expert Group on Pediatric TB, Central TB Division
- Member, National Technical Working Group (NTWG) on HIV/TB
- Member, Board of Studies on Research, Tamil Nadu Dr. MGR Medical University

Dr. Alamelu Raja

- Member, Editorial Board, Indian Journal of Medical Research

Dr. P. Venkatesan

- Chairman- Board of Studies-MSc (Bioinformatics), B.Tech (Medical informatics) -Sri Ramachandra University, Chennai.
- Chairman- Institute Ethics Committee (Institute Review Board), Sri Ramachandra Medical University, Chennai.
- Member- Institute Ethics Committee (Institute Review Board), SRM University, Chennai and ESI-PGIMS, Govt. of India, Chennai.
- Expert-Member- Sai's Biosciences Research Institute, Chennai
- Expert-Member- Sri Ramachandra University ICMR Advanced Centre for Environmental Health-Air Quality, Chennai

Member- Board of Studies:
- MSc (Statistics), M.Sc (Biostatistics), University of Madras, Chennai
- MSc, M.Phil (Statistics), M.Sc (Biostatistics), MPH, Manormaniam Sundaranar University, Tirunelveli
- M.C.A., M.Phil (Computer Science), MSc, MPhil (Mathematics), Periyar University, Salem
- M.Tech (Bioinformatics) and B.Tech (Bioinformatics) of Sathyabama University, Chennai
- M.Sc (Mathematics), Meenakhi College (Autonomous), Chennai
- M.Sc (Bioinformatics)), Stella Maris College (Autonomous) Chennai
- External Examiner – 9 Universities
- Editorial Board- 5 Journals
- Member-Secretary-Data Safety and Monitoring Board
- General Secretary- Indian Society for Medical Statistics. NIRT
- President- International Biometric Society-Indian Region
- Adjuvant Professor –Manipal University
- Honorary Visiting Professor-Sri Ramachandra University

Dr. Beena E Thomas:
- Editorial Board Member for Journal of Health Sciences
- Member of National Advisory Committee on Advocacy Communication Social Mobilization (ACSM)
- Member of Project review committee on sexual and reproductive health of persons with disability
- Member of Institutional Abstract and Web committee
- Member of International Association of Schools of Social Work
- Member of International Federation for social workers

Dr. N. S. Gomathi:
- Nominated as member of the ICMR Expert Group on TB Diagnostics; Represented NIRT as SNRL member for lab up gradation of Madurai Medical College and BMHRC, Bhopal.

Dr.Balaji:
- Nominated as a representative of NIRT for "Regional Review Meeting on Programmatic Management of Drug Resistant TB South States and Delhi" 11th April 2014

Dr.Radhakrishnan:
- Nominated as a representative of NIRT for Central Internal Evaluation of Punjab, West Bengal on 23-29 September 2013 and on 9-13 December 2013 respectively.
- Participated NRL Coordination Committee meeting to RMRC Bhubaneswar on 17th -19th February 2014 for mentoring the Institute for functioning as National Reference Laboratory under RNTCP.

Dr.Radhakrishnan & Dr.Samson:
- Nominated as a representative of NIRT for the Central Internal Evaluation of TamilNadu state on 20-24 January 2014.

ADVOCACY

Dr. Sujatha Narayanan:

- Patent has been applied for a lichen compound which has antimicrobial properties collaborative project between MSSRF and NIRT (ICMR).

- Ph.D. thesis examiner for Mr. Srinivasan Vijay, Indian Institute of Science, both thesis correction and Viva which was Bangalore on March 4th 2014.

- PhD Thesis examiner for Joseph Biljo (RCGB) University of Kerala, Trivandrum.

Dr. Beena E Thomas:

- Commemorate for World TB day, TB Awareness Programme at East Cemetery Road PHC, Annai Sathya Nagar, Government Higher Secondary School – Basin Bridge, Kodungaiyur PHC, Slum area in Basin Bridge zone, Neyveli village in Thiruvallur District, Quiz and drawing competition held at NIRT.

- NSS Programme for School teachers and Lecturer's, professors, NSS coordinators for Tamilnadu and Puducherry – Total No. of programmes during the period – 20.

CAPACITY BUILDING

Dr.C. Padmapriyadarsini:

- Attended the 15-days training course - "V International Course in Nutritional Research Methods" jointly orgnaised by St.John's Research Insitute, Bangalore and Tufts University, Boston (Bangalore-Boston Nutrition Collaboration) during January, 2014.

