thsti

ट्रांसलेशनल स्वास्थ्य विज्ञान
एवं प्रौद्योगिकी संस्थान

TRANSLATIONAL HEALTH SCIENCE
AND TECHNOLOGY INSTITUTE

ANNUAL REPORT 2018 - 2019

OUR MISSION

By integrating the fields of medicine, science, engineering and technology into translational knowledge, we aim to make the resulting biomedical innovations accessible to public health, to improve the health of the most disadvantaged people in India and throughout the world.

OUR VISION

THSTI is a collective of physicians and scientists who work to improve health in India by creation of new knowledge for innovation, development of innovative solutions based on existing knowledge, and new strategies for implementation of existing solutions. THSTI complements the discovery, design and development of interventions by building rigorous research capacity through high quality training.

ANNUAL REPORT 2018 - 2019

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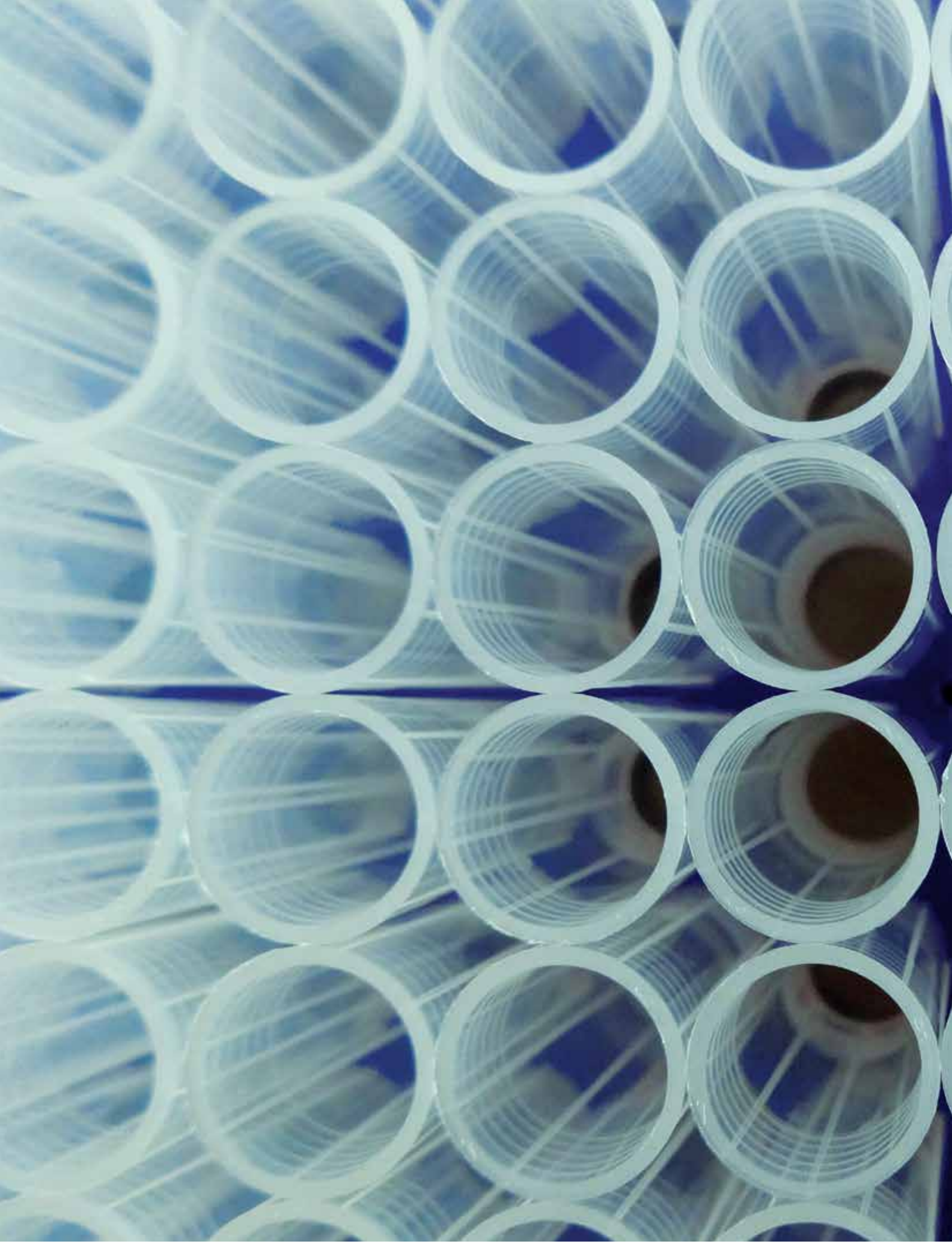
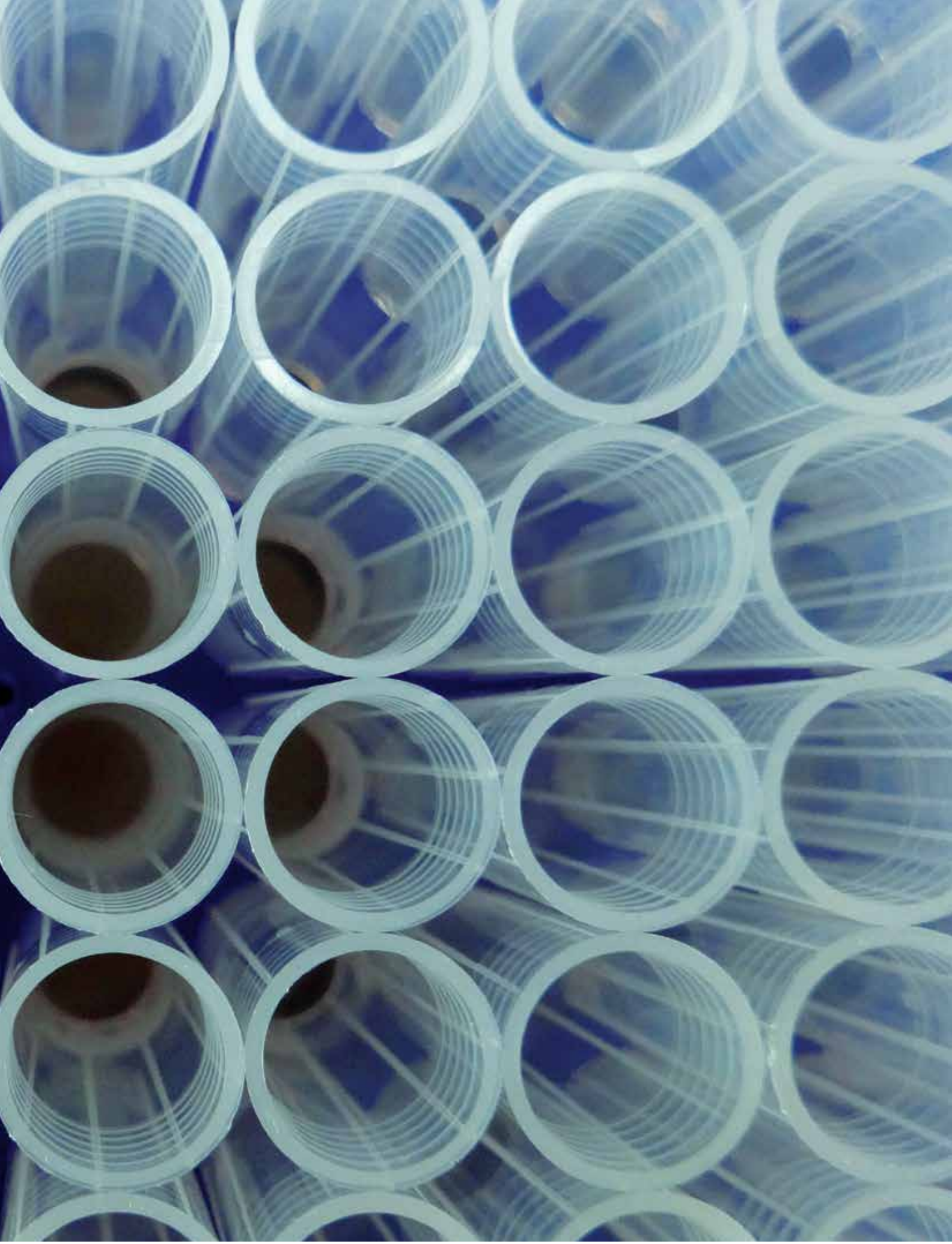




THSTI SOCIETY

THSTI was registered as a Society under the Societies Registration Act, 1860 on 15th July 2009 with distinguished scientists as nominated and ex-officio members. The President of the Society and most members are nominated by the Honorable Minister of Science and Technology, Government of India. The Society is the apex authority of THSTI and manages, administers, directs and controls the affairs of THSTI through the Governing Body as per the relevant rules, bye-laws and various orders issued by the Government of India. In 2018-19, the Society underwent a reconstitution in its composition.

PRESIDENT

Ms. Shailaja Chandra

Dr. Renu Swarup

Dr. Balram Bhargava

Mr. B. Anand

Dr. Alka Sharma

Dr. Amulya K Panda

Dr. Apurva Sarin

Dr. M. Radhakrishna Pillai

Dr. Randeep Guleria

Dr. Vineeta Bal

Dr. Uday Yaragatti

Dr. Ashalatha

MEMBER SECRETARY

Prof. Gagandeep Kang

THSTI GOVERNING BODY

The Governing Body of THSTI has been functional since the institute's establishment and is chaired by the Secretary, Department of Biotechnology and comprises of ex-officio members and eminent scientists who, like THSTI Society members, are nominated by the Honorable Minister of Science and Technology, Government of India. It carries out and pursues the objectives of the Society in accordance with the Memorandum of Association.

CHAIRPERSON

Dr. Renu Swarup

EX OFFICIO MEMBERS

Prof. Gagandeep Kang

Dr. Balram Bhargava

Mr. B. Anand

Mr. C. P. Goyal

Dr. Alka Sharma

Dr. Neeraj Jain

Dr. Sudhanshu Vrati

Dr. Amulya K Panda

Dr. Ramesh V. Sonti

Prof. Shinjini Bhatnagar

NOMINATED MEMBERS

Dr. T.S. Balganesh

Prof. P. N. Tandon

Prof. G. Padmanabhan

Dr. Ashutosh Sharma

Dr. Vaskar Saha

Mr. Utkarsh Palnitkar

Dr. Mahima Datla

Dr. Sangeeta Bhatia

FROM THE EXECUTIVE DIRECTOR'S DESK

At the end of 2018-2019, the Translational Health Science and Technology Institute (THSTI) is approaching the completion of its first decade since its inception. The last nine years have seen comprehensive research programs being pursued in each area as 'centre' grants, all of which have now completed their terms with approved extensions until 31st December 2018. The 'centres' have been evaluated by DBT and the research programmes have been recommended for continuation since they have significant near-term and long-term outcomes for public health. Based on DBT's advice, we have restructured THSTI's research programmes optimally utilize scientific and technical expertise required for the continuation of research and support services for the National Capital Region Biotech Science Cluster (NCR-BSC).

As a result of this restructuring, there are three main components, one staffed by permanent staff and the others in project mode for the continuation of research programmes and facilities at THSTI.

The Health Science Technology Centre (HSTC) is staffed by faculty chosen to establish and develop research programs that integrate engineering, science, medicine, and business to improve human health. It is organized to focus on four broad themes, i) Infection and Immunology, ii) Maternal & Child Health, iii) Non-Communicable Diseases and Multidisciplinary Clinical & Translational Research.

The Translational Research Program (TRP), in long-term project mode, is intended to build capacity for research, clinical product evaluation and regulation of support and technology transfer to complement the basic discovery goals of HSTC. The TRP is organized to complement high-end health science technology creation by providing support for preclinical testing and clinical product development. In the restructuring, we developed the TRP as four facilities that will work in alignment with the translational mandate of the institute. These are the i) Biorepository, ii) Bioassay Laboratory, iii) Data Management Centre and iv) Small Animal Facility.

In addition, the Clinical Development Service Agency, established as a separate entity, serves a critical training and clinical support role for projects within and outside THSTI.

The summary of the THSTI's activities provided in this Annual Report provides a brief overview of the year's activities, but I thought that it would be useful

to provide a few summary highlights of the work of THSTI, that demonstrate the ability and commitment of our scientists.

