Studies Completed:

(i) A Phase II, placebo-controlled, double-blind, randomized trial to evaluate the anti-bacterial activity, safety and tolerability of Tibotec medicinal compound (TMC) 207 in participants with sputum smear-positive pulmonary infection with multi-drug resistant *M. tuberculosis*

(Principal Investigator: Dr. Aleyamma Thomas; Funding: Tibotec BVBA)

**Background:** Development of new TB drugs is a priority. Tibotec medicinal compound (TMC) 207 is a methoxyquinoline with potential value in combination treatment of TB. It has a unique structure with high *in vitro* activity against wild-type and drug-resistant (DR) strains of *M. tuberculosis*. TMC207 was found to be generally safe and well tolerated in phase I studies. In the exploratory Phase I of the trial completed in South Africa, results of the interim analysis showed that addition of TMC207 to a 5-drug multi-drug resistant TB (MDR-TB) regimen resulted in significantly shorter time to culture conversion compared to placebo.

**Aim:** To demonstrate the anti-bacterial activity of TMC207 compared to placebo when added for 24 weeks to a background regimen in participants with newly diagnosed sputum smear-positive pulmonary MDR-TB infection

**Methods:**

**Study design:** National Institute for Research in Tuberculosis (NIRT) was involved in Phase II of the trial. This was a stratified, randomized, double-blind, multi-centric, placebo-controlled Phase II trial.

TMC 207 was given at 400mg dosage once a day for first two weeks followed by 200 mg thrice a week for the next 22 weeks along with the background regimen consisting of kanamycin, ethionamide (Eth), pyrazinamide (PZA), ofloxacin (OFX) and cycloserine (Cs) in doses based on body weight. The patients were monitored weekly for two months, fortnightly for four months and monthly for 18 months.
**Results:** The study was initiated in August 2009. A total of 3 patients were enrolled, among whom one was a diabetic. All the patients had culture conversion. The patients were followed up for two years; this was completed in March 2012. A total of 150 patients were admitted to this study from different centres. Analysis of the data is in progress.

**Studies in progress:**

(i) 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 pulmonary TB (CTRI Registration No: 476/09, NCT No: 933790)

(Principal Investigator: Dr. G. Narendran, Co-PI: Dr. Soumya Swaminathan; Funding Agency: Intramural or United States Agency for International Development (USAID) through WHO Model DOTS Project)

This study comparing daily vs. intermittent therapy of anti-TB treatment (ATT) is a unique opportunity to compare various aspects of TB outcome in HIV-pulmonary TB (PTB) such as sputum conversion, immune reconstitution inflammatory syndrome (IRIS), emergence of acquired rifampicin resistance (ARR), radiological improvement and toxicity profile. The three regimens are: (i) daily regimen (2EHRZ₇/4HR₇), (ii) partly intermittent (2EHRZ₇/4HR₃) and (iii) a fully intermittent regimen (2EHRZ₃/4HR₃), given for 6 months duration and followed up for a further period of one year.

As on 01.03.12, 125 patients (33 from Madurai and 92 from Chennai) have been allocated to the three regimens randomly (Daily - 41, Part daily - 44, Intermittent - 40). Mean age and weight of the study participants were 39 years and 43 kg respectively. The median CD4 cell counts was 135 cells /mm3 (IQR-70-252) and the median viral load (VL) being 211000 copies/ml (IQR – 39450 -592913); most patients were in the advanced stage of HIV. At present, all the regimens are equally efficacious and the study is ongoing.
(ii) Study to evaluate the effect of Physician’s advice in quitting smoking in HIV and TB patients in south India - A pilot study

(Principal Investigator: Dr. S. Ramesh Kumar (Funding: Fogarty grant, Miriam Hospital, Brown University, USA)


Background: The burden of HIV and TB in India is high. The association of TB and smoking is evident. Smoking in HIV poses additional risks. Smoking cessation initiation by Physician’s advice has been shown to be useful in previous studies.

Aims: (i) to determine the efficacy of Physician’s advice using “modified 5 A” strategy in quitting smoking in patients with HIV and patients with TB and (ii) to compare the effectiveness of Physician’s advice with administering a brochure containing smoking cessation information and a counselor’s counseling to brochure and counselor counseling alone in quit rate in patients with HIV and patients with TB

Sample size: HIV patients (smokers) = 80; TB patients (smokers) = 80

Methods: Patients with TB or HIV with history of current smoking referred to the clinic will be randomized to receive Group A (Physician’s advice + Counselors counseling + Brochure / educative material) or Group B (Counselors counseling + Brochure / educative material) strategy of smoking cessation, stratified based on Nicotine dependence assessed by using Fagerstrom dependence scale. In Group A, in addition to educating using brochures and a standard counseling by counselor (strategy in Group B), Physician’s advice using 'modified 5 A' strategy will be systematically approached in the five standard steps namely "Ask, Advise, Assess, Assist and Arrange". In addition, the physician will deliver a brief structured advice to subject and his/her family member. This is the change in the ‘Assist’ step of the standard “5 A” approach and hence we call the strategy as 'modified 5 A' approach.

Recruitment: Patient recruitment to this study was initiated in March 2010. At the end of March 2012, recruitment was completed; 160 subjects (80 TB and 80
HIV) were recruited. Among them 80 each were allocated to Groups A (Physician’s arm) and B. These patients are being followed up.

The study has been registered in Clinical trials registry of India.

(iii) 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 pulmonary TB

(Principal Investigator: Dr. M.S. Jawahar)

Shortening the duration of TB treatment is a research priority. To address this issue, the NIRT is investigating the efficacy and safety of 3- and 4-month regimens in comparison with that of the standard 6-month regimen for the treatment of patients with sputum positive pulmonary TB. In this randomized clinical trial, the standard 4-drug TB regimen is supplemented with moxifloxacin (MFX), a fluoroquinolone with potent bactericidal and sterilising activities against *M. tuberculosis*. Patients with newly diagnosed sputum positive, HIV sero-negative pulmonary TB, resident in Chennai and Madurai are randomly allocated to 3-month or 4-month MFX, regimens, or a 6-month regimen as control. 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 which are critically documented. Patients with successful treatment outcome are followed up for 24 months with monthly evaluations for assessing recurrence of TB. The regimens are shown in table 1.