Dr.S. Ramesh Kumar:

- Completed Operational Research course by International Union against TB and Lung Diseases (The UNION) at Chennai, Module -2 during 9 - 13 May, 2013.

Dr. Prabu Seenivasan:

- Conducted LT &STLS training in Patiala, Punjab from 5th Nov to 19th Nov 2013
- Conducted EQA Training for the District Microbiologists at Chennai, Tamilnadu during 25 - 27 Nov. 2013.

Silambu Chelvi, B. Mahizhaveni, Michel Prem Kumar:

- Training Routine Laboratory procedures & Operations of the Liquid Culture Training at NIRT during 17 - 21 June, 2013.

Conference(s) / Workshop(s) /Symposium(s) organized:

I. Standard Operating Procedures for Institutional Ethics Committee members by ICMR-FERCAP

Date: 3 - 5 June, 2013

The National Institute for Research in Tuberculosis organized and conducted the "ICMR-FERCAP Standard Operating Procedures training course" for Ethics Committee Members during June 3 – 5, 2013 and was held at NIRT, Chennai,

The FERCAP (Forum for Ethical review Committees in the Asian & Western Pacific Region) is a project of the World Health Organization (WHO) Special Training and Research Programme in Tropical Diseases (TDR) and is a regional forum under the umbrella of the Strategic initiative for Developing Capacity in Ethical Review (SIDCER).

This training was a step towards the process of obtaining SIDCER Recognition Ethics Committees (EC), the purpose of which is to assist the ECs in improving their review processes and functioning.

A total of 57 IEC members of NIRT, NIE, Madras Diabetes Research Foundation, Y R Gaitonde Centre for AIDS Research and Education (YRG Care) Sri Ramachandra University and Dr K M Cherian's Frontier Lifeline Heart Foundation and Hospital attended the training course.

II. Improving the quality of care for children with TB and Drug Resistant TB

Date: 17-19 June, 2013

A workshop on "Improving the Quality of Care for Children with TB and Drug- Resistant TB" was jointly organised by the National Institute for Research in Tuberculosis and the Sentinel Project on Pediatric Drug –Resistant Tuberculosis with financial support from the Global TB programme, WHO. This was held at NIRT, Chennai during June 17-19, 2013.

This workshop was the first step of the Sentinel Project learning collaborative towards a mentorship process for ongoing activities to improve the quality of services provided to children in the country. The workshop was attended by paediatricians and DOTS Plus medical officers from all over the country involved in the management of Pediatric TB and DR-TB in the RNTCP. The faculty members included senior specialists (including three international faculty), pediatricians, microbiologists and programme managers from various institutions, with expertise in the field of pediatric TB/DRTB.

The objective of this workshop was to provide locally relevant baseline training for state teams on diagnosis and management of pediatric TB/DRTB and to form a group of national

experts, who will serve as trainers and mentors in their respective states. The other objectives were to identify the high priority operational research issues in the field of DR-TB in children and also to identify barriers and facilitators in including children in PMDT in India and to provide feedback to Central TB division on the actions required.

III. Tuberculosis and Diabetes Mellitus

Date: 2 - 3 August, 2013

The NIRT conducted a workshop on "Tuberculosis and Diabetes Mellitus" during August 2 – 3, 2013 at ICGEB in New Delhi, to bring together basic researchers and clinicians. This workshop was funded by Department of Biotechnology, Govt. of India. The goal of this workshop was to review the knowledge base and identify research priorities to combat the dual burden of diabetes and TB in India.

The following project goals were agreed upon during the workshop:

- ➢ Determine the incidence of TB in diabetics and understand how diabetes duration and severity influence this incidence.
- ➢ Determine the outcome of TB therapy in diabetics, including relapse and death.
- ➢ Develop protocols for diagnosis of TB in diabetics, as well as protocols for case management including endpoints of TB care.
- ➢ Generate a bio-repository that includes serum, DNA, and blood-derived RNA, for future genetic and molecular investigation.
- ➢ Begin to identify clinical and gene expression biomarkers to identify latently infected diabetic individuals with highest risk of developing TB.