Products developed (not including the celiac disease test and the Rotavac vaccine where development was initiated prior to the founding of THSTI):

- Poorti-Breast prosthesis for post-mastectomy patients
- TB filtration device-for improved smear microscopy
- AptaDx TB-semi-automated aptamer-based diagnostic for TB

Products in proof of concept/validation

- Second generation dengue point-of-care diagnostic
- Fever of unknown origin point-of-care diagnostic
- Hepatitis B point-of-care testing for blood safety screening-comparable to nucleic acid testing

Products transferred to companies for development

- Anti-TB compound SPR113 to enter phase 1 studies in humans in 2019
- Green carbon technology for cheaper manufacture of drugs near patent expiry
- Typhoid detection kit transferred to startup

Entrepreneurial ventures

- Aptabharat Innovation Ltd
- Tritek Innovation Ltd
- Aarna Biomedical Products Ltd

Contributions to policy/process

- Road map for Cholera submitted to NTAGI
- Impact assessment of rotavirus vaccine introduction into the national programme
- Development of common ethics forms and process for multi-site clinical trial review
- Support to major public health/clinical research through conduct / monitoring / design of programmes

Training

- Development of online training for Good Clinical Practice with CDSCO
- Training programmes with BIRAC/DBT/CDSCO
- Training programmes with ICMR

Development of common infrastructure

- Biorepository for data and biological samples
- BSL-3 facility for animal experimentation

Key scientific areas being taken forward

Maternal and child health

- One-third of Indian babies are small and one in seven is born too soon (three times higher than developed countries)
- One in four preterm births can be prevented by improving maternal nutrition and air quality
- Diagnostic biomarkers and interventions are being tested

Diagnostics

- Programme on point-of-care diagnostics for fevers, blood-borne viruses, and snakebite

Non-communicable diseases

- Diabetes prediction from blood samples of healthy individuals
- Non-alcoholic fatty liver disease prediction and testing of high purity compounds from Indian plant product manufacturers

Vaccines, infections, and immunology

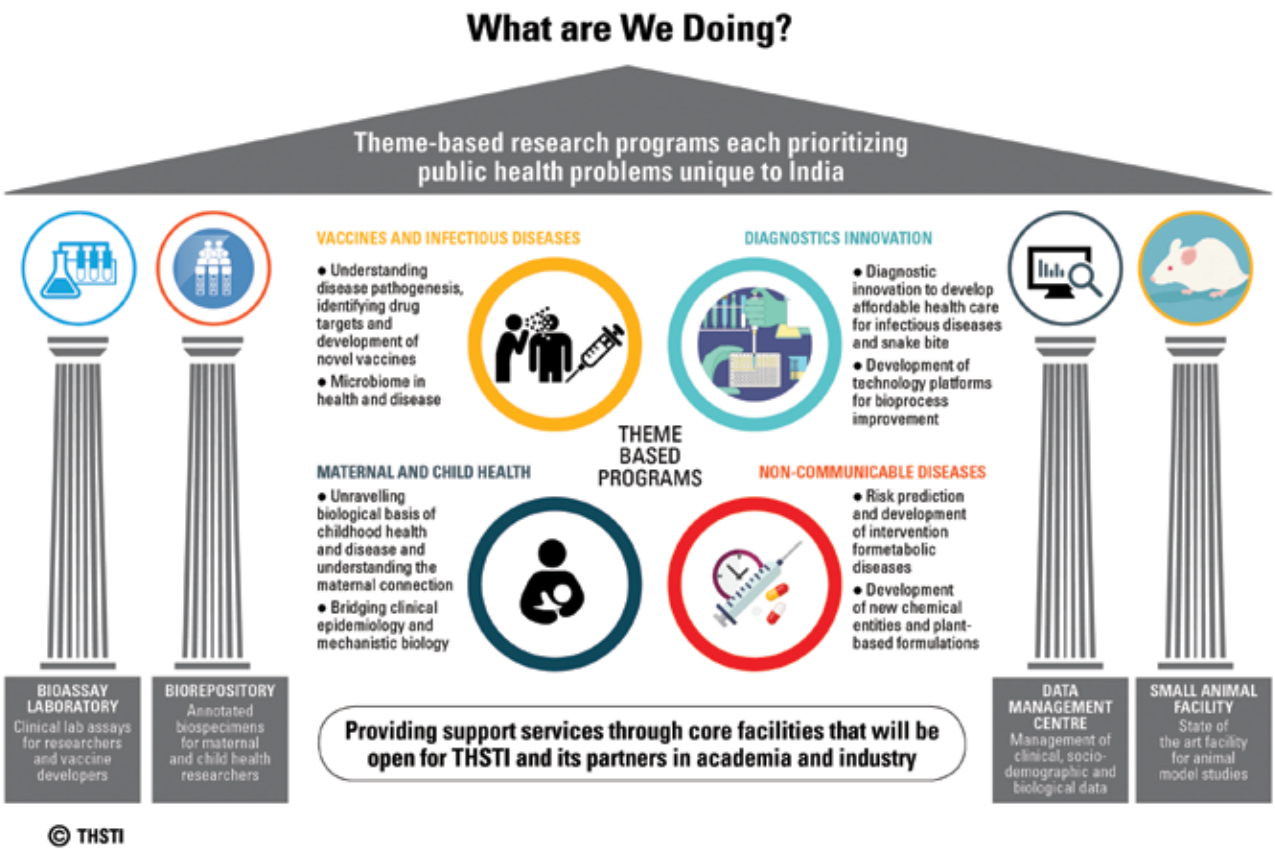
- TB drug development through rational drug design
- Cholera vaccine development with BIBCOL under National Biopharma Mission
- Dengue translational research consortium under National Biopharma Mission
- IndCEPI infrastructure development for industry partnership
- Bioassay lab supporting dengue vaccine development

All of these demonstrate THSTI's commitment to serving public health needs in India. The commitment is not restricted to just the scientific staff but extends across everyone, as demonstrated by the active participation in the many outreach and community engagement activities that are a critical part of our calendar. With DBT's support and governmental and non-government project funding, we hope to amplify our contributions in years to come.

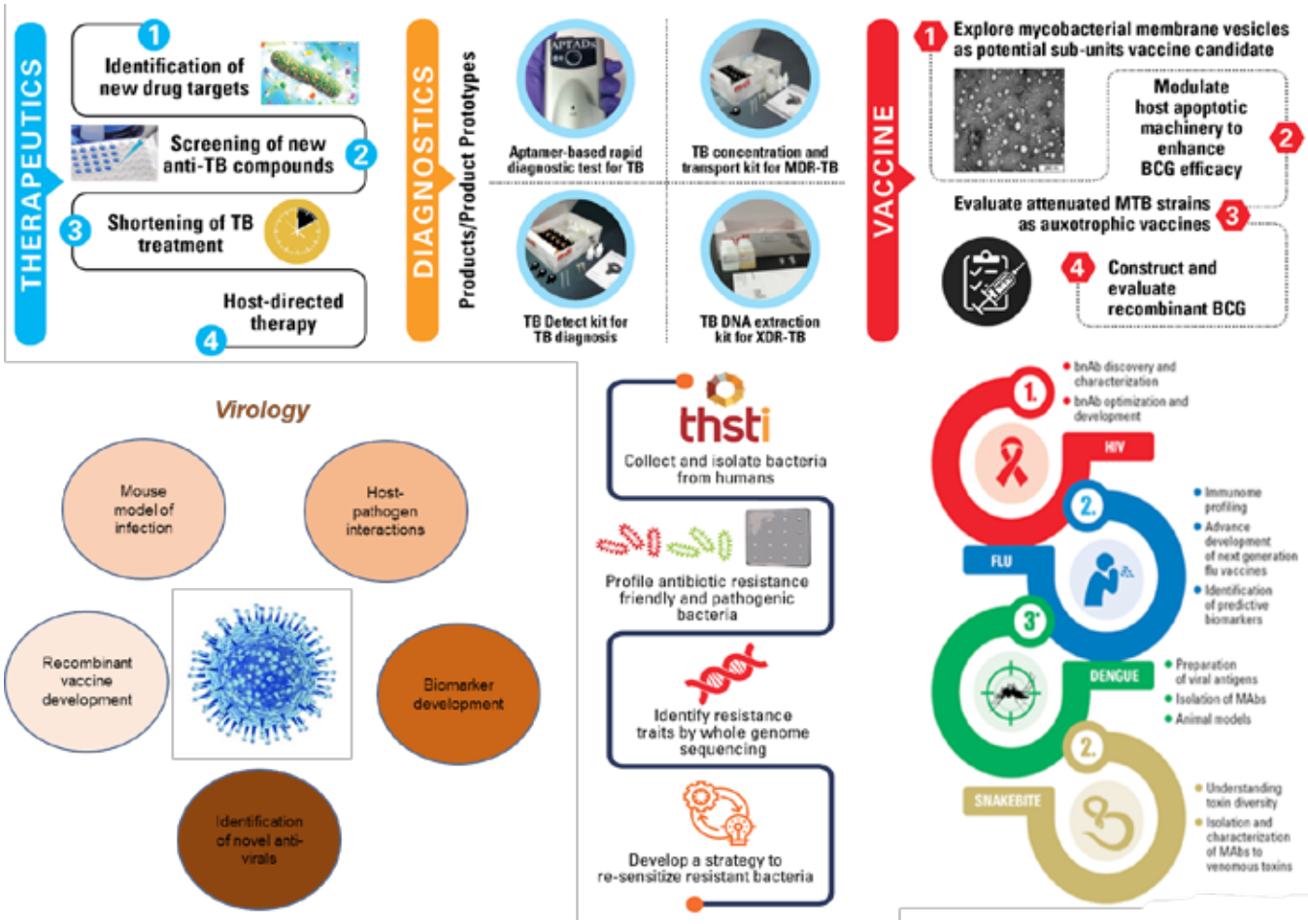
SUMMARY OF ACTIVITIES 2018-19

This year's Annual Report marks close to a decade of efforts of the Translational Health Science and Technology Institute (THSTI) in research and innovation on challenges affecting public health in India. Groups of talented faculty members, scientists, and young researchers helped the institute navigate uncharted territory as the first translational research institute in India, under the guidance of the senior advisors and academics in the THSTI Society and Governing Body, supported by the Department of Biotechnology, Government of India. With much initial exploration, in the past year, we identified areas that both need attention and where THSTI has built unique expertise. This annual report, has thus, been structured according to the 2018-2019 re-organization of THSTI – as four theme-based programs (1. Infection and Immunology, 2. Multidisciplinary Clinical and Translational Research, 3. Maternal and Child Health and 4. Non-Communicable Diseases) and four core facilities (1. Biorepository, 2. Data Management Centre, 3. Bioassay Laboratory, 4. Small Animal Facility).

The Infection and Immunology group is pursuing TB, virology and microbiome research. Efforts of the TB group have been focused on understanding the biology of the host and the pathogen, to subsequently use the knowledge generated to inform the development of effective, safer vaccines, diagnostics, and drugs. The nationwide support facilitating TB researchers in the country through the provision of reagents is now also catering to international researchers. Host-pathogen interactions in dengue infection, understanding dengue virus behavior inside host cells, as well as studying aspects of Hepatitis E virus biology are all directed to find drugs for controlling these viral infections. A new area of research is being developed, in providing better ways for studying influenza and developing new influenza vaccines for which we hope to have new collaborations and support mechanisms. The HIV program has reduced its focus on the development of antigens and has expanded the scope of their broadly neutralizing anti-HIV antibody research to cover snake envenomation. The research on enteric bacteria is



The reorganized structure of THSTI



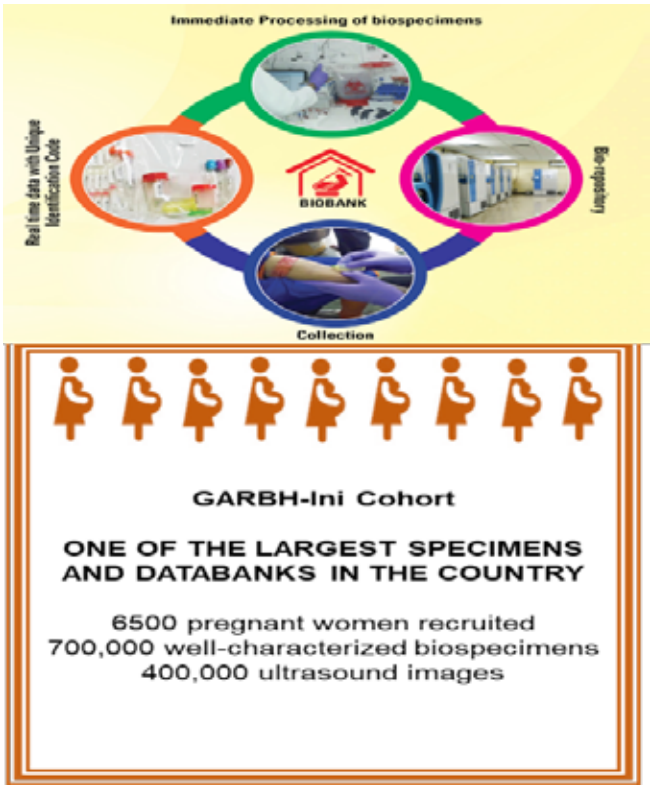
Infection and Immunology program comprises groups investigating the underlying biology, development of vaccines, diagnostics and therapeutics for TB and viral illnesses. Also included is the work on understanding antimicrobial resistance.

on how genes that code for antimicrobial resistance (AMR) in bacterial pathogens are acquired and lost, as well as how the gut flora aids or prevents AMR gene movement. This knowledge will help develop new treatments and we will be better equipped to eliminate the factors that enable AMR in bacterial pathogens.

Infection and Immunology program comprises groups investigating the underlying biology, development of vaccines, diagnostics and therapeutics for TB and viral illnesses. Also included is the work on understanding antimicrobial resistance.

The MCTR team identifies unmet needs and develops diagnostics customized for India. During the past year, the aptamer-based assays for TB detection which have been developed in partnership with AIIMS were tested further and showed better performance than previously available tests. The aptamer technology is also being used for a snake bite diagnostic program, paralleling the neutralizing antibody approaches to developing new ani-venoms, to also consider diagnostics to identify the species of snake, so that appropriate anti-venom can be used. This is important, because snake bites are under-recognized as a public health problem, with India accounting for half the snake bite-related deaths occurring globally. Diagnostic assays for blood-borne illnesses and tropical fevers continue to be refined for point-of-care testing. The absence of a rapid test for pathogen identification and antimicrobial susceptibility testing leads to irrational antibiotic use and THSTI has begun its efforts to address this issue by developing rapid tests to guide treatment.

The Atal JaiAnusandhan Biotech Mission with five mission programs was announced by the Union Minister for Science & Technology at the 33rd Foundation Day ceremony of DBT. The Biotech Mission includes IndCEPI which is aimed to develop affordable vaccines for endemic diseases and GARBH-Ini, to promote maternal and child health and develop prediction tools for preterm birth. Both of these are programs in which THSTI is playing a large role.