### Table 1: Study regimens

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Months</th>
<th>Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Reg. 1</td>
<td>RHZEM</td>
<td>3</td>
</tr>
<tr>
<td>Test Reg. 2</td>
<td>RHZEM</td>
<td>RHM</td>
</tr>
<tr>
<td>Test Reg. 3</td>
<td>RHZEM</td>
<td>RHM$_3$</td>
</tr>
<tr>
<td>Test Reg. 4</td>
<td>RHZEM</td>
<td>RHEM$_3$</td>
</tr>
<tr>
<td>Control Reg.</td>
<td>RHZE$_3$</td>
<td>RH$_3$</td>
</tr>
</tbody>
</table>

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

[Green] Intensive phase  [Yellow] Continuation phase
As of 31st March 2012, a total of 618 patients have been enrolled in the study. The baseline characteristics of these patients are shown in table 2.

Table 2: Baseline characteristics of 618 patients enrolled in study

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Test Reg. 1 (n = 111)</th>
<th>Test Reg. 2 (n = 121)</th>
<th>Test Reg. 3 (n = 139)</th>
<th>Test Reg. 4 (n = 122)</th>
<th>Control Reg. (n = 125)</th>
<th>Total (n = 618)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>88 (79%)</td>
<td>91 (75%)</td>
<td>105 (76%)</td>
<td>86 (71%)</td>
<td>99 (79%)</td>
<td>469 (76%)</td>
</tr>
<tr>
<td>Female</td>
<td>23 (21%)</td>
<td>30 (25%)</td>
<td>34 (24%)</td>
<td>36 (29%)</td>
<td>26 (21%)</td>
<td>149</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40 years</td>
<td>77 (69%)</td>
<td>73 (60%)</td>
<td>94 (68%)</td>
<td>81 (66%)</td>
<td>80 (64%)</td>
<td>405 (66%)</td>
</tr>
<tr>
<td>≥40 years</td>
<td>34 (31%)</td>
<td>48 (40%)</td>
<td>45 (32%)</td>
<td>41 (34%)</td>
<td>45 (36%)</td>
<td>213</td>
</tr>
<tr>
<td>Initial sputum culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+</td>
<td>3 (3%)</td>
<td>6 (5%)</td>
<td>8 (6%)</td>
<td>4 (3%)</td>
<td>2 (2%)</td>
<td>23</td>
</tr>
<tr>
<td>2+ or 3+</td>
<td>108 (97%)</td>
<td>115 (95%)</td>
<td>131 (94%)</td>
<td>118 (96%)</td>
<td>123 (98%)</td>
<td>595 (96%)</td>
</tr>
<tr>
<td>Extent of Initial X-ray involvement (zones)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 2</td>
<td>24 (22%)</td>
<td>26 (21%)</td>
<td>31 (22%)</td>
<td>28 (23%)</td>
<td>25 (20%)</td>
<td>134</td>
</tr>
<tr>
<td>&gt; 2</td>
<td>87 (78%)</td>
<td>95 (78%)</td>
<td>108 (78%)</td>
<td>94 (77%)</td>
<td>100 (80%)</td>
<td>484 (78%)</td>
</tr>
</tbody>
</table>

Sputum culture conversion with treatment

A salient finding of this study is that the proportion of patients who became sputum culture negative after the initial 2 months of treatment was significantly higher (94%) in the MFX arm (consolidated for all four test regimens) compared to the control arm (75%). This observation which was made earlier (Annual Reports 2009-2010, 2010-2011) is sustained even with the larger population. Fig. 1 illustrates the proportion of patients with positive sputum cultures at 15, 30, 45 and 60 days of treatment. This is a significant finding as it shows that patients treated with the test regimens become less infectious earlier and to a greater degree compared to those treated with the control regimen.
Results at end of treatment

Table 3 describes the results at the end of treatment in 555 patients. Among patients treated with MFX regimens, 90 to 95% had all cultures negative at the end of treatment compared to 83% in the control regimen. Six patients required change of treatment for either drug toxicity or pregnancy.

Table 3: Response at the end of treatment

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Patients</th>
<th>Favourable</th>
<th>Unfavourable</th>
<th>Defaulted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bacteriological</td>
<td>Treatment changed</td>
</tr>
<tr>
<td>Test Reg. 1</td>
<td>106(^{^\circ})</td>
<td>97 (91%)</td>
<td>2*</td>
<td>7</td>
</tr>
<tr>
<td>Test Reg. 2</td>
<td>113</td>
<td>107 (95%)</td>
<td>1*</td>
<td>1</td>
</tr>
<tr>
<td>Test Reg. 3</td>
<td>119(^{s})</td>
<td>107 (90%)</td>
<td>4</td>
<td>1*</td>
</tr>
<tr>
<td>Test Reg. 4</td>
<td>107</td>
<td>100 (93%)</td>
<td>1***</td>
<td>1</td>
</tr>
<tr>
<td>Control Reg.</td>
<td>110</td>
<td>91 (83%)</td>
<td>4</td>
<td>1**</td>
</tr>
</tbody>
</table>

\(^{^\circ}\) 3 patients who received < 80% of treatment excluded
1 patient who received < 80% of treatment excluded
* for jaundice
** for rifampicin induced skin lesions
*** for pregnancy

**Recurrence of TB**
Patients with a successful outcome at the end of treatment are being followed up for 24 months. Of 502 such patients 47 had recurrence of TB (Table 4). TB recurrence was significantly higher in 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) recommended the temporary suspension of intake to this regimen, pending a more detailed review later. Intake to the other regimens is continuing.

**Table 4: Recurrence of TB according to regimen**

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Patients</th>
<th>Recurrence of TB</th>
<th>Recurrence of TB / 100 Person-years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Reg. 1</td>
<td>97</td>
<td>18*</td>
<td>9.99</td>
</tr>
<tr>
<td>Test Reg. 2</td>
<td>107</td>
<td>7</td>
<td>3.84</td>
</tr>
<tr>
<td>Test Reg. 3</td>
<td>107</td>
<td>9</td>
<td>4.58</td>
</tr>
<tr>
<td>Test Reg. 4</td>
<td>100</td>
<td>7</td>
<td>3.51</td>
</tr>
<tr>
<td>Control Reg.</td>
<td>91</td>
<td>6</td>
<td>3.74</td>
</tr>
</tbody>
</table>

* 1 patient relapsed with TB meningitis

The Study is registered in the Clinical Trials Registry of India (CTRI) (ctri.nic.in) (PROVCTRI/2008/091/000024)
(iv) **HIV associated lipodystrophy syndrome in children: Role of nutrition, anti retroviral treatment and genes**
[Principal Investigators: Dr. Soumya Swaminathan, Dr. Padmapriyadarsini; Funding Agency: National Institute of Health (RO1 grant)]