IV. Sensitization for the RNTCP TB Xpert Project

Date: 10 - 11 September, 2013

The Sensitization Workshop for the RNTCP TB Xpert Project was held during September 10 – 11, 2013. This was organised by NIRT, Chennai with support from WHO, CTD, STOP TB Partnership and UNITAID. Central, State level officials and key technical staff from different states (Delhi, Karnataka, Chhattisgarh, Rajasthan, West Bengal, Maharashtra, Madhya Pradesh, Gujarat, Tamil Nadu and Andaman & Nicobar islands) participated in the work shop. The objectives of the workshop were to develop innovative models for private sector engagement, understand technical aspects in handling and trouble shooting on the Xpert machines get oriented on the recording and reporting requirements for the project.

V. Intellectual Property Rights in Medical Research

Date: 10 -11 September, 2013

A series of workshops on "Intellectual Property Rights in Medical Research" was jointly organized by the Indian Council of Medical Research and Department of Health Research to create awareness on intellectual property rights (IPR) issues, problems and opportunities pertaining to medical research. The first of this series of workshops was held at the NIRT, Chennai, during 10 - 11 September, 2013. There were 60 registered participants at this workshop, mostly scientists and research scholars from the state of Tamil Nadu, who were actively involved in medical research.

VI. Developing an innovative Tribal Health System Model to estimate the burden of TB, Co-infections and improve the effectiveness of RNTCP in India – a Multi-Centric Study

Date: 4 October 2013

A workshop on "Developing an innovative Tribal Health System Model to estimate the burden of TB, Co-infections and improve the effectiveness of RNTCP in India – a Multi-Centric Study" was conducted at NIRT on 4th October, 2014. Principal investigators from 14 ICMR institutes participated.

The NIRT has been assigned the task of coordinating a Multi-centric Task Force Study on assessing burden of TB (and co-infections) among the tribal population. A common protocol was developed in this regard by NIRT.

VII. Recent Trends in HIV/TB issues and Challenges in Nursing

Date: 30 November, 2013

The National Institute for Research in Tuberculosis conducted a workshop on "Recent Trends in HIV/TB issues and Challenges in Nursing" on 30th November 2013. The aim of the workshop was to create extensive knowledge and awareness among nursing faculties and students on early diagnosis and treatment of TB, management of HIV-TB, MDR-TB and proper implementation of RNTCP at the DOTS centre.

The workshop highlighted TB management during pregnancy, and in geriatric and pediatric populations. The workshop also highlighted the importance of cleaning & disinfection of patient care equipment and personal protective equipment.

Nursing students from various colleges in Chennai participated in the workshop.

VIII. Clinical Research Methodology and Biostatistics

Date: 13 – 15 March, 2014

The NIRT organized a 2.5 days interactive workshop on "Clinical Research Methodology and Biostatistics" from 13 to 15 March, 2014 for medical postgraduates from Government and private Medical colleges in Tamil Nadu, with the aim to promote medical research in India. The workshop was attended by 45 participants, MD students from various faculties such as, General Medicine, Chest medicine, Pediatrics, Preventive and Social Medicine from both Government and Private medical colleges.

Workshop on "Recent Trends in HIV/TB issues and Challenges in Nursing" held on 30 November, 2013.

Interactive workshop on "Clinical Research Methodology and Biostatistics" held on 13 – 15 March, 2014.

A workshop on "Improving the Quality of Care for Children with TB and Drug-Resistant TB" held on 17-19 June, 2013.

Ph.D. Scholars

List of staff / students who have obtained their Ph.D. degree (Part time/Full time)

from the University of Madras

Sl. No.	Name of the candidate	Title of the Ph.D. thesis	Part time / Full time	Supervisor/ Guide
1.	Ms. Neema Bourai	Penicillin binding protein from *M. tuberculosis* & *M. smegmatis*	Full time	Dr. Sujatha Narayanan
2.	Mr.P. Dinesh Kumar	A molecular approach to the role of serine/ threonine kinase PknE in signal transduction involved in host pathogen interactions	Full time	Dr. Sujatha Narayanan
3.	Mr.M. Radhakrishnan	Anti-TB drugs from actinomycetes	Full time	Dr. Vanaja Kumar
4.	Mr. Sameer Hassan	Genome analysis of phages and viruses	Full time	Dr. Vanaja Kumar
5.	Mr.M. Muthusamy	Antimicrobial and antimycobacterial agents	Part time	Dr. Vanaja Kumar
6.	Mr.R. Srinivasan	Bayesian spatial models for disease mapping of HIV and tuberculosis	Part time	Dr.P. Venkatesan
7.	Ms.N. Yamuna	Classification and regression trees	Full time	Dr.P. Venkatesan