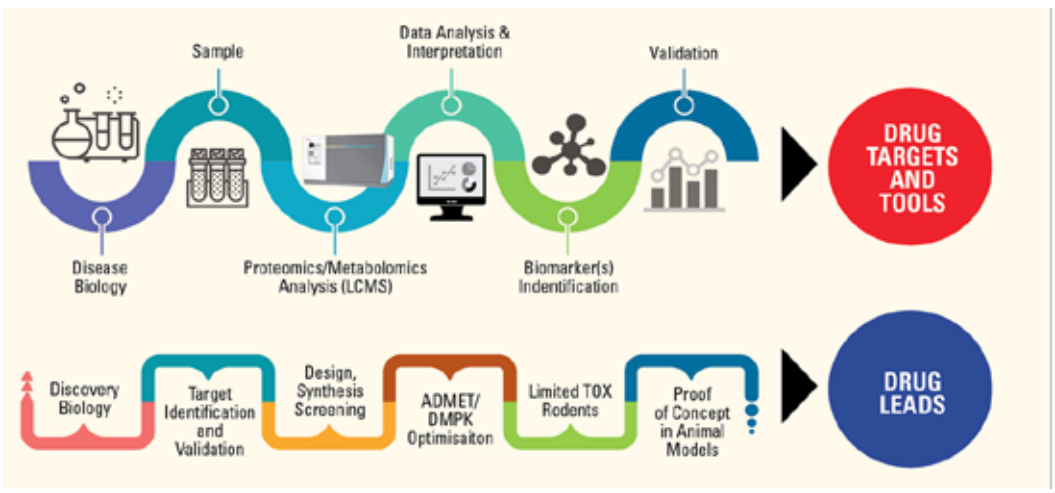
The GARBH-Ini cohort, which follows pregnant women from early pregnancy through delivery has grown to 6500 pregnant women; 700,000 well-characterized biospecimens and 400,000 ultrasound images form one of the largest specimens and databanks in the country. This repository will serve as a global resource to answer important biological questions related to maternal and child health. A collaboration has been initiated with the Indian Institute of Technology-Madras (IIT-M) to apply machine learning and artificial intelligence approaches for predicting pregnancy outcomes and childhood mortality and morbidity.

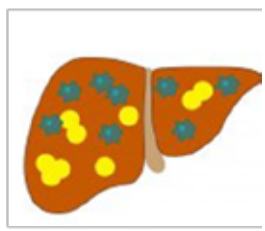
In the non-communicable disease program, fatty liver, a large and increasing problem in India, is a new area being developed. Animal models for this condition, also known as Nonalcoholic fatty liver disease/Nonalcoholic steatohepatitis (NAFLD/NASH) have been characterised and are being used for experimentation. The non-communicable disease program has developed a collaboration with Dabur, a company known for its quality herbal products, and is evaluating its products to understand how they work in well-characterized cell-based systems and animal models. In work published during the year, vitamin D deficiency was shown to be a risk factor for heart failure.

Nonalcoholic fatty liver disease (NAFLD), a problem on rise in India, is the focus of research in the non-communicable disease program

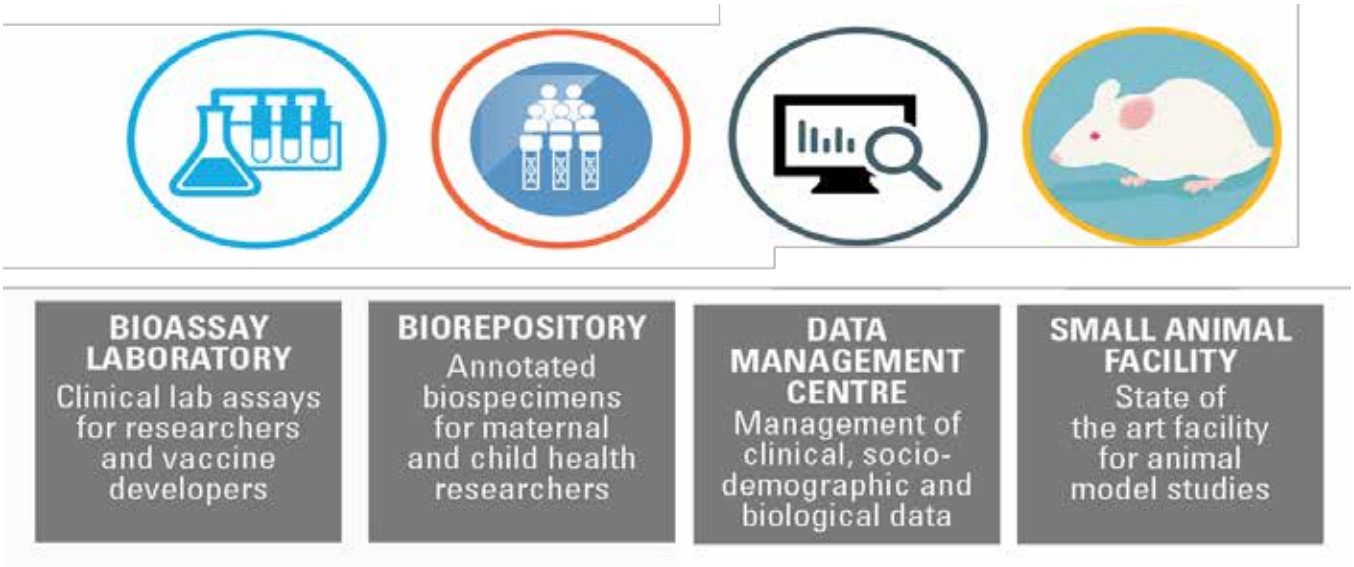
The Data Management Centre (DMC) has developed an in-house Laboratory Management System (LMS) using barcodes to track the journey of a biospecimen from collection till the time it is analyzed or stored in the biorepository. The bioassay lab has acquired necessary equipment, optimized laboratory procedures and trained a core team. The Infectious Disease Research Facility (IDRF), a specialized animal biosafety level III containment facility was made functional at the Small Animal Facility to aid research on TB and HIV. Mandatory training is being conducted for users within and outside THSTI.

In 2018-19, the Clinical Development Services Agency (CDSA) conducted 19 programs across 14 different cities to train biomedical researchers, clinicians, scientists and ethics committee members. CDSA also recently launched India's first online courses on drug





Nonalcoholic fatty liver disease (NAFLD), a problem on rise in India, is the focus of research in the non-communicable disease program



regulations with technical support from the National Programme on Technology Enhanced Learning (NPTEL).

Research groups at THSTI published 100 peer-reviewed publications during the past year. Six students were awarded doctoral degrees in the last academic year. Thirteen undergraduate and postgraduate students from institutes across the country were trained through the Short-term training program (STTP). An exciting new very short-term training program (Shadow A Scientist) was arranged and 22 students from 6 colleges across NCR participated. An open day was conducted as a pre-event of the India International Science Festival (IISF), 2018 and attended by more than 200 students from seven schools and colleges; eight visits were organized wherein faculty members, scientists and PhD students visited colleges in NCR for delivering lectures. The outreach and the open day programs received excellent feedback from students.

The institute organized scientific meetings and other events that hosted national and international researchers to aid collaborative ventures, exchange of ideas and training.

The institute is deeply engaged in what was previously largely uncharted territory of translational health science research in India. At the end of its first decade, THSTI is now in a position to be an example for institutions to not only strive for excellence in research and innovation but also reach the last mile to deliver solutions to public health problems.

INFECTION AND IMMUNOLOGY

INFECTION AND IMMUNOLOGY

Tuberculosis

TB is still a major health crisis that necessitates the need to develop better vaccines, drugs and diagnostic tools. Annually, the current incident rates of TB are ~ 10.4 million and 1.6 million deaths. World Health Organization (WHO) estimates 600,000 cases of rifampicin-resistant TB, of which, 490,000 were multidrug-resistant (2016).

At least a quarter of the world's population is latently infected with *Mycobacterium tuberculosis* (*M. tuberculosis*) bacilli. As a result, when any of such latently infected individuals reach an immunocompromised state, the pathogen resurfaces to cause active TB. In addition, over the last decade, drug-resistant TB (DR-TB) has emerged as a major challenge to treatment. It is a challenge to cure DR-TB with the combination of existing first-line drugs and demands the intervention of new chemotherapeutic agents.

Tuberculosis pathogenesis and novel anti-TB therapeutic targets

Dr. Nisheeth Agarwal's lab is pursuing the long-term goal to understand the molecular mechanism of TB pathogenesis and the emergence of drug resistance and persisters. They specifically focus on the characterization of essential metabolic pathways in mycobacteria, understanding the host response to mycobacterial infection and identification of new druggable targets in *M. tuberculosis* and host macrophages. These objectives are being accomplished through two projects with a goal of investigating molecular changes underlying *M. tuberculosis* infection. In one they adopt a systems approach to decipher the modification of phosphorylation status of host proteins brought about following infection by virulent and avirulent strains of mycobacterium. The second one is aimed at identifying and characterizing novel metabolic pathways essential for *M. tuberculosis* to thrive in the host tissue as well as in a synthetic medium. The information generated through these efforts is applied while screening small molecule inhibitors as an early attempt towards new TB drug development.

The team continued its work on three aspects: 1) comparative analysis of the effect of infection with virulent and avirulent mycobacteria on host phosphoproteome profile 2) characterization of the *in vivo* functioning of DNA gyrase in *M. tuberculosis* and 3) understanding the mechanism of proteostasis by emphasizing the role of Clp proteases. They also initiated a nationwide program to facilitate TB researchers in the country by providing them defined CRISPRi-based knockdown plasmid constructs and mutant strains. DBT is considering a joint proposal with CSIR-IMTech (Institute of Microbial Technology, Chandigarh) for funding against a call for proposal on "Genome Engineering Technologies and Their Applications".

Last year, the team's analysis of the host proteome and phosphoproteome unraveled the role of host inflammasome pathway in the intracellular survival of *M. bovis* BCG. Further analysis by hierarchical clustering and ingenuity pathway analysis tool revealed significant changes in the phosphorylation status of host proteins upon infection with *M. tuberculosis* and BCG such as those associated with actin metabolism, nitric oxide synthesis, dendritic cell maturation, and protein degradation. Apart from these, mycobacterial infection also modulated phosphorylation of many signaling proteins involved in mTOR, GPCR, integrin, MAPK, AMPK signaling pathways, etc. **(Figure 1.1)**.

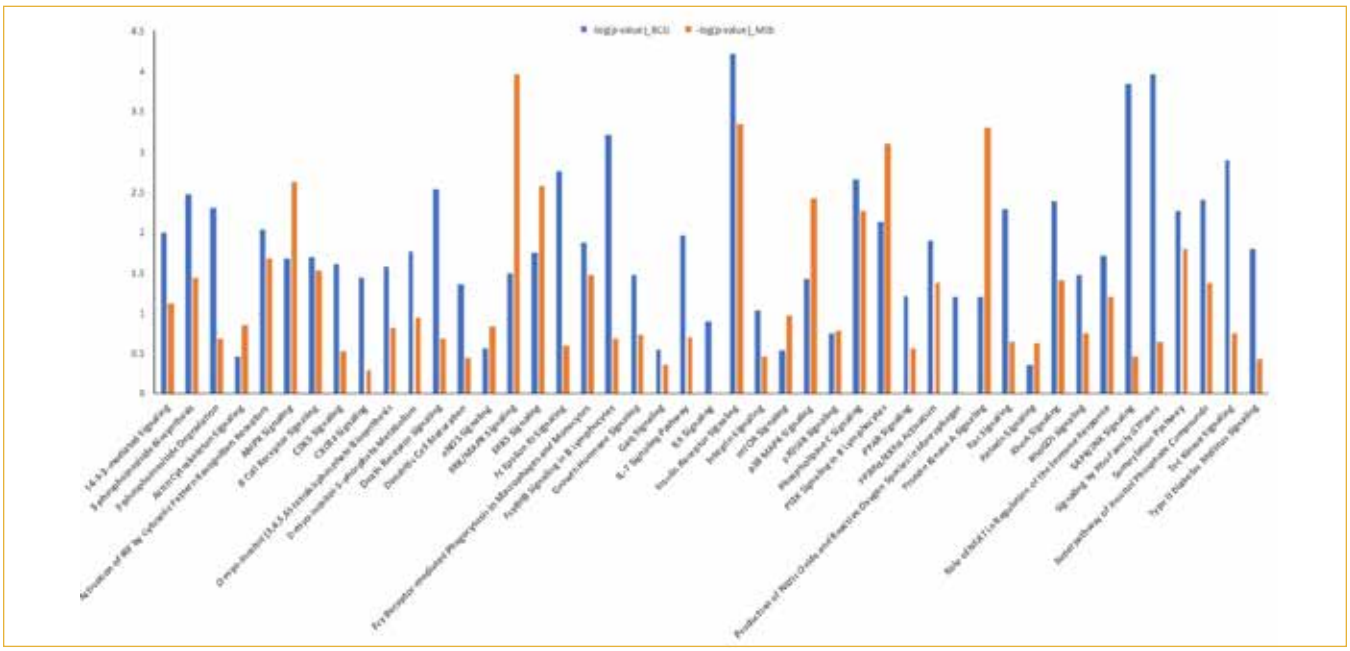


Figure 1.1: Analysis of canonical pathways that are differentially phosphorylated upon infection with M. tuberculosis and BCG. Shown in this graph are - log p values for each pathway which is significantly modulated.