**Background:** This is a prospective multi-centric study undertaken at NIRT, Chennai and St. John’s National Academy of health sciences, Bangalore.
**Aims:** (i) 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 10 years after 12 months after initiating anti-retroviral treatment (ART) and (ii) to determine the role of genetic factors in the development of fat redistribution, insulin resistance and dyslipidemia in HIV-infected children after 12 months of ART in south India

**Methods:** HIV-infected children who are about to initiate ART are recruited. Details about their demographics, clinical & dietary history (Food security questionnaire, 24 hour dietary recall), physical examination, including anthropometric measurements are collected at baseline. They are followed every 3 months upto 12 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, 6 and 12 months. The following single nucleotide polymorphisms are studied: APOA5 (64G>C & -1131T>C), APOC3 (-482 C>T, -455 T>C & 3238 C>G), APOE (E2, E4 alleles), CETP (279 G>A, -629 C>A) and PLIN (6209T>C, 11482G>A, 13041A>G & 14995A>T)

The study was initiated in June 2011. As on 31st July 2012, 87 children have been recruited to the study. The sample size is 440 (220 at each site) and the study is ongoing.
HIV Vaccine Trial

Analysis of DNA prime-MVA boost Phase I HIV vaccine trial
(Principal Investigator: Dr.V.D. Ramanathan)

A randomized, double-blind, placebo controlled phase I trial was conducted in 32 HIV-uninfected healthy volunteers to assess the safety and immunogenicity of prime-boost vaccination regimens with either 2 doses of DNA (ADVAX) prime and 2 doses of recombinant MVA (TBC-M4) boost (Group A) or 3 doses of TBC-M4 alone (Group B). Both vaccine regimens were found to be generally safe and well tolerated. The breadth of anti-HIV binding antibodies and the titres of anti-HIV neutralizing antibodies were significantly higher (p<0.05) in Group B volunteers at 14 days post last vaccination. Neutralizing antibodies were detected mainly against Tier-1 subtype B and C viruses. HIV-specific IFN-γ ELISPOT responses were infrequent after ADVAX vaccinations, but were detected in all 12 Group A vaccinees after 1st and 2nd TBC-M4 injections. Six volunteers from Group B showed positive IFN-γ ELISPOT responses after 1st and 2nd TBC-M4 injections and 11/12 after the 3rd vaccination. The IFN-γ ELISPOT responses were directed mostly to Env and Gag proteins. The response rate, breadth and magnitude of IFN-γ ELISPOT responses did not differ significantly between the groups at the end of vaccination schedules. The responses persisted in 8/11, 8/12, 3/10 volunteers from Group A and 7/12, 4/10 and 4/11 volunteers from Group B at 3, 6 and 9 months post-last vaccination to TBC-M4 matched peptides, respectively. Enhancement of T-cell immune responses to DNA priming deserves further investigation using new technologies such as DNA administration by electroporation and/or molecular adjuvants.
Socio-behavioral studies

Studies in progress:

(i) HIV prevention through mobile phone technology among male sex workers in India (International collaborative studies)
(Principal Investigator: Dr. Beena E. Thomas, Source of Funding- Indo-US Joint Working group)

Background: This proposal is an outcome from a collaborative intervention study done in NIRT with the Harvard Medical School to address psychosocial needs of men having sex with men (MSM). Sex work is a matter of concern among this group, with elevated levels of sexual risk behaviour, inconsistent condom use and high prevalence of HIV and sexually transmitted infections (STIs). Our prior formative work revealed that the vast majority of sex workers in India 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.

Aims: (i) to develop a HIV risk reduction counseling intervention for MLSWs in India using mobile phone technology,
(ii) to examine, in a pilot randomized controlled trial, the feasibility, acceptability and potential impact of the proposed intervention and
(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)

The study has been initiated.

(ii) Health seeking behaviour and awareness of TB among migrants – Brick kiln workers - A study from Tiruvallur District, Tamil Nadu, India
(Principal Investigator: Mrs. Niruparani Charles, Source of Funding- Model DOTS Project)

Background: Migration has been reported as one of the main reasons for default. Brick kiln workers are one such group in Tiruvallur district who come here to work from adjoining areas between January to July each year. They are
hard to reach as they are confined in their brick kiln for long hours and these kilns are usually located on the outskirts. Furthermore, these workers are exposed to smoke and dust, have problems with regard to smoking and alcohol and little is known about their health care-seeking behavior and their awareness on TB.

**Aims:** (i) to understand the knowledge, attitude and perceptions on TB among brick kiln workers,
(ii) to identify the prevalence of chest symptomatics and health seeking behavior among brick kiln workers and
(iii) to find out the perceptions of the health providers in providing treatment and management of TB with special reference to brick kiln migrant workers

**Methods:** This is a cross sectional study which has been planned in 2 phases following a sequential approach. We have completed the first phase of the study which is,
- Enumeration of the recent list of brick kiln chambers in the Tiruvallur district
- Random selection of chambers and calculation of sample size (Sample size=3850 )

**Progress of the study:** We have completed 40 chambers covering a population of 2800 brick kiln workers. We have also conducted seven in-depth interviews with Medical officers and two focus group discussions with health care providers. The study is ongoing.

(iii) **An experimental study to enhance treatment adherence in TB patients who consume alcohol**
(Principal Investigator: Dr. Mohanarani Suhadev, Source of Funding- Model DOTS Project)

**Background:** This study is an outcome of a previous pilot study conducted during 2009-2010 to explore suitable intervention measures for TB patients with Alcohol Use Disorders (AUD) in Chennai Corporation, Tamil Nadu. The pilot study revealed that 29% of the 490 TB patients consumed alcohol. The prevalence of AUD among them was 52%. The qualitative component of the study highlighted the need for an intervention among TB patients with AUD and
the feasibility and acceptability of an intervention. This Randomized Controlled Trial (RCT) experimental intervention study has been planned to enhance treatment adherence in TB patients who consume alcohol.

**Objectives:**

(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 by comparing the treatment adherence of TB patients with disorders related to alcohol use in the experimental area with those TB patients with disorders related to alcohol use in the control area

The findings will provide the necessary information on enhanced treatment adherence due to alcohol intervention measures and help the Revised Nationalontrol Programme (RNTCP). This information could help develop a standard intervention model that could be feasible and acceptable in programmatic conditions to improve adherence among TB patients.