List of staff/students who have submitted their Thesis and waiting for their Ph.D. degree from the University of Madras (Full time and Part time)

Sl.No.	Name of the candidate	Title of the Ph.D. thesis	Part / Full time	Supervisor/Guide
1.	Ms.R. Lakshmi	Molecular studies on mycobacteria	Full time	Dr. Vanaja Kumar
2.	Ms.V. Malini	Functional chacracterization of FtsY, a singla recognition particle receptor from M. tb	Full time	Dr. Sujatha Narayanan
3.	Ms.S. Suba	Characterization of lipoproteins of M. tb	Full time	Dr. Sujatha Narayanan
4.	Mr. Brijendra Singh	Chemokine gene polymorphism and chemokine expression in PTB	Full time	Dr.P. Selvaraj
5.	Mr. Jagdish Chandra Bose	Immunodominant epitopes against HIV subtype C	Full time	Dr. Luke E Hanna
6.	Mr.N. Pavan Kumar	Paediatric TB	Full time	Dr. Luke E Hanna
7.	Ms.R. Anuradha	Role of TLR in filarial pathology	Full time	Dr. Luke E Hanna

List of students who have registered (full-time) for their Ph.D. programme with the University of Madras

Sl.No.	Name of the Candidate	Source of Funding	Title of the Ph.D. thesis	Supervisor/ Guide
1.	Mr.P. Pugazhvendhan	ICMR	Immunoproteomic identification of B-cell antigens of *M. tuberculosis*	Dr. Alamelu Raja
2.	Ms.D. Santhi	ICMR-TASK FORCE	Novel subunit vaccine targets from *M. tuberculosis*	Dr. Alamelu Raja
3.	Ms.Maddineni Prabhavathi	CSIR	Comparative genomics and pathogenesis of TB	Dr. Alamelu Raja
4.	Mr.S. Balaji	ICMR	Diagnostic evaluation of novel T-cell (Rv2204c, Rv2394) antigens of M. tb	Dr. Alamelu Raja
5.	Ms.G. Akilandeswari	INSPIRE FELLOW	Structural characterization of 3 essential genes from M. tb	Dr. Alamelu Raja
6.	Mr.K. Srinivasan	ICMR	Comparative genomics and pathogenesis of TB	Dr.Sujatha Narayanan
7.	Ms. Ahmed Kabir Refaya	ICMR	Mycobacterial transcriptional regulators in pathogenesis	Dr. Sujatha Narayanan
8.	M.V. Arunkumar	ICMR	Gene regulation of mycobacteria	Dr. Sujatha Narayanan
9.	Mr.K. Afsal	ICMR	Effect of vitamin D3 on innate and adaptive immunity in pulmonary TB	Dr.P. Selvaraj
10.	Ms.J. Nancy Hilda	ICMR	Neutrophil mediated innate immune response in TB	Dr.D. Sulochana
11.	Mr. Narayanaiah Cheedarla	UGC	Comparative studies between HIV-1 and HIV-2 cases in Inda	Dr. Luke Elizabeth Hanna
12.	Mr. Jovvian George	ICER	Helminth Immunology	Dr. Luke Elizabeth Hanna

Staff registered (part-time) for their Ph.D. programme
with the University of Madras, Chennai

Sl.No.	Name of the staff	Title of the Ph.D. thesis	Supervisor/Guide
1.	Ms. Amudha N.	Antimycobacterial compounds	Dr. Vanaja Kumar
2.	Ms. A.S. Shainaba	Phage based drug target identification and anti-mycobacterial drug discovery	Dr. Vanaja Kumar
3.	Mr. Anbalagan S.	Innate & adaptive immunity in HIV	Dr. Luke Elizabeth Hanna
4.	Mr. Harishankar M.	Role of vitamin D receptor promoter & 3'UTR gene variants on vitamin D modulated immune functions in TB	Dr.P. Selvaraj
5.	Mr. Sekar L.	Survival analysis	Dr.P. Venkatesan
6.	Mr. Sivakumar S.	Molecular epidemiology of TB	Dr. Sujatha Narayanan
7.	Mr. Sukumar B.*	Statistical methods for micro array data analysis	Dr.P. Venkatesan
8.	Ms. Vasantha M.	Structural equation modeling	Dr.P. Venkatesan

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