Interestingly, they also found many phosphoproteins that are enriched specifically in response to BCG and *M. tuberculosis* infections **(Figure 1.2)**. Phosphoproteins that regulate host immune response and cell death are modulated during infection

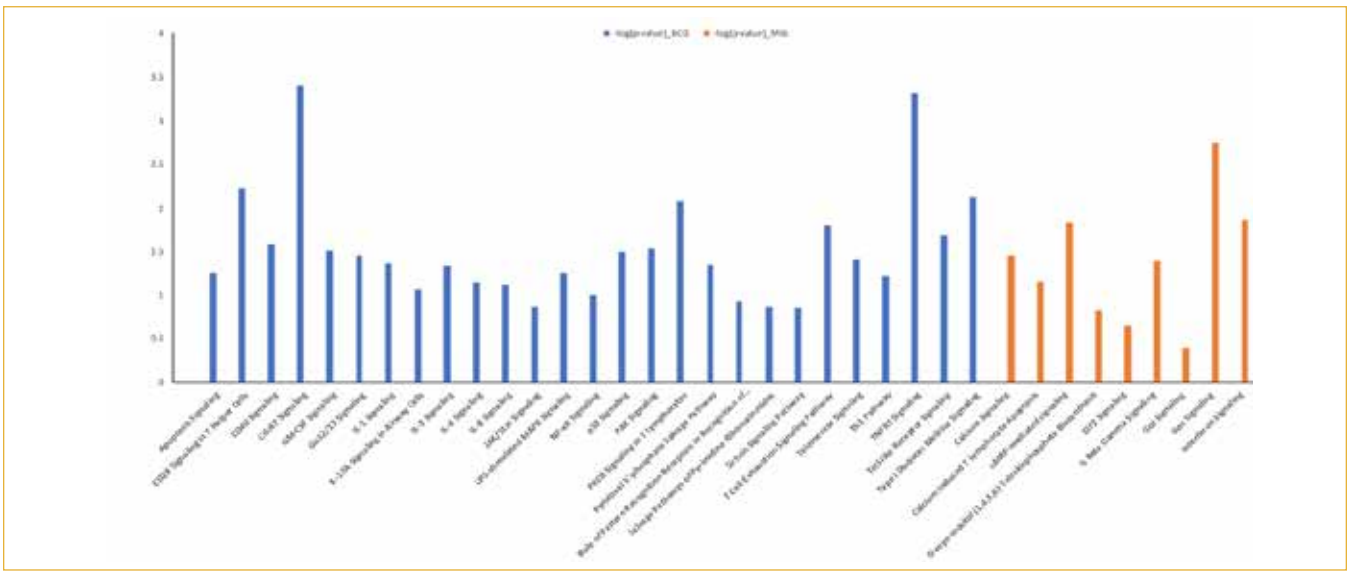


Figure 1.2: Analysis of canonical pathways that are differentially phosphorylated specifically to M. tuberculosis and BCG infections. Shown in this graph are - log p values for each pathway which is significantly modulated.

by avirulent BCG whereas *M. tuberculosis* infection affects phosphorylation status of proteins involved in calcium signaling, cAMP-signaling, GPCRs and interferon signaling **(Figure 1.2)**.

In regard to the characterization of the role of essential metabolic pathways in *M. tuberculosis*, the group had previously identified how genetic suppression of DNA gyrase in *M. tuberculosis* affects phenotypic tolerance to first-line anti-TB drugs attributed to DNA damage response mediated by poor gyrase activity. Subsequently, they performed analysis of metabolites by mass spectrometry (MS) which identified accumulation of several nucleotides such as thymidine 5'-monophosphate (TMP, 3.0-fold), adenosine 5'-monophosphate (AMP, 2.18-fold), guanidine 5'-monophosphate (GMP, 3.42-fold) and uridine monophosphate (UMP, 2.19-fold) in DNA gyrase-depleted bacteria. Moreover, they also found significant increase in the levels of other metabolites in *gyr(-)* such as coenzymes nicotinic acid (NA, 3.61-fold) and nicotinic acid mononucleotide (NAMN, 2.58-fold) that are key regulators of energy metabolic pathways; and amino acids such as L-(-)-methionine (L-Met, 2.46-fold), L-glutamine (L-Gln, 1.38-fold) and L-pyroglutamic acid (PCA, 1.37-fold), respectively (**Figure 1.3**). Overall, the accumulation of these metabolites suggested that suppression of DNA gyrase results in perturbation of the central metabolic pathways in *M. tuberculosis*.

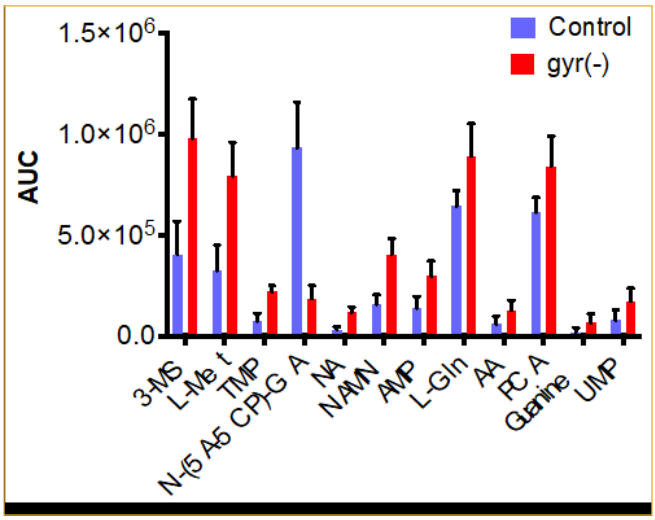


Figure 1.3: Analysis of metabolites that are modulated in M. tuberculosis upon suppression of DNA gyrase

Overall, the accumulation of these metabolites suggested that suppression of DNA gyrase results in perturbation of the central metabolic pathways in *M. tuberculosis*.

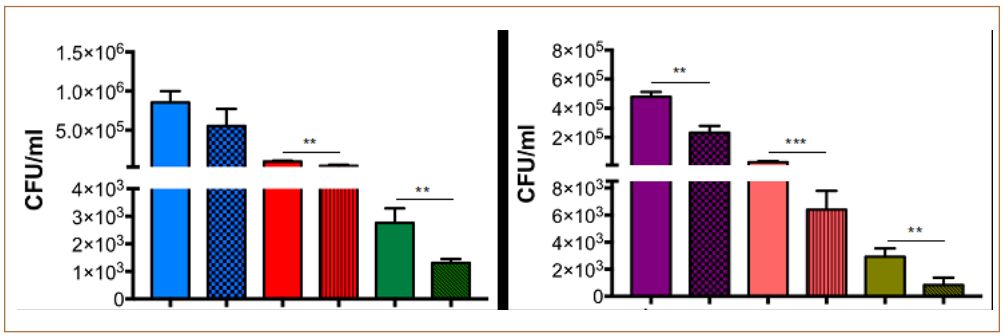


Figure 1.4: Effect of suramin on killing effects of Rif and INH against wild-type (left) and gyr (-) knockdown (right) strains.

Next, they tested if the suppression of the SOS response affects the bacterial susceptibility to drugs. RecA was inhibited in vivo by incubating wild-type and *gyr(-)* strains with suramin, and subsequently bacterial susceptibility to Inh and Rif was determined. As depicted in **Figure 1.4**, they observed a marked improvement in the bactericidal effect of Rif and INH against both the strains. Since proportion of persisters was relatively more in *gyr(-)*, they found better effect of suramin against it compared to wild-type strain of *M. tuberculosis*.

In another project on understanding the regulation of proteostasis by Clp proteases in *M. tuberculosis*, they earlier showed how these proteases are activated by selective degradation. Concurrently, they also set up an *in vitro* assay and performed screening of small molecule inhibitors against Clp proteins. Subsequently, they performed studies to characterize the underlying mechanism of degradation of prospective substrates by Clp proteases. The results showed that proteins are

degraded irrespective of the molecular mass of a single subunit. However, proteins forming intramolecular complexes resist proteolysis. Importantly, degradation is sequence-nonspecific and relies on the conformation of target proteins.

The overall focus of the laboratory has been to identify host factors that cause differential susceptibility to TB (both infection and disease) in humans and identify new drug targets. It is interesting to note that even the close contacts exhibit varying degrees of symptoms or no symptoms at all. Dr. Agarwal's team feels that there is a strong need to understand the host responses to *M. tuberculosis* infection so that new molecular targets can be identified in the host. In addition, we urgently require new chemical inhibitors for tackling DR-TB cases. The lab is working to characterize novel host pathways that are differentially regulated upon infection and control mycobacterial growth in the host cells to accomplish these goals.

They are also working on potential target genes in *M. tuberculosis* itself to understand the cause(s) of the emergence of drug resistance in bacteria and to come up with potential drug targets and new scaffolds for future evaluation against drug-resistant as well as susceptible population. While suramin has shown some promise in improving the activity of first-line anti-TB drugs against persisters *in vitro*, its effect in mouse model of infection is yet to be determined. In the last few years, the team has made significant progress in recognizing a handful of target genes in *M. tuberculosis* that show promise for screening small molecule inhibitors. Also, efforts to identify host pathways will help to better comprehend the differential levels of susceptibility in humans towards this 'difficult to cure' disease.

There is an urgent need (i) to understand the metabolic pathways that enable *M. tuberculosis* to persist in the host and (ii) for the development of shorter course, more effective, safer and better-tolerated treatment regimens. **Dr. Ramandeep Singh's** lab is working to improve our understanding of the role of various metabolic pathways such as toxin-antitoxin (TA) systems, inorganic polyphosphate metabolism, reductive TCA cycle, GntR family of transcription factors in *M. tuberculosis* physiology and pathogenesis. Another aspect of the research activities is to identify small molecules that possess a novel mechanism of action, are compatible with the current regimen and active against both drug-resistant and susceptible bacteria. To address this, they are performing (i) phenotypic screening (ii) target-based screening and (iii) modulation of host anti-microbial pathways to develop novel interventions against *M. tuberculosis*.

Toxin-antitoxin (TA) systems are widespread in prokaryotes and are involved in diverse physiological processes such as stress adaptation, plasmid maintenance, phage protection, virulence, and biofilm formation. TA systems exist as cognate pairs of either protein: protein or protein: RNA molecules. Based on the nature of antitoxins and the mechanism of toxicity neutralization, TA systems have been broadly classified into six types - Type I to VI. Among these six subtypes, Type II is the most well-characterized TA systems in prokaryotes. The genome of *M. tuberculosis* encodes for 90 TA systems and among these 50 belong to the Virulence-associated protein B and C (VapBC) family of TA systems. VapC, the toxin, inhibits protein translation by cleaving either tRNA or the sarcin-ricin (SRL) loop of 23S rRNA or mRNA, and this activity is neutralized by their cognate VapB antitoxins. Several DNA-binding motifs such as ribbon-helix-helix (RHH), helix-turn-helix (HTH), AbrB and Phd/YefM have been mapped to the N-terminal domain of antitoxins. Dr. Singh's team had previously reported that VapBC TA systems are differentially expressed, regulated in a post-transcriptional manner and their overexpression

results in bacteriostasis. In the last year, they performed the structural and functional characterization of VapBC11 TA systems in *M. tuberculosis*. Previous work had shown that VapC11 is differentially expressed upon exposure to various stress conditions and antibiotics, suggesting its crucial role in stress adaptation and pathogenesis. Using the LIVE/DEAD BacLight bacterial viability kit, they observed that *M. bovis* BCG overexpressing VapC11 was viable in accordance with previous reports. They also showed that recombinant VapC11 cleaves $tRNA^{Leu}$ CAG in an Mg^{2+} dependent manner in ribonuclease assays. They further investigated the effect of VapC11 overexpression on the *M. tuberculosis* transcriptome. The ectopic expression of VapC11 was found to result in significant changes in the transcriptional profiles (**Figure 1.5**). The transcriptional profiles obtained were strikingly similar to those reported for nutritionally-starved bacteria, those undergoing the enduring hypoxic response, nonreplicating persisters and drug-induced persisters. They had reported that VapBC TA systems are differentially expressed and are also regulated in a post-transcriptional manner. Both MazF and VapC ribonucleases were shown to be essential for establishing infection in guinea pigs. Next, they evaluated contribution of the VapBC11 TA system in *M. tuberculosis* physiology and virulence. They generated a *vapBC11* knockout strain (*ΔvapBC11*) of *M. tuberculosis* using temperature-sensitive mycobacteriophages and the replacement of *vapBC11* locus with hygromycin resistance genes was confirmed by Southern blot and PCR analysis. They observed that the *vapBC11* TA locus was dispensable for *M. tuberculosis* survival upon exposure to *in vitro* stress conditions, such as nitrosative stress, hypoxia, nutritional stress, macrophage and exposure to antimycobacterial drugs. Further, they compared the survival of parental and *ΔvapBC11* strains in aerosol-infected guinea pigs at both 4 weeks and 8 weeks post-infection. In comparison to the parental strain, lung bacillary loads of *ΔvapBC11*-infected guinea pigs were reduced by 80-fold and 100-fold at 4 weeks and 8 weeks post-infection respectively. In concordance, a statistically significant 100-fold reduction in splenic bacillary loads was observed in mutant strain-infected guinea pigs. This difference in splenic bacillary load further increased to 1000-fold eight weeks post-infection. They also observed less pathological damage in lung section from *ΔvapBC11*-infected