**Progress of the study:** We have screened 1212 TB patients and recruited 540 patients. The study is ongoing.

(iv) **A pilot study to test the feasibility and impact of an intervention for mothers living with HIV/AIDS**

(Principal Investigator: Dr. Beena E. Thomas)

**Background**

This was an outcome of a previously conducted Indo-US study in which a community-based approach to design an HIV/AIDS program for mothers living with HIV (MLH) was used.

The previous study had brought out key areas that challenge MLH which included barriers to accessing care, stigma from health providers, disclosure issues, legal issues, nutrition and coping with the illness. The present pilot experimental intervention study was initiated keeping in mind the felt needs of the MLH. The strategies adopted were based on the suggestions of the MLH.

**Aim:** To compare the Intervention Care group with the Standard Care group with respect to
• Physical health status
• Mental well being
• Social and family support
• Quality of life

**Methods:** This community-based, prospective, randomized, experimental two-group design randomly assigned 32 MLH to intervention and 32 MLH to a standard program. The intervention program designed through a community participatory approach covered topics on stigma, disclosure issues, adherence to ART, nutrition, legal issues, coping strategies and sharing personal experiences. There were five monthly sessions delivered over five months. Eighty one percent of the participants have completed the 3-month assessment and 72% of the participants completed the 6-month assessment.

**Progress of the study:** The study has been completed and data analysis is in progress. The study findings would help to plan a larger experimental study to assess the impact of the intervention model on the quality of life of MLH.
Laboratory studies

Clinical Pharmacology

Completed studies:

(i) Pharmacokinetics of first-line anti-TB drugs in children
(Principal Investigator: Dr. Geetha Ramachandran)
(Collaboration: Institute of Child Health, Chennai, Govt. Hospital of
Thoracic Medicine, Chennai, Kilpauk Medical College & Hospital, Chennai
and Govt. Rajaji Hospital, Madurai)
(Funding: ICMR Task force on Pediatric HIV)

Background: Current recommendations for pediatric dosages of anti-TB drugs
are based on a small number of pharmacokinetic studies, few of which include
younger children. In the RNTCP in India, ATT is given thrice-weekly and dosages
of drugs are based on body weight. The importance of adequate dosing and
therapeutic blood levels of anti-TB drugs has received attention in the light of
recent reports suggesting that the currently recommended dosages of rifampicin
(RMP), isoniazid (INH), PZA and ethambutol (EMB) are inadequate in children.
During early life, children experience significant changes in the relative sizes of
their body compartments and their ability to absorb, distribute, metabolize and
excrete drugs. Genetic polymorphisms could also have a profound influence on
INH and RMP drug levels. Not much is known about the impact of nutritional
status on anti-TB drug pharmacokinetics.

Aims: (i) to evaluate the pharmacokinetics of RMP, INH and PZA in HIV
uninfected children with TB who were receiving ATT according to the
RNTCP guidelines in India and
(ii) to relate drug pharmacokinetic parameters with treatment outcomes

Methods: HIV seronegative children aged 1 to 12 years attending the RNTCP
(TB treatment) centres at the Institute of Child Health, Chennai, Government
Hospital of Thoracic Medicine, Chennai, and Government Rajaji Hospital,
Madurai, meeting the study criteria were recruited. All the children were
diagnosed of TB (pulmonary & extra pulmonary) using programme definition.
They were receiving ATT according to RNTCP guidelines from pediatric patient-wise boxes for a minimum of 2 weeks (six doses). The study commenced after obtaining approval from the Institutional Ethics Committees of all the study sites. Phenotypic INH acetylator status was determined. Nutritional status was assessed using z scores. During the intensive phase of ATT, a complete pharmacokinetic study was performed after directly observed administration of drugs. At two and six months, drug levels were checked 2 hours post-dosing. Plasma concentrations of RMP, INH and PZA were measured by High performance liquid chromatography (HPLC) and pharmacokinetic variables calculated. Multivariable regression analysis was done to explore factors impacting drug levels and treatment outcomes.

**Results:** A total of 84 children took part in the study. Table 5 shows the demographic and clinical characteristics of these children. The numbers of slow and rapid acetylators of INH were 57 and 27 respectively.

**Impact of age:** Peak concentration and exposure of all RMP, INH and PZA were significantly lower in children in the age range of 1 - 3 years compared to the other age groups (p < 0.01); however, drug pharmacokinetics were similar in the three older age groups. Ninety percent (76 of 84) of children had sub-therapeutic RMP peak concentration (< 8µg/ml) across all the age groups (all children under 3 years had RMP concentrations below 8µg/ml). In the case of INH and PZA, 10 / 84 (12%) and 31 / 84 (37%) respectively had sub-therapeutic peak concentrations (INH < 3µg/ml; PZA < 35µg/ml). There were a significantly higher number of children aged 1 – 3 years with sub-therapeutic concentrations than those aged 3.1 – 12 years (INH: 6 /10 vs. 10 / 74; p = 0.003 & PZA: 14 / 31 vs. 2 / 53; p < 0.001).

**Impact of Nutritional Status:** Peak concentration and exposure of RMP, INH and PZA were lower in children with stunting and underweight compared to normal children, the differences being statistically significant for RMP, INH and PZA for stunting and RMP and PZA for underweight (p < 0.05) (Fig. 2). No significant difference in peak concentration and exposure of RMP, INH and PZA was observed in children with wasting compared to normal children (Fig. 2).
**Treatment Outcome:** TB treatment outcomes were available in 70 children; 14 children had migrated and could not be followed up. Among the 70 children, there were 15 with unfavourable outcomes (death – 1; relapse – 1; failure - 13). Peak concentration, exposure, 2nd and 6th month concentrations of RMP and INH were significantly lower in children with unfavourable outcomes compared to those with favourable outcomes (Table 6). There was a delay in the time to attain peak concentration of RMP in the unfavourable responders compared to favourable responders; \( p = 0.03 \). Favourable outcome was achieved in 75% of cases when RMP, INH and PZA peak concentrations were within the therapeutic range. This dropped to 56% when all the drugs were below the therapeutic range; this difference however was not significant. A higher proportion of children > 3.1 years had a favourable outcome compared to those aged 1 – 3 years (84% vs. 54%; \( p=0.016 \)). There was also a significantly higher proportion of slow acetylators of INH among children with favourable outcome (89% vs. 60%; \( p = 0.005 \)).