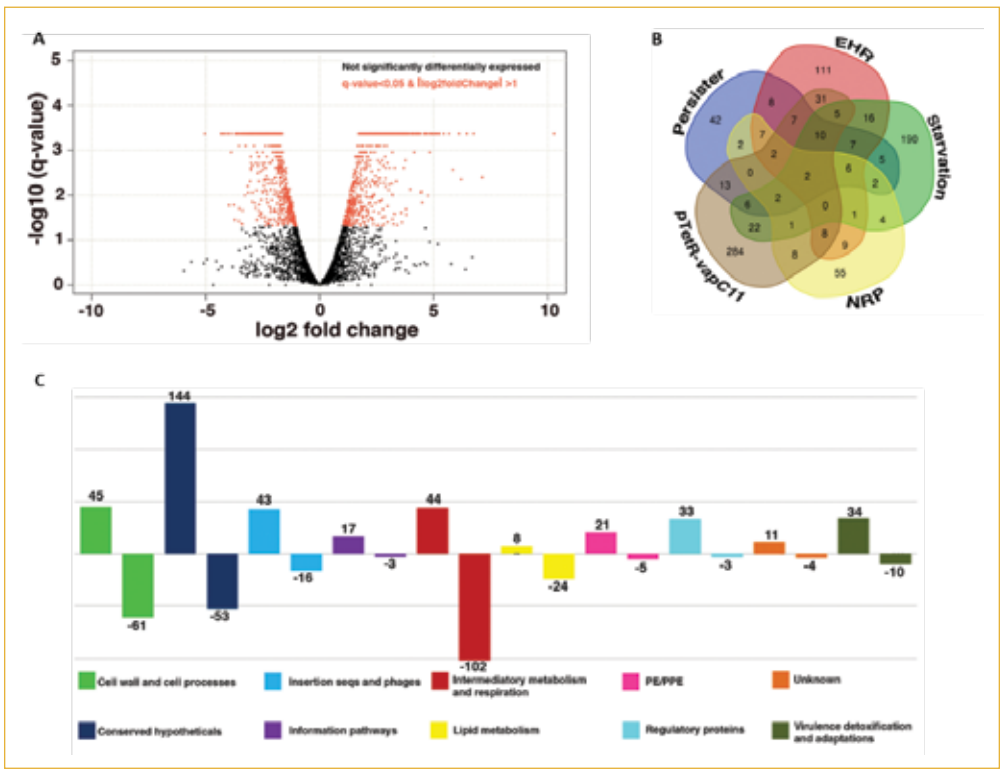


Figure 1.5: (A) This panel depicts volcano plot displaying the gene expression profile from 24 hrs post VapC11 induced samples. The Y-axis depicts q-values for each gene and the X-axis depicts fold change in either direction. (B) Venn diagram showing the correlation of differentially upregulated genes in M. tuberculosis overexpressing VapC11 (pTetR-vapC11) versus nutrient-starved (starvation), persister, nonreplicating persisters (NRP), and enduring hypoxia response (EHR) transcriptome. (C) Bar plot showing the number of differentially expressed genes with their pathway category/involvement, as annotated in the Mycobrowser https://mycobrowser.epfl.ch/

guinea pigs in comparison to tissue sections from parental strain infected guinea pigs. In collaboration with Dr. Krishan Gopal Thakur of CSIR-IMTech, Chandigarh, they solved the structure of VapBC11 at a resolution of 1.80 Å. Although the overall structure of VapC11 closely resembled the other known VapC structures, there were subtle differences in the C-terminal regions. They showed that in addition to PIN domains, the Arg94 residue is important for the catalytic activity of VapC11 endoribonuclease. The group further designed VapB11-derived peptides which were able to inhibit VapC11 ribonuclease activity. They observed that these peptides inhibited $tRNA^{Leu}$ CAG cleavage by VapC11. *In summary, they have structurally and functionally characterized VapBC11 TA systems from M. tuberculosis and designed peptide-based inhibitors against VapC11 ribonuclease.*

The tricarboxylic acid (TCA) cycle plays an essential role in cellular metabolism by providing reducing equivalents for energy generation and precursors for lipids and amino acids biosynthesis. The *M. tuberculosis* genome also encodes for enzymes involved in reverse TCA cycle. Most of the enzymes are common in forward and reverse TCA cycle except for ATP citrate lyase, α-ketoglutarate:ferredoxin reductase and fumarate reductase. Bacterial citrate lyase comprises six copies of each of the α, β and γ subunits. The γ-subunit (CitD) functions as an acyl carrier protein (ACP) and contains coenzyme A (CoA) derivative as a prosthetic group. The α-subunit (CitF) functions as an acyltransferase and is responsible for the formation of citryl-ACP intermediate. CitE, the β-subunit cleaves citryl-CoA into oxaloacetate and acetyl-CoA. The *M. tuberculosis* genome lacks homologs for α and γ subunits of citrate lyase but encodes for two homologs of β-subunit of citrate lyase, Rv2498c (CitE1) and Rv3075c (CitE2). Dr. Singh's team characterized both Rv2498c and Rv3075c from *M. tuberculosis*. They observed that citE1 and citE2 transcript levels increased by 60-fold and 5-fold upon exposure to low oxygen growth conditions. The exposure to nitrosative stress resulted in 100-fold and 20-fold upregulation of citE1 and citE2 respectively. Further, citE1 transcript levels were upregulated by 4-fold and 10-fold upon *M. tuberculosis* exposure to oxidative or nutritional stress respectively.

Next, to comprehend the role of these enzymes in stress adaptation and virulence of *M. tuberculosis*, they generated a mutant strain of *M. tuberculosis* devoid of both citE1 and citE2. The replacement of citE1 and citE2 with hygromycin and kanamycin resistance gene, respectively, in the mutant strain, was confirmed by both Southern blot and PCR. They observed that inactivation of CitE enzymes increased the susceptibility of *M. tuberculosis* towards oxidative and detergent stress. In comparison to the parental strain, the double mutant strain was more susceptible to killing by approximately 5-fold upon exposure to oxidative stress conditions. This was a cumulative effect as the single mutant strains were susceptible at levels that were comparable to those observed for the parental strain. They observed that in comparison to the parental strain, the double mutant strain exhibited a 4-fold decrease in bacterial counts upon exposure to 0.1% sodium dodecyl sulfate. The double mutant strain, *ΔcitE-DM* survived at rates comparable to those observed for the wild-type strain upon exposure to other stress conditions tested. They further observed that simultaneous deletion of both CitE1 and CitE2 rendered *M. tuberculosis* incapable of growing in macrophages. Also, *ΔcitE-DM* was severely attenuated for growth in guinea pigs at both 4 weeks and 8 weeks post-infection. The bacterial loads were lower by 75 – 90 folds in lungs and spleens of *ΔcitE-DM* infected guinea pigs in comparison to guinea pigs infected with the parental strain at 4 weeks post-infection. This

difference in bacterial loads increased by 90 folds and 1000 folds in lungs and spleens respectively, at 8 weeks post-infection. These observations showed that CitE enzymes contribute cumulatively to the ability of *M. tuberculosis* to replicate and cause infection in guinea pigs.

In collaboration with Dr. Bichitra Biswal of National Institute of Immunology, (NII) Delhi, Dr. Singh's carried out the structural and biochemical characterization of Rv3137, histidinol phosphate phosphatase from *M. tuberculosis*. The absence of enzymes involved in L-histidine biosynthesis and their essentiality in the *M. tuberculosis* genome makes them attractive targets. Dr. Biswal's team demonstrated that Rv3137 functions as the HolPase belonging to Inositol monophosphatase (IMPase) family and specifically dephosphorylates histidinol phosphate. The structure of Rv3137 was solved by Dr. Biswal to provide mechanistic insights into the activity of this enzyme. Dr. Singh's team performed target-based screening in a 96-well format to identify *M. tuberculosis* HolPase inhibitors. The screening experiments were performed in a reaction buffer containing 50 mM Tris pH-7.4, 5 mM dithiothreitol, 5 mM MgCl2, 100 µM L-Histidinol phosphate, 0.5 µM recombinant *M. tuberculosis* HolPase. The amount of Pi released in enzymatic assay was quantified using malachite green reagent as per manufacturer's recommendations. Next, they screened small molecules belonging to the NIH Diversity Set V to identify HolPase specific inhibitors. The initial screening was performed at 100 µM concentration and they identified five compounds (NSC65238, NSC67436, NSC311153, NSC608210 and NSC756645 that inhibited HisN dependent dephosphorylation activity by >40%. NSC 311153, the most potent inhibitor in our *in vitro M. tuberculosis* HolPase inhibition assays, displayed an IC50 value of 94.25 µM. This compound will be explored further for its efficacy *in vitro*, *ex vivo* and *in vivo* in future studies in the laboratory.

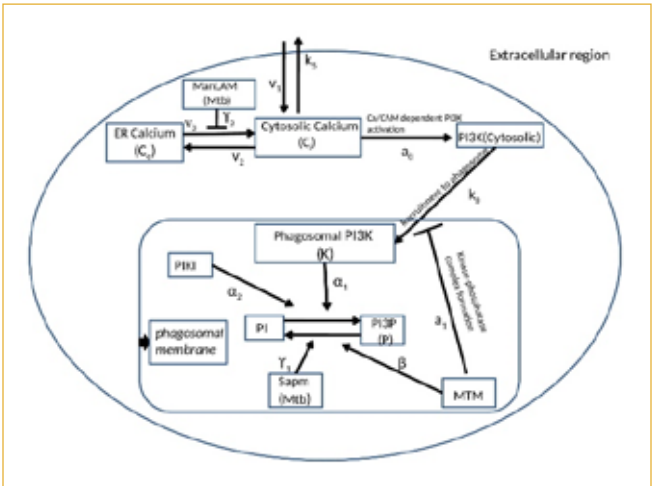


Figure 1.6: Schematic diagram of the host-pathogen interaction model under Mycobacterium tuberculosis infection. The variables are written in the first parentheses.

Mathematical model of host-pathogen interactions during Mycobacterium tuberculosis infections:

Mycobacterium tuberculosis's (*M. tuberculosis*) ability to modulate the host's anti-microbial pathways makes it a highly successful intracellular pathogen. Phagocytosis acts as the first line of defence against microbial infection. However, *M. tuberculosis* inhibits Phosphatidylinositol 3-phosphate (PI3P) oscillations which is required for phagolysosomal fusion. **Dr. Samrat Chatterjee's** team attempted to decipher the mechanisms underlying *M. tuberculosis's* elimination of phagosome-lysosome fusion. To address this, they built a four-dimensional ordinary differential equation model (**Figure 1.6**) and explored the contribution of PI3P during *M. tuberculosis* phagocytosis. Using this model, they identified some sensitive parameters that influence the dynamics of host-pathogen interactions. They observed that PI3P dynamics can be controlled by regulating the intracellular calcium oscillations. Some plausible methods to restore PI3P oscillations are endoplasmic reticulum (ER) flux rate, recruitment rate of proteins, like Rab GTPase, and cooperativity coefficient of calcium-dependent consumption of calmodulin. They further investigated whether modulation of these pathways is a potential therapeutic intervention strategy.

Their model analysis revealed that the calcium flux from ER to cytoplasm could be a possible therapeutic strategy to clean bacteria from the host cell. To validate it experimentally, they showed that the RyR2 agonist caffeine stimulated calcium influx and inhibited the growth of intracellular *M. tuberculosis* in macrophages. Taken together, they demonstrate that the modulation of host calcium level is a plausible strategy for killing intracellular *M. tuberculosis*. The experimental validation was done in collaboration with Dr. Singh.

They plan to understand and capture other interactions between *M. tuberculosis* with its host using mathematical models. They are also working with the collaborators to validate the results through *in vitro* experiments and then, if possible, through *in vivo* experiments.

Mechanistic understanding of persistence in mycobacteria

One of the most challenging aspects of TB treatment is the presence of a slow-growing, non-replicating, metabolically inactive "persister" population of bacilli inside host cells, that requires an extremely long treatment regimen. The capacity of *M. tuberculosis* to enter a dormant state leading to latent infection is the key to the survival of *M. tuberculosis* inside the host, thus delaying the efficacy of currently available therapies. **Dr. Amit Pandey** believes that inhibiting dormancy or altering the metabolic state of dormant *M. tuberculosis* could increase the effectiveness of antibiotics and shorten treatment duration. His lab has been working on the hypothesis that the differentially regulated critical metabolic pathways triggered by the intracellular nutrient availability and requirements contribute significantly towards the generation of *M. tuberculosis* persisters. They earlier demonstrated that *M. tuberculosis* could metabolize and survive on media containing cholesterol as a sole carbon source and that cholesterol metabolism is very critical for *M. tuberculosis* persistence. Utilizing genetic and high-dimensional informatics approach, they have identified nutrient-specific pathways critical for the generation of persisters in mycobacteria. Their efforts could aid 1) an improved understanding of host-pathogen symbiosis and 2) designing novel intervention strategies targeting persisters (**Figure 1.7**).

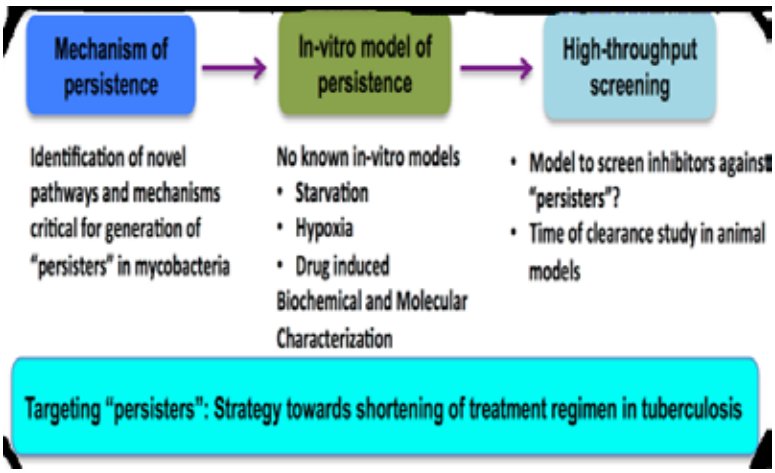


Figure 1.7: Schematic representation of the strategy employed towards shortening of treatment regimen in tuberculosis.

Dr. Pandey's lab identified a novel pathway in *M. tuberculosis* essential for the generation of persister during infection. Further mechanistic understanding studies led to the identification of *M. tuberculosis* proteins that could potentially be used as a target against persisters.

Cholesterol utilization pathway

Being an intracellular pathogen, *M. tuberculosis* is completely dependent on the host for its nutritional requirements. Inside the host, *M. tuberculosis* subsists mainly on host-derived fatty acid and cholesterol as a preferred carbon source. Although *M. tuberculosis* ingests host-

derived cholesterol throughout the infection process, cholesterol becomes essential only during the later stage of chronic infection. Using cholesterol-rich media, Dr. Pandey's lab has identified one of *M. tuberculosis* pathways critical for the generation and enrichment of persisters during mycobacterial infections (manuscript under review). Genetic and molecular understanding of cholesterol utilization, its mechanism and relevance would contribute significantly not only towards designing novel intervention strategies in the treatment of tuberculosis but could provide interesting target against "persisters".

Pathways regulating Iron (Fe) homeostasis

Although iron is essential for most of the bacteria, excess of intracellular free iron is toxic. Failure to do so might lead to death either due to iron deficiency or toxicity. Hence, the acquisition and storage of iron in bacteria are tightly regulated. Since iron deprivation is also one of the anti-microbial strategies that the host adopts, both pathogen and the host compete for the limited iron during infection. Dr. Pandey's team is the first to demonstrate that the *M. tuberculosis* transcription repressor protein $sufR_{TB}$ regulates the *Isc* operon and has a role in controlling the intracellular iron homeostasis in *M. tuberculosis*. Disruption of the iron homeostasis in $\Delta sufR_{TB}$ decreased the fitness of the mutant strain to grow inside mouse bone marrow-derived macrophages. The transcription repressor protein $sufR_{TB}$ was also required for growth of *M. tuberculosis* under oxidative and nitrosative stress conditions. The enhanced biofilm production phenotype observed *in* $\Delta sufR_{TB}$ is intriguing and suggests a role of intracellular iron homeostasis in the generation of biofilms in mycobacteria. Finally, they have demonstrated that the $sufR_{TB}$ protein-mediated regulation of Fe homeostasis (in *M. tuberculosis*) is required for *M. tuberculosis* to persist inside the host.

Designing novel intervention strategies against "persisters"

Based on the information derived out of the above studies, Dr. Pandey's lab has developed recombinant *M. tuberculosis* and *M. bovis* BCG strains, that could potentially be used as an *in vitro* model of persistence in mycobacteria (patent under process). Currently, the lab is developing protocols for high-throughput screening of compounds that could potentially be inhibiting the generation persisters. Both target and phenotype-based screening approaches will be used to identify potential hits. Their goal is to validate these molecules in both *in vitro* and *in vivo* models of tuberculosis infection.

Feasibility and efficacy testing of a live recombinant M. bovis BCG strain as a novel vaccine candidate against tuberculosis

Despite the availability of the BCG vaccine, tuberculosis has been very difficult to eradicate. Since *M. tuberculosis* is an obligate intracellular pathogen, a timely activation of the cell-mediated immune response by the host is very important for its prevention. Although subunit vaccines against tuberculosis have done well in animal models, they fail to demonstrate similar outcomes and have failed badly in early human trials. On the contrary, the use of live attenuated strains seems to be very promising. These strains could either be genetically modified BCG or a very attenuated strain of *M. tuberculosis*. The former being more attenuated and in use certainly has an edge over the latter as far as the safety and regulatory

issues are concerned. The challenge is to design a vaccine strain that is safe, immunogenic, long-lasting and protects against tuberculosis caused by all strains. Dr. Pandey's lab is currently working on the hypothesis that some of the pathways critical for the generation of persisters also help *M. tuberculosis* mask itself from a very robust host immune response. Based on the information derived out of the persister studies, his team has generated recombinant *M. bovis* BCG strains that demonstrate enhanced antigenicity which, in turn, could eventually translate into better and longer protection against tuberculosis.

Vaccines' development for Tuberculosis

Despite global use of the TB vaccine BCG (Bacillus Calmette Guerin), every year at least 10 million people emerge as fresh TB patients. This is because BCG's protective ability is extremely variable and wanes rapidly within the first 5-10 years of life. By 2025/30, the Government of India (GoI) and WHO aim to reduce TB deaths by ~90% and TB incidence by >80%. Though improved diagnosis and accurate antibiotics therapy might slow down the current burden rates, achieving the targets would undoubtedly require administration of the alternate vaccine(s) that are superior to BCG.

Given BCG's mediocre rating and its ability to fail as a booster per se, the first long-term goal of this group is to design a subunit vaccine that (i) enhances BCG's protective efficacy; (ii) extends protection into several decades of life; (iii) prevent latently-infected individuals progress to active TB disease state; and (iv) steer immune responses in TB patients (especially with either multi and extremely drug resistance and/or extrapulmonary TB) from Th2 to Th1 pathway for rapid recovery (therapeutic vaccine).

Exploration of membrane vesicles of mycobacteria as a subunit vaccine candidate: All mycobacteria, similar to Gram-negative and Gram-positive bacteria, constitutively make and release extracellular membrane vesicles (mEVs). In general, EVs are 10-350 nm diametered circular structures that bud out of microbes' surface. They constitute a subset of the bacterial proteins, lipids, sugars, nucleic acids, and small molecules. Because of their natural packaging, the contents are considered to be in their native conformations thus providing the opportunity for us to explore them as immune priming agents (vaccine antigens). It is in this context, EVs of attenuated *Neisseria meningitidis* were packaged into an effective vaccine against Meningococcus B infections.

Dr. Krishnamohan Atmakuri's group had, in the past, standardized a protocol for enriching mEVs from large volumes of *in vitro* grown pathogenic and non-pathogenic mycobacteria. Later on, employing mass spectrometry (MS), they identified the vesicle proteomes. Comparative analyses indicated a few common and several distinct proteins. Pathogenic mEVs contained several proteins established to play a role in virulence/pathogenesis. Interestingly, the EVs proteome was vastly different between lab virulent and clinically-isolated mycobacteria indicating perhaps too much conditioning to laboratory media altered lab virulent EVs content. Lastly, they had also reported presence of mycobacterial nucleic acids (NAs) in the enriched mEVs preparations and how those NAs broadly influence mEVs protein content.

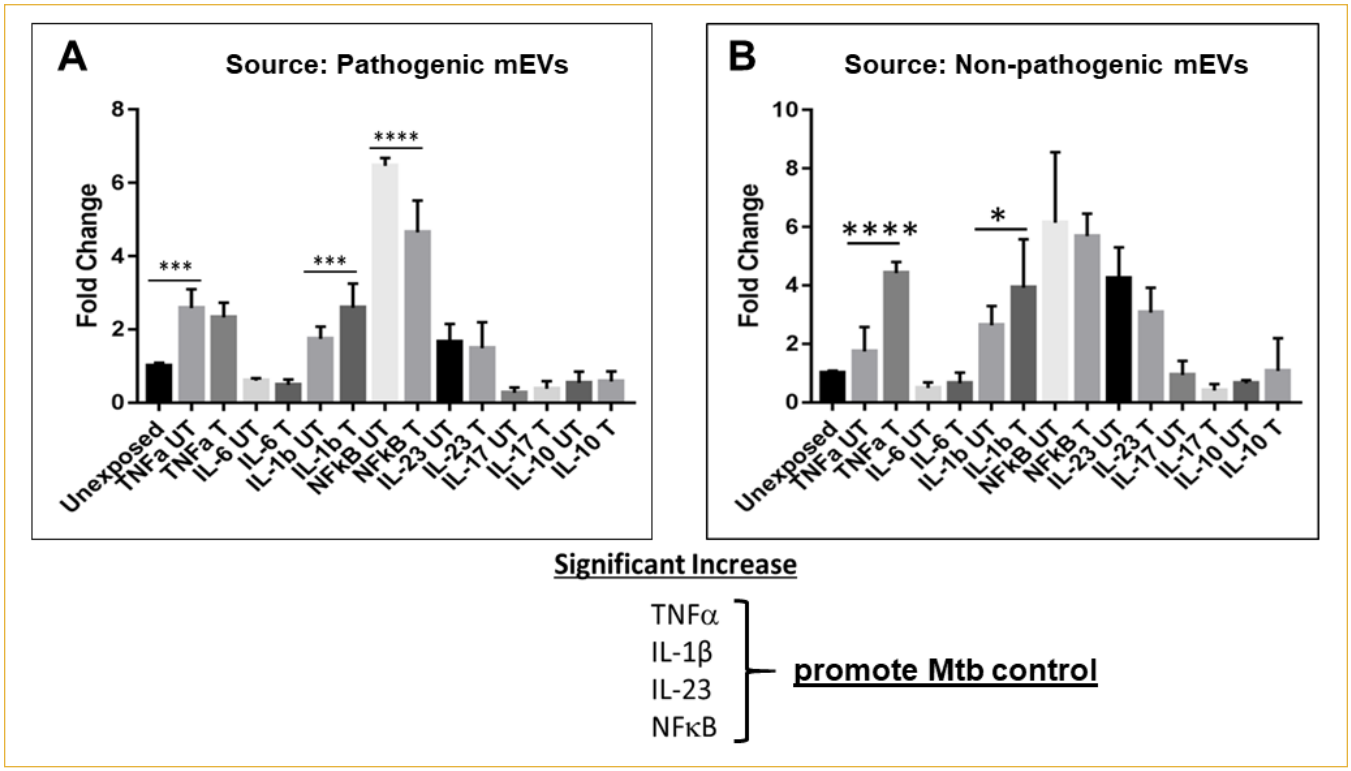
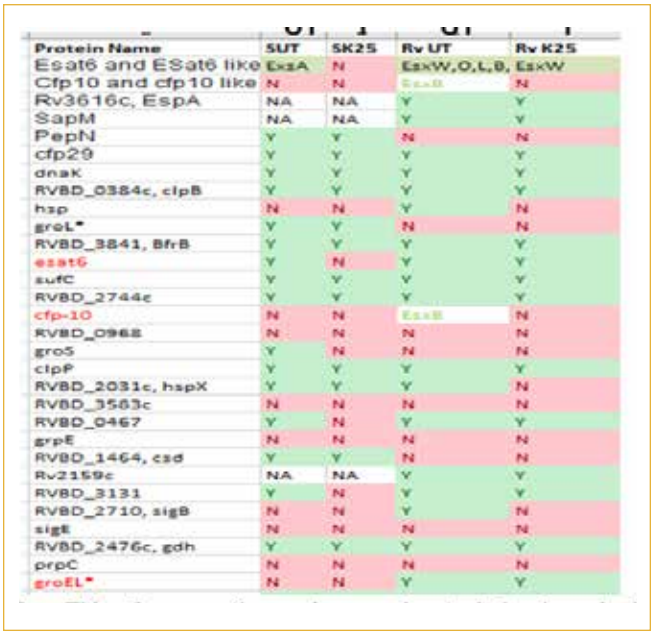


Figure 1.8: mEVs trigger proinflammatory cytokines. Treated (T) and untreated (UT) mEVs from Pathogenic (A; M. tuberculosis) and non-pathogenic (B; M. smegmatis) mycobacteria were added to differentiated macrophages (THP1) at 100:1 MOI. Five days later, cells were lysed, total RNA extracted and cytokine transcripts quantified by RT-PCR. House-keeping genes were used as control. Unexposed macrophages cytokine transcript levels were used for determining fold exchange. Each assay was performed thrice (biological triplicates) technical triplicates were employed each time and technical duplicate RT-PCRs were performed for each biological sample. Significantly increased cytokines are indicated at the bottom of A and B panels.

Around the same time, another research group from the USA reported that mEVs of the lab virulent mycobacterial strain when used as a vaccine candidate barely protects mice from pathogen challenge. Dr. Atmakuri's group predicted that perhaps the EVs of pathogenic mycobacteria suppress innate immunity pathways in mice leading to pathogen sustenance. To understand this better, they analyzed mEVs content in more detail and as expected found several virulent proteins of the pathogen. It is well established that pathogenic mycobacteria use virulent proteins to subdue host innate immune pathways and establish infection. Hence, this group proposes that in mice, while the EVs of pathogenic mycobacteria may trigger required proinflammatory immune responses, the virulent proteins that they harbor simultaneously subdue those triggers with sum total response leading to pathogen sustenance.

To test this directly, in the last year, Dr. Atmakuri's group evaluated if the pathogenic mEVs trigger or suppress the innate immunity in a model macrophage cell-line (THP1) infection assay. They had also shown earlier that because of the simultaneous enrichment of NAs, around 20-30% of mEVs proteome constitute NAs associating proteins (NAaPs). To make sure they don't influence the outcome of the THP1 infection assay, they first treated the enriched mEVs with nucleases and Proteinase K, cleaned the treated mEVs off the nucleases and Proteinase K and then compared the treated and untreated samples for cytokines trigger. Interestingly, both the treated and untreated mEVs triggered pro-inflammatory cytokines including TNFα, IL1β, IL23 and NFκB to similar levels (**Figure 1.8**). IL10, a representative of anti-inflammatory cytokines was however not upregulated by both EVs. They conclude that (i) mEVs of pathogenic mycobacteria indeed



Protein Name	SUT	SK25	Rv UT	Rv K25
Esat6 and ESat6 like	EsxA	N	EsxW,O,L,8,	EsxW
Cfp10 and cfp10 like	N	N		N
Rv3616c, EspA	NA	NA	Y	Y
SapM	NA	NA	Y	Y
PepN	Y	Y	N	N
cfp29	Y	Y	Y	Y
dnaK	Y	Y	Y	Y
RVBD_0384c, clpB	Y	Y	Y	Y
hsp	N	N	Y	N
groL*	Y	Y	N	N
RVBD_3841, BfrB	Y	Y	Y	Y
esat6	Y	N	Y	Y
sufC	Y	Y	Y	Y
RVBD_2744c	Y	Y	Y	Y
cfp-10	N	N		N
RVBD_0968	N	N	N	N
groS	Y	N	N	N
clpP	Y	Y	Y	Y
RVBD_2031c, hspX	Y	Y	Y	N
RVBD_3563c	N	N	N	N
RVBD_0467	Y	N	Y	Y
grpE	N	N	N	N
RVBD_1464, csd	Y	Y	N	N
Rv2159c	NA	NA	Y	Y
RVBD_3131	Y	N	Y	Y
RVBD_2710, sigB	N	N	Y	N
sigE	N	N	N	N
RVBD_2476c, gdh	Y	Y	Y	Y
prpC	N	N	N	N
groEL*	N	N	Y	Y

Figure 1.9: mEVs of non-pathogenic mycobacteria harbor virulent proteins homologs. Treated (T) and untreated (UT) mEVs from pathogenic (M. tuberculosis) and non-pathogenic (non-path; M. smegmatis) mycobacteria were processed by mass spectrometry to determine their proteomes. A subset of the virulent proteins identified in the Pathogenic mEVs are indicated as protein names to the left. SUT, M. smegmatis UT Evs; SK25, M. Smegmatis T Evs; Rv UT, M. tuberculosis UT Evs; and Rv K25, M. tuberculosis T EVs.

trigger proinflammatory pathways; (ii) their trigger seems undisrupted by mEVs treatment; and (iii) the associating NAs and NAaPs do not significantly alter the trigger. A similar outcome was also reported by the US group. Therefore, Dr. Atmakuri's group concludes that, in mice, though mEVs of pathogenic mycobacteria are capable of triggering proinflammatory cytokine responses, perhaps the accompanying virulence proteins subdue the overall immune responses in mice.