**Factors influencing drug levels:** Multiple regression analysis was performed to test the influence of factors such as age, body mass index (BMI), serum albumin, INH acetylator status, stunting, underweight and wasting on peak concentration and exposure of RMP, INH and PZA. Results showed that age and WAZ (underweight) significantly influenced peak concentration and exposure of RMP, INH and PZA (\( p < 0.05 \)). In addition, acetylator status had a significant influence on the pharmacokinetics of INH (\( p < 0.05 \)).

**Factors influencing treatment outcome:** In univariate analysis, age, INH acetylator status, peak concentration, exposure, 2- and 6-month levels of RMP and INH were found to be significantly associated with treatment outcomes, \( p < 0.05 \). Multivariate logistic regression by stepwise method showed that only peak concentration of RMP (AOR 1.7; \( p=0.03 \)) and 6 month INH concentration (AOR 2.2; \( p=0.005 \)) significantly impacted treatment outcome.

**Conclusions:** This is the first study to report on the pharmacokinetics of RMP, INH and PZA in children with TB being treated with short-course, intermittent regimens, and also the first to relate drug levels with treatment outcomes.
study has demonstrated that age (<3 years) and nutritional status (stunting and underweight) could influence drug pharmacokinetics, and that these could in turn influence treatment outcome. Regular monitoring of drug levels particularly in younger children and those with malnutrition could help in better patient management. Future recommendations for anti-TB treatment in children should consider age and nutritional status in order to achieve optimal treatment outcomes. The study findings of low anti-TB drug levels in children receiving standard short course chemotherapy (SCC) according to RNTCP guidelines in India and its impact on treatment outcomes, highlights the importance of considering children’s dosage requirements separately.

Table 5: Demographic & clinical features of study participants (n = 84)

<table>
<thead>
<tr>
<th>Details</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>7.1 ± 3.3</td>
</tr>
<tr>
<td>Males (n)</td>
<td>40</td>
</tr>
<tr>
<td>Body wt (kg)</td>
<td>18.8 ± 7.2</td>
</tr>
<tr>
<td><strong>Nutritional status</strong></td>
<td></td>
</tr>
<tr>
<td>Height for age z score (HAZ)</td>
<td>- 1.2 ± 1.3</td>
</tr>
<tr>
<td>Weight for age z score (WAZ)</td>
<td>- 1.7 ± 1.0</td>
</tr>
<tr>
<td>Weight for Height z score (WHZ)</td>
<td>- 1.2 ± 1.1</td>
</tr>
<tr>
<td>Mid-arm circumference (cm)</td>
<td></td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td></td>
</tr>
<tr>
<td><strong>Thrice weekly treatment dose mg/kg</strong></td>
<td></td>
</tr>
<tr>
<td>RMP</td>
<td>9.7 ± 2.2</td>
</tr>
<tr>
<td>INH</td>
<td>9.7 ± 2.2</td>
</tr>
<tr>
<td>PZA</td>
<td>32.0 ± 8.9</td>
</tr>
<tr>
<td><strong>Duration of ATT (months)</strong></td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td><strong>Regimen (n)</strong></td>
<td></td>
</tr>
<tr>
<td>Category I</td>
<td>48</td>
</tr>
<tr>
<td>Category II</td>
<td>3</td>
</tr>
<tr>
<td>Category III</td>
<td>33</td>
</tr>
<tr>
<td><strong>Type of TB (n)</strong></td>
<td></td>
</tr>
<tr>
<td>Pulmonary</td>
<td>19</td>
</tr>
<tr>
<td>Extrapulmonary</td>
<td>63</td>
</tr>
<tr>
<td>Both</td>
<td>2</td>
</tr>
<tr>
<td>Rapid acetylators of INH (n)</td>
<td>27</td>
</tr>
</tbody>
</table>
Table 6: Comparison of drug levels between children with favourable and unfavourable TB treatment outcome

<table>
<thead>
<tr>
<th>Drug</th>
<th>N</th>
<th>Unfavourable outcome</th>
<th>N</th>
<th>Favourable outcome</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 month (2-hr)</td>
<td></td>
<td>6 month (2-hr)</td>
<td></td>
</tr>
<tr>
<td>RMP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>3.8 ± 2.3</td>
<td>55</td>
<td>5.7 ± 2.2</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>17.5 ± 10.3</td>
<td>55</td>
<td>27.5 ± 11.8</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>3.6 ± 1.5</td>
<td>55</td>
<td>2.6 ± 1.1</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>2.9 ± 2.0</td>
<td>48</td>
<td>4.4 ± 2.3</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1.0 ± 1.0</td>
<td>47</td>
<td>3.6 ± 2.4</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>4.7 ± 2.6</td>
<td>55</td>
<td>6.4 ± 2.7</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>17.3 ± 9.7</td>
<td>55</td>
<td>26.0 ± 11.9</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>2.8 ± 1.5</td>
<td>54</td>
<td>2.3 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>2.7 ± 2.1</td>
<td>48</td>
<td>6.4 ± 3.3</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1.6 ± 1.4</td>
<td>47</td>
<td>4.9 ± 2.7</td>
<td>0.001</td>
</tr>
<tr>
<td>PZA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>32.8 ± 12.8</td>
<td>55</td>
<td>37.8 ± 11.3</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>188.7 ± 70.0</td>
<td>55</td>
<td>215.5 ± 67.4</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>3.2 ± 1.7</td>
<td>55</td>
<td>2.9 ± 1.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

$C_{\text{max}}$ – Peak concentration; $T_{\text{max}}$ - Time to attain peak concentration;
AUC – Exposure; NS – non-significant
Fig. 2: Stunted and underweight children have significantly lower peak levels of anti-TB drugs (Values are mean; vertical bars denote SD)

* P < 0.05 vs. normal group

- Normal;  - Stunted;  - Under weight;  - Wasted
(ii) Determination of content of anti-TB drugs stored in different treatment centres in the RNTCP in Tamil Nadu: An observational study

(Principal Investigator: Dr. Geetha Ramachandran)

Funding: Model DOTS Project

**Background:** In India, the RNTCP provides decentralised treatment through a network of more than 400,000 DOT providers, to provide TB treatment to the patients as near to their home as possible. Adverse storage conditions could affect drug content. EMB is known to become hygroscopic if not stored properly. In the case of Cs, a second-line anti-TB drug, gross deterioration under tropical climatic conditions has been reported from NIRT. It is important to ensure that patients receive good quality drugs, since poor quality of anti-TB drugs is one of the reasons for development of MDR-TB. Systematic quality control analysis of anti-TB drugs are required to improve cure rates and have a successful TB control programme.

**Aim:** To determine the content of certain anti-TB drugs available at different TB treatment centers of the RNTCP in the state of Tamil Nadu

**Methods:** This study was undertaken in eight districts in the state of Tamil Nadu, India. These districts were spread across the state with varying geographical terrain, accessibility and availability of DOTS plus drugs. In each district, drugs were collected at different settings such as, District TB centre, one TB Unit (TU), two designated microscopy centres (DMC) and DOT providers. A maximum of 10 each of the following drugs were collected from each setting:

(i) RMP(150mg & 450mg)
(ii) INH (300mg)
(iii) PZA (500mg & 750mg)
(iv) EMB (400mg & 600mg)
(v) Ethionamide (Eth) (250mg)
(vi) Levofloxacin (LFX) (500mg)
(vii) Cs (250mg)

At the time of drug collection, details such as manufacturing source, dates of manufacture & expiry, batch number and storage conditions were collected. For
analysis, drugs stored in the State drug stores (SDS) and drugs purchased and used in NIRT were also included in the study.

Drug estimations for their active ingredient were undertaken according to validated spectro-photometric methods after coding the tablets. The acceptable limits for content of the active ingredient applied to the individual drugs were taken as 90% - 110% (WHO report, 2011).

**Results:** Table 7 shows the mean drug content and % of drugs within the acceptable range in all the districts. The content of RMP 450mg, INH 300mg, PZA 750mg & 500mg, EMB 600mg & 400mg and Eth 250mg were within acceptable limits in all the districts. The drug content did not differ among districts, and that the geographical location of the districts did not influence the drug content. The number of tablets not within acceptable range was higher for RMP 150mg, Levofloxacin (LFX) 500mg and Cs 250mg.

**Rifampicin 150mg:** A total of 80 capsules from eight districts were analysed. Fig. 3 shows the distribution of individual RMP 150mg capsule content in each district. The mean drug content was 154mg (range: 102 – 277mg). Overall, 80% of capsules were within the acceptable range; the values were below the acceptable limits in five out of eight districts (70% in four districts & 80% in one district). About 83% of capsules from NIRT were within acceptable limits.

**Cycloserine 250mg:** A total of 70 Cs tablets from eight districts were analysed. Fig. 3 shows the distribution of individual Cs tablet content in each district. The mean drug content was 200mg (range: 108 - 245mg). Overall, only 21% of tablets from the districts were within acceptable limits; the values were below acceptable limits in all the districts. The percent of tablets falling within the acceptable limits ranged from 0 to 40%. About 40% and 68% of tablets from the SDS and NIRT respectively were within acceptable limits.

**Levofloxacin 500mg:** A total of 67 tablets from seven districts were analysed. Fig. 3 shows the distribution of individual LFX tablet content in each district. The mean drug content was 475mg (range: 365 - 515mg). Overall, 87% of tablets were within acceptable limits; the values were below acceptable limits in three
out of seven districts. About 77% of tablets from the SDS were within acceptable limits.

**Conclusions:** This is the first systematic study to report on the content of anti-TB drugs available in the RNTCP centres in the state of Tamil Nadu. Most of the drugs had their content within the acceptable range, which is quite encouraging. Low content of Cs observed in all the districts is a matter of concern. Although the RNTCP takes every effort to procure good quality drugs, varying storage conditions in different settings, particularly in DOT providers’ homes (most of them lived in thatched huts) could have a bearing on the quality of drugs, particularly Cs. Periodic quality checks of anti-TB drugs available at the RNTCP centres are necessary to ensure that the programme continues to provide good quality anti-TB drugs to patients.

**Table 7: Mean drug content & % adherence to stated content in 8 districts**

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of tablets</th>
<th>Mean (mg)</th>
<th>Min (mg)</th>
<th>Max (mg)</th>
<th>% drugs within acceptable range (90 – 110%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin 450mg</td>
<td>398</td>
<td>445</td>
<td>291</td>
<td>508</td>
<td>92</td>
</tr>
<tr>
<td>Rifampicin 150mg</td>
<td>80</td>
<td>154</td>
<td>102</td>
<td>277</td>
<td>80</td>
</tr>
<tr>
<td>Isoniazid 300mg</td>
<td>406</td>
<td>295</td>
<td>216</td>
<td>350</td>
<td>96</td>
</tr>
<tr>
<td>Ethambutol 600mg</td>
<td>346</td>
<td>601</td>
<td>406</td>
<td>842</td>
<td>97</td>
</tr>
<tr>
<td>Ethambutol 400mg</td>
<td>94</td>
<td>404</td>
<td>344</td>
<td>458</td>
<td>97</td>
</tr>
<tr>
<td>Pyrazinamide 750mg</td>
<td>406</td>
<td>722</td>
<td>137</td>
<td>857</td>
<td>94</td>
</tr>
<tr>
<td>Pyrazinamide 500mg</td>
<td>17</td>
<td>500</td>
<td>480</td>
<td>514</td>
<td>100</td>
</tr>
<tr>
<td>Cycloserine 250mg</td>
<td>70</td>
<td>200</td>
<td>108</td>
<td>245</td>
<td>21</td>
</tr>
<tr>
<td>Ethionamide 250mg</td>
<td>64</td>
<td>241</td>
<td>230</td>
<td>254</td>
<td>100</td>
</tr>
<tr>
<td>Levofloxacin 500mg</td>
<td>67</td>
<td>475</td>
<td>365</td>
<td>515</td>
<td>87</td>
</tr>
</tbody>
</table>
Fig. 3: Percent of cycloserine 250mg tablets within acceptable limits. The shaded area represents the acceptable range (90% - 110%)
Studies in progress:

(ii) Pharmacokinetics of anti-TB drugs in HIV-infected children with TB  
(Principal Investigator: Dr. Geetha Ramachandran)

Collaboration: Institute of Child Health, Chennai, Govt. Hospital of Thoracic Medicine, Chennai, Kilpauk Medical College & Hospital, Chennai and Govt. Rajaji Hospital, Madurai; Funding: ICMR Task force on Pediatric HIV)

Background: There is a paucity of pharmacokinetic data on existing anti-TB drugs given to children with HIV-TB co-infection, who also exhibit age-related differences in drug absorption, metabolism and clearance. HIV infection combined with intestinal dysfunction and malnutrition could cause impaired absorption of anti-TB drugs leading to reduction in blood levels of the drugs that can result in poor treatment response.