Interestingly, this group also observed homologs of several virulence proteins present in the EVs generated by non-pathogenic mycobacteria (**Figure 1.9**). Of course, these are barely homologs but with no innate virulent capabilities. Since pathogenic EVs barely protect mice from pathogen challenge, this group tested if EVs from a non-pathogenic mycobacterium would support a similar cytokine trigger response (as in **Figure 1.8A**). After treating the non-pathogenic mEVs, they performed similar macrophage cell-line (THP1) infection assay and quantified the induced cytokines transcripts. Surprisingly, the EVs from the non-pathogenic mycobacteria also exhibited a similar trigger response of the listed (**Figure 1.8B**) pro- and anti-inflammatory cytokines. As a consequence, they now hypothesize that EVs of non-pathogenic mycobacteria might be better vaccine candidates as (i) they trigger immune responses; (ii) lack the ability to cause virulence; and (iii) harbor virulent protein homologs that might be good immunogens but not subdue host immune responses. Such EVs may thus trigger protective immune responses that recognize and inactivate the pathogen and its virulent proteins during infections. In the coming year, they will test if these EVs, unlike those from pathogenic source, will be protective in an animal model of TB.

Design and development of recombinant MVs: In addition to simply exploring the EVs of non-pathogenic mycobacteria for their protective ability, this group hypothesizes that packing them with mutant forms of pathogenic virulent proteins might significantly enhance pathogen control when used as a vaccine candidate. Towards that, last year they designed and constructed a recombinant method to deliver heterologous proteins of interest. In the current year, they generated several recombinant EVs harboring well-established pathogenic immunogens including Ag85 complex. These will be tested in the next year in the *in vitro* macrophage cell-line (THP1) infection assay and in an *in vivo* animal model for TB. Additionally, the corresponding active site mutants will also be constructed.

B. Current long-term therapeutic treatment regimens (from 6-9 months to 2-3 years) lead to poor adherence rates. Poor adherence leads to the emergence of pathogenic multi- and extremely drug-resistant strains. Such mutant strains emerge because the drugs used for treatment target essential genes of pathogen growth.

The second long-term goal of Dr. Atmakuri's group is to identify small molecule inhibitors against secreted virulence factors of the pathogen that are essential for pathogenesis in the host. They hypothesize that targeting such factors post their

release from the pathogen helps not only attenuate the pathogen but also target the pathogen for elimination by host immune surveillance. Since the targeting of the secreted virulence factors occurs outside the pathogen, emergence/selection of multi- and extremely drug-resistant strains is highly remote.

Secreted virulence proteins of Mycobacterium tuberculosis – Targets for therapeutic application: To explore this, Dr. Atmakuri's group and his collaborators targeted ESAT6, a highly essential secreted virulent protein. As indicated earlier, to survive and establish infection, the TB causing pathogen delivers a battery of arsenal including ESAT6. Upon entry into the host environment, ESAT6 inhibits the host's autophagy flux, modulates phagosome maturation, promotes granulomas formation and drives recruitment of naïve alveolar macrophages to the site of infection. It is so important that in animal models, an *esat6* knockout is as attenuated as BCG. ESAT6 is secreted together with a chaperone CFP10 (culture filtrate protein of 10 kDa size) before it gets separated in the host. These are known to form a 1:1 dimer whose crystal structure is available.

Using the available ESAT6+CFP10 structure, and exploiting the comparative modeling and loop building methods, they first built ESAT6 only structure. They found that ESAT6, when alone, stabilized as a dimer. ESAT6 had been demonstrated by others to interact with at least three host proteins viz. β-2M (part of MHC Class I proteins), Syntenin1 and ADAM9, all interacting with ESAT6's C-terminal 11 amino acids region. Due to lack of co-crystal structure of ESAT6-host proteins complex, they performed protein-protein docking to obtain the most likely binding conformations for ESAT6-β-2M and ESAT6-Syntenin1 interaction. Then, using molecular dynamics (MD) simulations, they performed extensive conformation sampling of this complex. Following that, they conducted quantitative and thermodynamics analysis of the complex trajectory and calculated the binding free energy of the complex. They further calculated the free energy contributed by each amino acid in the complex association and identified the "hot-spot" amino acids critical for these pathogen and host proteins complexes to be stable. Through extensive computational, structural and biophysical analyses, they then narrowed down (*in silico*) seven different peptides with high binding efficiencies (*in silico*) that can potentially interact at the same site as the host proteins to ESAT6. The overall workflow of the approach is represented in **Figure 1.10**. To evaluate *in vitro* if one or more of these seven peptides indeed bind to purified ESAT6, they cloned *esat6* gene into an *Escherichia coli* expression vector, fused a 6X Histidine amino acid-tag to its N-terminus and purified it to homogeneity after affinity, size-exclusion, and ion-exchange

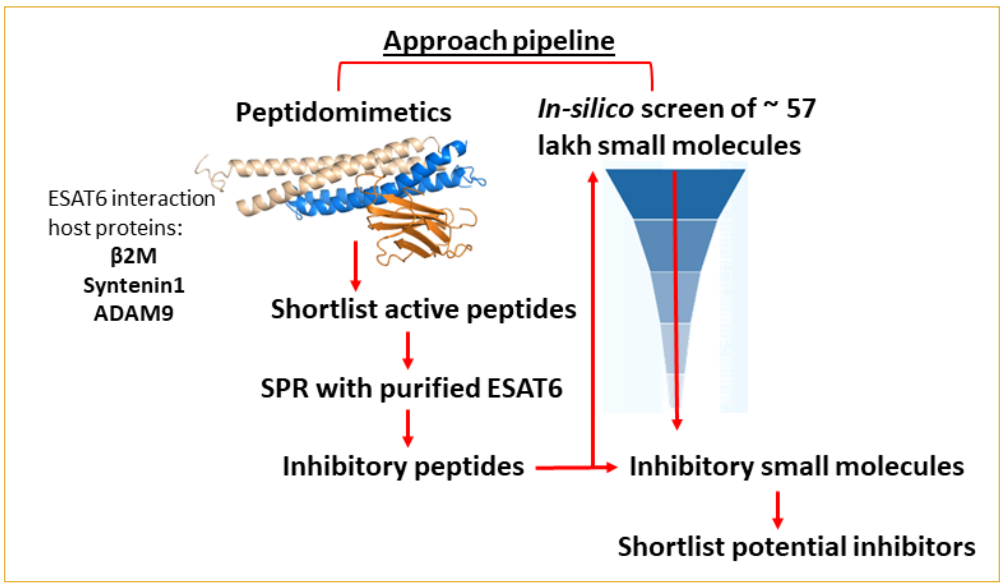


Figure 1.10: Overall work flow for shortlisting peptides and small molecule inhibitors against ESAT6

chromatography steps. After confirming the purified His-ESAT6 confirmation (with Circular Dichroism Spectroscopy) to be close to the reported crystal structure, they are currently testing the binding efficiencies of these peptides to ESAT6 by exploiting Surface Plasmon Resonance platform.

Identification of peptides from PPI interface to block ESAT6: Dr. Shailendra Asthana's team is using peptide/small molecule inhibitors to block secreted protein ESAT6 to attenuate the pathogen thus facilitating its elimination by the host immune system. For the same, few computationally active peptides were identified against ESAT6. These peptides were identified from among the possible host-interactors of ESAT6 using vast computational algorithms and resources. A set of few peptides appeared as ESAT6 binders to modulate its activity and confirmed through Surface Plasmon Resonance (SPR) binding analysis (Intramural Project with Dr. Atmakuri, THSTI).

The team successfully determined that EVs of pathogenic and non-pathogenic mycobacteria have equally good immunostimulant properties. In summary they,

- Determined that EVs of non-pathogenic mycobacteria also harbor several virulent protein homologs that might perhaps be good immunogens but poor virulent proteins.
- Determined that mEVs trigger pro- and not anti-inflammatory cytokines in host macrophages
- Determined that mEVs trigger the Th1 pathway that helps control *M. tuberculosis*-mediated pathogenesis.
- Generate recombinant EVs from non-pathogenic mycobacteria that harbor immunogens.
- Built an *in silico* structure to ESAT6, determined the potential binding sites of the host proteins to ESAT6 and identified potential inhibitory peptides to the interacting site.

As discussed previously, the current TB vaccine BCG continues to be a poor protector against pulmonary TB in adolescents and adults. The discovery of mycobacterial EVs incites a new and exciting possibility of exploring and manipulating them for as a potential subunit vaccine candidate or as a novel, subunit vaccine vehicle. Employing Guinea pig (GP) TB model, they soon intend to perform comparative analyses of protective abilities of mEVs obtained from pathogenic (both clinical (pulmonary and extrapulmonary isolates) and laboratory sources); attenuated; non-pathogenic and recombinant mycobacteria. They will also test the number of vesicles required for prime/boost BCG, test the route of delivery and the number of boosters necessary to achieve protection. Such an exercise will help narrow down the mEVs that need to be taken forward to furthering protection studies and mode of protection, both necessary for translation.

Also, mEVs of different sources will be evaluated in an animal model of TB for their rifampicin-resistant and -protective properties, immune pathways they trigger and their boosting ability as a subunit vaccine for BCG. In the ESAT6 front, the synthesized peptides will be tested for their binding efficiencies. Those that bind will be evaluated in the *in vitro* macrophage infection model for bacterial survival rates.

FLAVIVIRUS INFECTIONS

Host-pathogen interactions in Dengue

Zinc homeostasis in RNA virus infection

There is increasing recognition that nutritional deficiencies including micronutrients are important determinants of infection and their outcomes. Zinc is an acute phase reactant, and, therefore, the serum zinc levels are reduced during infections and conditions such as sepsis. Supplementation with zinc has been documented to provide protection against common childhood infections such as diarrhea but the same is not conclusively shown for respiratory infections. The basic molecular mechanism behind the maintenance of zinc homeostasis in normal versus disease conditions has not been characterized. It is possible that infections affect zinc homeostasis by altering relative concentrations of zinc within cells of specific tissues. **Dr. Guruprasad Medigeshi's team is investigating modulation of zinc homeostasis in virus infections using dengue virus (DENV), respiratory syncytial virus (RSV) -and rotavirus (RV) as models of infection.**

The team showed that transient zinc depletion inhibited dengue virus (DENV) and Japanese encephalitis virus (JEV) replication enhanced respiratory syncytial virus (RSV) infection but rotavirus (RV) replication was unaffected (**Figure 1.11A-D**). Zinc deficiency-induced oxidative stress and induction of oxidative stress had the same effect on virus infection as zinc chelation. They observed a strong induction of oxidative stress in RSV infection but no oxidative stress in both primary endothelial cells, epithelial cells or hepatoma cells infected with DENV. DENV infection appeared to suppress induction of oxidative stress as leukocyte populations isolated from dengue patients showed down-regulation of reactive oxygen species (ROS) levels (**Figure 1.12**). Their results provide interesting insights into the effect of zinc deficiency on RNA virus infections and contrasting susceptibility of RNA viruses to oxidative stress and possible link between the antioxidant role of zinc and antiviral pathways.

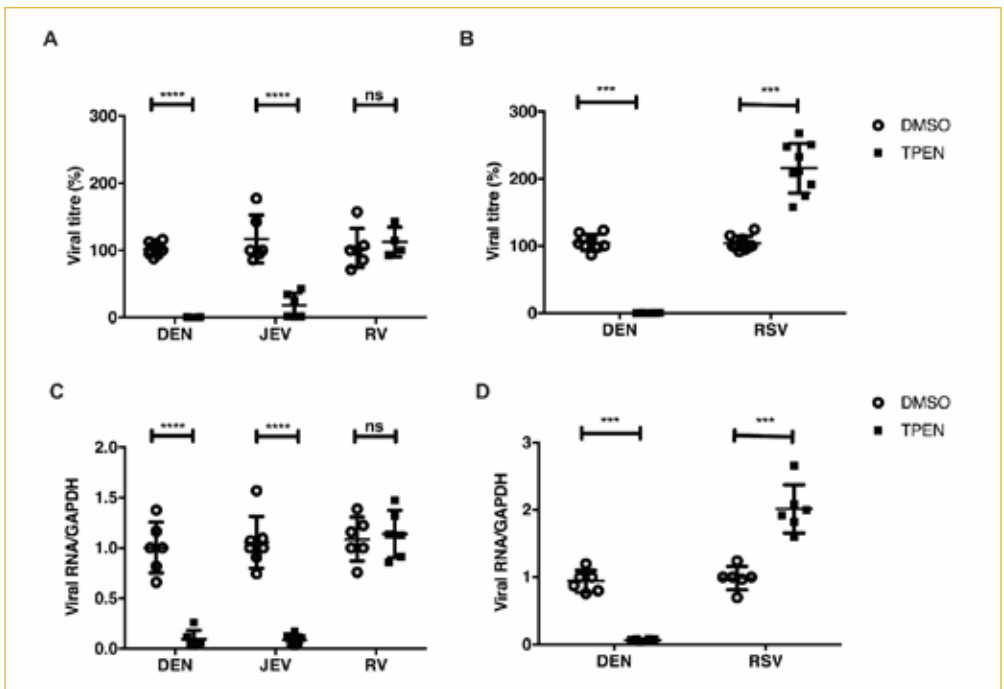


Figure 1.11(A-D): Zinc depletion specifically inhibits flavivirus infection.