Aim: To study the influence of HIV infection on the pharmacokinetics of RMP, INH, PZA and EMB in children with HIV & TB

Methods: The study population includes two groups of children.
Group 1: TB
Group 2: HIV-TB

The required sample size is 80 in each group.

Children aged 1 to 12 years receiving treatment for TB from the RNTCP (TB treatment) centres at the Government Hospital of Thoracic Medicine, Chennai, Kilpauk Medical College & Hospital, Chennai, Institute of Child Health, Chennai and Government Rajaji Hospital, Madurai are recruited.

The INH acetylator status using saliva is determined. The nutritional status is assessed using z scores. The pharmacokinetic study is undertaken in the respective hospitals. On the day of the study, serial blood samples at pre-dosing and at 2, 4, 6 and 8 hours of blood (2ml) is collected followed by supervised drug administration. Wherever possible, RMP and INH concentrations at two and end of treatment (2 hour post-dosing) are determined. Plasma concentrations of RMP, INH and PZA are measured by HPLC and pharmacokinetic variables calculated. TB treatment outcomes are noted from the RNTCP card.
We have recruited 84 and 52 children respectively with TB and HIV-TB. Recruitment of HIV-infected children with TB is in progress.

(iii) Comparative pharmacokinetics of RMP during daily and intermittent dosing in HIV-TB patients

(Principal Investigator: Dr. A. K. Hemanth Kumar)

Collaboration: Govt. Hospital of Thoracic Medicine, Chennai

**Background:** This is a prospective pharmacokinetic study nested within a randomized controlled clinical trial in which HIV-infected patients with TB receive RMP along with other medications either daily or intermittently. It has been reported that intermittent RMP therapy in HIV-infected patients increases the risk of acquired RMP resistance among patients who fail. Sub-therapeutic plasma RMP could lead to unfavourable TB treatment outcome.

**Aim:** To study the pharmacokinetics of RMP in HIV-infected patients with TB who are receiving daily and intermittent anti-TB regimens

**Methods:** The study is conducted in a sub-set of 48 patients (24 each in the daily and intermittent dosing arms) who are getting recruited to the clinical trial. Eligible subjects are identified by the Clinicians involved in the trial. On the day of the study, blood samples are collected at 0, 1, 2, 6, 8, 12 and 24 hours after dosing. Plasma concentrations of RMP are estimated by HPLC according to a validated method. So far, 21 patients have been recruited to the study. The study is in progress.

(iv) Pharmacokinetics of rifabutin in HIV-infected TB patients

(Principal Investigator: Dr. Geetha Ramachandran)

(Collaboration: Govt. Hospital of Thoracic Medicine, Chennai)

**Background:** TB is the most common opportunistic infection and is an entry point for a significant proportion of HIV-infected patients who are eligible for ART. It is therefore a common scenario that HIV-infected patients eligible for ART also have concomitant TB and require treatment for both infections. Current recommendations are to treat patients with HIV-related TB with a regimen including a rifamycin for the full course of ATT. The rifamycin, rifabutin (RBT) is recommended for use in HIV-infected TB patients who receive second-line ART
regimens containing lopinavir / ritonavir. Concomitant use of RBT with PI-based antiretroviral therapy has been reported to lead to successful treatment outcomes. However, ritonavir (RTV), being a CYP3A4 inhibitor markedly increases serum concentrations and toxicity of RBT. It therefore becomes necessary to decrease the dose of RBT when co-administered with RTV. There have been conflicting reports regarding the dose of RBT during concomitant RTV administration. In India, the current practice is to reduce the dose of RBT by 50% in patients receiving concomitant RTV. There is not much pharmacokinetic data available to support this reduction.

**Aim:** To study the pharmacokinetics of RBT at the dose of 150mg thrice weekly during concomitant RTV administration in HIV-infected TB patients

**Methods:** This study is conducted at the Government Hospital of Thoracic Medicine, Chennai. Patients meeting the following inclusion criteria are recruited to the study:

**Inclusion criteria**
1. HIV-infected TB patients with a diagnosis of PTB based on sputum smear positive for *M. tuberculosis*
2. Receiving standard ATT with RBT-containing regimens regularly for minimum 2 weeks
3. Should have failed virologically to the first-line ART regimens and be receiving second-line regimens containing LPV/RTV regularly for minimum 2 weeks before enrolment
4. Should be adults ≥ 20 years
5. Body weight ≥ 30 kg
6. Willing to accept hospitalization
7. Willing to give informed written consent

On the day of the pharmacokinetic study, serial blood samples are collected at pre-dosing and at 1, 2, 4, 6, 8, 12 and 24 hours after drug administration. Estimation of plasma RBT, LPV and RTV are undertaken by HPLC according to validated methods. So far, 12 patients have been studied. The study is in progress.
(v) Monitoring plasma RMP and INH in HIV-infected patients with TB  
(Principal Investigator: Dr. A. K. Hemanth Kumar)  

**Background:** Treatment of TB in HIV-infected patients is an important issue in terms of both duration as well as frequency of administration. This becomes more complicated due to malabsorption of anti-TB drugs, especially RMP during HIV infection, resulting in subsequent treatment failure. This raises an important question of whether HIV-infected patients require therapeutic drug monitoring and, if required, increase the dosages of anti-TB drugs, in order to overcome the effect of malabsorption.  

**Aim:** To determine plasma RMP and INH concentrations in HIV-infected patients with TB and correlate with TB treatment outcome  

**Methods:** This study is done in all patients who are participating in an ongoing randomized controlled clinical trial at NIRT. Blood samples at 2-hour post dosing are collected at months 1 and 5 of ATT. Plasma RMP and INH estimations are undertaken by HPLC in a blinded manner. Patients are followed up according to the trial protocol. So far, plasma samples at both time points have been collected from 41 patients. The study is in progress.  

(Principal Investigator: Dr. A. K. Hemanth Kumar)  

Accurate and sensitive methods to estimate anti-TB drugs in plasma are necessary for the conduct of pharmacokinetic studies in adult and pediatric groups of patients. Methods by Liquid Chromatography Mass Spectrometry (LCMS) have an advantage of being highly sensitive requiring a low plasma volume for estimation. This would be useful, especially in young children from whom it is difficult to collect blood at multiple time points. At present, we run most of the drug estimations by HPLC. We are in the process of standardizing methods for estimation of RMP, INH, PZA and EMB by LCMS.
HIV studies

Studies in progress

(i) Predictors and immunologic characterization of TB-associated IRIS in a prospective clinical trial cohort in Chennai, India

(Principal Investigator: Dr. Soumya Swaminathan; Funding Agency: Intramural to India Grant (NIAID), National Institute of Health)

IRIS or paradoxical worsening of infection, following the initiation of ART is particularly common in AIDS patients with co-existent TB. In our efforts to predict this phenomenon, a prospective cohort study nested within a parent TB-treatment trial has been undertaken by NIRT in collaboration with the NIH. In this study, clinical predictors and risk factors are evaluated and compared between patients who develop TB-IRIS and those who do not, based on baseline characteristics. Markers of T-cell activation (e.g., PD-1, CD69, intracellular Ki67, co-expression of HLA-DR and CD38) are being evaluated for predicting TB-IRIS by comparing values at baseline and during TB-IRIS episodes. The effector response of CD4 T-cells to TB is studied by longitudinal follow-up of T-cell stimulation assays and a panel of serum cytokines (Th1/Th2/Th17) is measured. The study was started in November 2009. As on 01.03.12, 57 patients were enrolled, out of which 19 experienced IRIS. The median time to ART after ATT was 22 days (14-34 IQR). The median time to IRIS occurrence was 9 days (7-15 IQR) and to resolve was 11 days (8-15 IQR). Low CD4 cell counts and high viral load were predictive of this syndrome. Analysis of laboratory markers in patients with and without IRIS is in progress.

(ii) Frequency of drug resistance mutations among HIV-infected adults failing first line antiretroviral therapy in southern India

(Principal Investigator: Dr. Luke Elizabeth Hanna)

The current standard first-line treatment for HIV in India consists of two nucleoside reverse transcriptase inhibitors (NRTIs), zidovudine or stavudine plus lamivudine, and one nonnucleoside reverse transcriptase inhibitor (NNRTI), nevirapine (NVP) or efavirenz (EFV). Regimens with protease inhibitors (PIs) are available as second-line treatment options upon failure of the first-line ART under
the national program. Surveillance for emergence of resistance in HIV against the drugs recommended by the National Programme has been undertaken by the WHO HIV Resistance Monitoring Programme (HIVResNet) for HIV Drug Resistance Prevention, Surveillance and Monitoring Programme. The information obtained will help us to review the efficacy of the current treatment regimens and to plan or modify the composition of ART in order to derive maximum benefit from this intervention.

The present study aimed at determining the prevalence of drug resistant mutations in 50 HIV-1-infected individuals from southern India who failed standard first line antiretroviral therapy. The observed frequency of mutations conferring resistance to lamivudine was 82%, stavudine 60%, zidovudine 56%, EFV 66%, NVP 94%, didanosine and abacavir 48%, delavirdine 68%, emtricitabine 82%, zalcitabine 70% and tenofovir 8%, indicating a high frequency of NRTI and NNRTI mutations among this group of patients.

We also sequenced the protease and integrase genes to look for the presence of naturally occurring resistance mutations to PIs and integrase inhibitors. None of these samples had major resistant mutations to PIs as well as integrase inhibitors. However, two samples showed the presence of minor mutations (L74M and E138D) in the integrase gene.

(iii) Molecular characterization of HIV-1 subtype C isolates circulating in India

(Principal Investigator: Dr. Luke Elizabeth Hanna)

The HIV-1 epidemic in India is predominated by the subtype C virus, which is not only distinct from other HIV-1 subtypes, but also from subtype C viruses present in other parts of the world. It is therefore imperative to understand the unique biological and molecular properties of these viruses. The present study aimed to characterize some of the unique features of HIV-1C isolates circulating in our population.

Molecular determinants of co-receptor usage are known to be present on the HIV envelope. Changes in cellular tropism by HIV-1 in vivo seems to be a key event in disease pathogenesis, and broadening of the co-receptor usage profile of HIV-
1 may be associated with accelerated CD4 T-cell loss, disease progression to AIDS. Published research suggests that HIV-1 subtype C isolates in different regions of the world isolated from various cohorts during different disease stages utilize CCR5 almost exclusively and show minimal conversion to CXCR4 tropism. Although the percentage of CXCR4 usage among HIV-1 subtype C strains is not as high as subtypes B and D (50–90%), there are indications that the frequency of subtype C CXCR4-utilizing viruses may be increasing with time. The present study is an attempt to determine the frequency of CCR5, CXCR4 and dual tropic phenotypes in HIV-1 isolates obtained from HIV-1 infected subjects in various stages of disease. Forty clinical isolates have been co-cultured and typed for co-receptor usage by means of a phenotypic assay using U87. CD4 cell lines expressing either CXCR4 or CCR5. Majority of the isolates used CCR5 as the co-receptor even during late stage disease. None of the isolates tested thus far were found to use CXCR4 exclusively. However, a few isolates were dual tropic and could use both co receptors. We also sequenced full length envelope genes of the viruses with a view to identifying molecular determinants for expanded coreceptor usage in HIV-1 subtype C isolates using more than one co-receptor.

HIV-1 Rev is an accessory protein which helps in the export of unspliced and partially spliced mRNA from the nucleus to the cytoplasm. A unique feature of HIV-1 subtype C is that it has rev genes of variable length, as a result of heterogeneity in the C-terminus. One of our objectives is to understand the functional significance of the C-terminus heterogeneity of HIV-1 subtype C Rev. Both exons of the rev genes of five different HIV-1 subtype C strains coding for Rev proteins ranging in size from 100 amino acids to 126 amino acids were cloned into a vector. A subtype B rev coding for a 116 amino acid long Rev protein was used as the control. The six constructs were assayed for rev activity by co-transfecting them into 293T cells along with a gagpol-RRE construct, first with a subtype B RRE belonging to NL4-3 (p-gagpol-RRE-B) and then with a subtype C RRE belonging to Indie-C1 (p-gagpol-RRE-C), and measuring gag production by measuring p24 antigen in the culture supernatant. We found that longer Revs appeared to function more efficiently than shorter ones, particularly
at low concentrations such as is seen *in vivo* in early stages of infection. We also found that subtype C RRE was a weaker activator of Rev activity than subtype B RRE, in spite of high levels of similarity between the two RRE sequences. HIV-1 LTR encodes regulatory elements that determine the infectivity and replicative fitness of the virus. Molecular characterization of the LTR of HIV-1C isolates in our population is also being undertaken. The study is ongoing.