Dr. Medigeshi's group is actively involved in running cohort studies with the Department of Pediatrics, All India

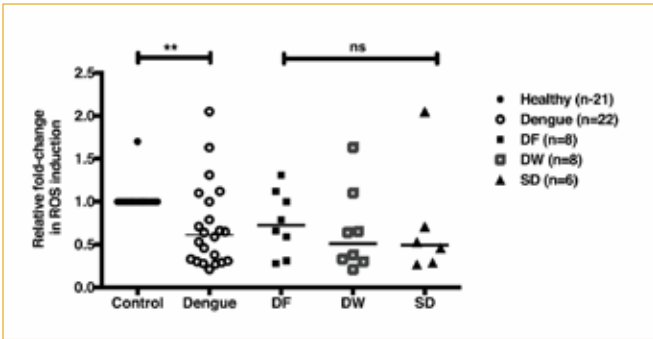


Figure 1.12: ROS induction in blood cell subsets from dengue patients

Institute of Medical Sciences, New Delhi and ESIC, Faridabad to investigate the role of specific subsets of blood cells and their zinc status in DENV and RSV infection. They hope to generate information on if and how viruses modulate zinc homeostasis and the molecular mechanisms involved in these alterations. They further hope to provide information that would rationalize zinc supplementation studies for specific viral infections. In addition to these studies, they are involved in the isolation of dengue viruses and molecular characterization of clinical isolates to gain insights into the sequence and phenotype of circulating dengue isolates.

Way ahead: The team intends to understand how different RNA viruses respond to conditions of zinc supplementation and depletion using *in vitro* models and identify pathways that are specifically altered under these situations. This information is expected to lead them to discover new drug targets. In addition to this, they plan to initiate animal studies to validate these results. They are also exploring community-based initiatives to further probe the effect of zinc sufficiency or deficiency on viral infections.

Pathobiology of Dengue virus

Dr. Sankar Bhattacharyya's team is focused on pathobiology studies of the Dengue virus (DENV) and particularly interested to comprehend the tropism of DENV in specific cell types found in the bone marrow. They are also contributing to, (i) discovery of either novel or repurposed drugs for use as anti-viral against DENV, (ii) elucidation of the cellular and molecular mechanism of function for traditional medications prevalent in India for management of Dengue Haemorrhagic Fever (DHF) and (iii) better management of critical symptoms associated with DHF.

Over the past year, Dr. Sankar's team added to our understanding of how dengue infection interferes with platelet biogenesis while also progressing in their search for the novel as well as repurposed drugs for therapeutic interventions in dengue. Mentioned here are few highlights:

- Dengue infection interferes with both JAK-STAT and MAPK signaling pathways which are crucial for megakaryopoiesis; megakaryocytes in the bone marrow serve as the initial reservoir for virus generation due to a promotion of its replication by the differentiation process.
- By reiterative efficacy assay, *in silico* modeling and design, they have developed a novel molecule that exhibited promising anti-viral activity in *ex vivo* models.

In collaboration with Dr. Prasenjit Guchhait of Regional Centre for Biotechnology, Faridabad, they were able to show the potential therapeutic effect of blocking specific cytokine receptor signaling by a repurposed drug in countering flavivirus pathology.

Way ahead: Dr. Sankar's team (with Dr. Asthana) is planning to patent the novel dengue inhibitor for its antiviral activity and develop novel and better molecules

for inhibiting signaling by cytokine receptors found to be beneficial for DENV replication.

Viral hepatitis

Dr. Milan Surjit's laboratory is investigating multiple aspects of hepatitis E virus (HEV) biology; (a) development of an animal model that mimics HEV infection in human and using this model to characterize the molecular mechanism of HEV pathogenesis, (b) understanding the host-pathogen interactions involved in HEV pathogenesis, (c) understanding the mechanism of viral translation, replication and release, (d) identification of novel anti-HEV compounds that can be used as therapeutic drugs and (e) development of a recombinant vaccine against HEV. The ultimate goal of the laboratory is to generate sufficient knowledge/resources for an in-depth molecular understanding of the lifecycle of the hepatitis E virus and development of efficient prophylactic and therapeutic products against the pathogen using the above knowledge/resources.

The team is identifying antivirals against HEV by targeting different stages of the viral life cycle. In the last year, they successfully identified two inhibitors against HEV and the same is summarized below.

- Inhibition of virus release is one possible antiviral development strategy, which limits the spread of the virus. Earlier studies have shown the interaction between the PSAP motif of the viral open reading frame 3 protein (ORF3-PSAP) and the UEV domain of host TSG101 protein (UEV-TSG101) essential in mediating HEV release. Dr. Milan's group has identified a cyclic peptide that blocks HEV release by inhibiting the interaction between the ORF3-PSAP and UEV-TSG101. The chemically synthesized cyclic peptide could inhibit virus release by approximately 90% in cell culture models.
- In another study, they identified the antiviral property of zinc against HEV. Earlier studies have reported broad-spectrum antimicrobial activity of zinc. Data generated by this team demonstrates that zinc salts directly inhibit the activity of HEV RNA-dependent RNA polymerase and block viral replication in mammalian cell culture models of HEV. Ongoing studies aim at developing an improved cyclic peptide that would be more efficient in preventing virus release as well as to characterize the mechanism(s) by which zinc inhibits HEV replication.

Way ahead: In the near future, in addition to working on the antiviral development against HEV (as described above), they intend to continue investigations on the molecular details of HEV life cycle and development of a recombinant vaccine against HEV.

Peptide discovery in Hepatitis E virus (HEV):

Cyclic peptide (CP) inhibitors of the interaction between the human immunodeficiency virus (HIV) gag-PTAP motif and UEV-TSG101 are known to block the release of HIV. **Dr. Shailendra Asthana's** team applied molecular dynamics simulations and structural bioinformatics approaches and observed that both gag-

PTAP and ORF3-PSAP motifs bind to the same site in UEV-TSG101. HIV-released inhibitory CPs also displayed binding to the same site in UEV-TSG101, indicating that they may compete with ORF3-PSAP or gag-PTAP for binding to UEV-TSG101. Two independent assays confirmed the ability of cyclic peptide 11 (CP11) to inhibit the ORF3-TSG101 interaction. CP11 treatment also reduced the release of both genotype 1 and genotype 3 HEV by approximately 90%, with a 50% inhibitory concentration (IC50) of 2 μM. Thus, CP11 appears to be an attractive candidate for further validation of its anti-HEV properties.

HIV - Antibody Translational Research and Drug Discovery efforts

The primary research goals of the HIV Vaccine Translational Research Laboratory or HVTRL under the ambit of the THSTI-International AIDS Vaccine Initiative (THSTI-IAVI) HIV Vaccine Design Programme were (a) Characterization of antigenic and immunogenic properties of HIV-1 envelope proteins with special reference to dominant Indian subtype and (b) Identification, isolation and characterization of broadly neutralizing antibodies (bNAbs) from HIV-1 Indian subtype C infected elite neutralizers.

The HVTR laboratory carries out early translational research and development following the principles of a center of excellence with unique strengths in the areas of virology, immunology, protein chemistry and structural biology towards accelerating the efforts towards development of broadly neutralizing antibodies (bnAbs) to HIV-1 and characterizing antigenic and immunogenic properties of HIV-1 envelope proteins (Env) in informing immunogen design and for their suitability to be used as antigen bait for bnAb isolation. With the experience and learning gained through the THSTI-IAVI HIV Vaccine partnership programme in last 5 years, the HVTR lab has also started planning to apply these skill sets in other disease areas of national importance namely Dengue, Flu, and snakebite.

Here are key achievements made at HVTR over the past one year.

Isolation and characterization of cross-neutralizing monoclonal antibodies to HIV-1

Investigators: Drs. Huma Qureshi, Suprit Deshpande, Rajesh Kumar, Ranajoy Mullick, Nitin Deshpande, and Jayanta Bhattacharya

The HVTR laboratory has been working to understand the neutralizing antibody response mounted in patients chronically infected with HIV¬1. This has led to the identification of rare individuals who have elicited potent and bNAbs in the course of infection. With that information, the laboratory has been working to isolate bNAbs from such individuals by antigen-specific single B cell sorting (**Figure 1.13**). The team has successfully built capacity in isolation and characterization of bNAbs from HIV-1 infected elite neutralizers in partnership with the IAVI Neutralizing Antibody Center (NAC) at the Scripps Research Institute, La Jolla, California. Towards the isolation of bNAbs from India Protocol G donors, antigen-

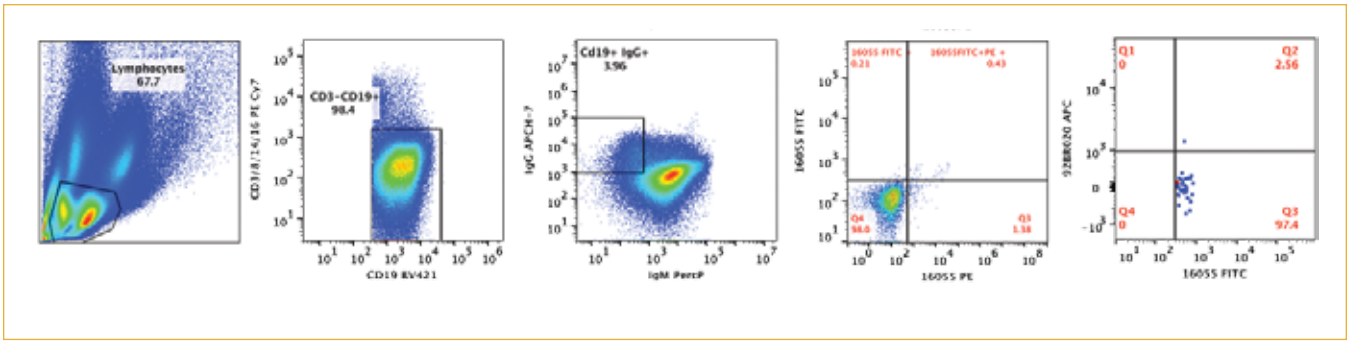


Figure 1.13

specific single-cell sorting has recently been done on three protocol G donors and screening neutralizing antibodies from these donors is in process.

The team has isolated two very potent and cross-neutralizing monoclonal antibodies (mAbs) from a well-defined HIV-1 clade A-infected Protocol G donor in collaboration with NAC by antigen-specific B cell sorting at the HVTR lab, which has been found to subtly differ in their variable IgG sequences from few well-characterized bnAbs isolated from this same donor by NAC. They are currently examining the two mAb clones for their potential to neutralize tier-2 and tier-3 clade C viruses with particular reference to Indian clade C viruses. The HVTR lab has an inventory of over 100 genetically different Env-pseudotyped viruses from Indian patients and from other clades built through collaboration with different institutions within and outside India. These viruses will be used for screening breadth and potency. The preliminary data suggests that several Indian clade C viruses are relatively resistant to well-defined bnAbs isolated from this donor, possibly due to the natural absence of the target epitopes or possibly due to variation in V1V2 loop length and/or glycosylation pattern. The team is currently examining attributes associated with resistance and will then use this information to optimize mAb sequences suitable for expanding its breadth and potency.

Characterization of antigenic and immunogenic properties of HIV-1 Env protein

Investigators: Drs. Rajesh Kumar, Shubbir Ahmed, Supratik Das, Jayanta Bhattacharya

A six-step method to identify efficiently cleaved, functional HIV-1 Envs suitable for immunogen design has been described before by this laboratory. Characterization of these Envs is necessary in order to understand their properties so that the suitability for immunogen design can be ascertained. Previously, a conserved hydrophobic domain in the C-terminal tail of a subset of these Envs was reported to determine their cell surface antigenicity and conformation. In addition, using confocal microscopy these Envs, whose cell surface antigenicity and conformation is determined by the CHD in their C-terminal tail, showed CHD-dependent altered retrograde transport. Using biochemical studies, the team now showed that retrograde transport from the early to late endosomes of a subset of these efficiently cleaved, functional HIV-1 Envs, mediated by a conserved hydrophilic domain in their C-terminal tail region, correlate with their cell surface antigenicity and conformational integrity (**Figure 1.14**). This is a new finding in HIV-1 Env biology and the part of work was led by Drs. Supratik Das and Sweety Samal.

To characterize antigenic properties of patient-derived primary Env protein, they prepared a stable and soluble trimeric protein of one of the autologous Envs that was sensitive to its autologous plasma antibodies i.e. HVTR-PG80v1.eJ19-SOSIP.664 (**Figure 1.13**). Two-dimensional negative EM studies have revealed that most of the trimers have closed native-like conformation and undergo minimal conformational change in the presence of CD4. In addition, the trimeric Env was found to bind to several known bNAbs including those elicited in HIV-1 clade C-infected Indian patients with unknown specificities. However, it did not bind to V3 and CD4i-epitope directed antibodies, indicating excellent antigenic properties. The ability of the well-ordered Env SOSIP trimers of PG80v1.eJ19 in inducing neutralizing antibody response in rabbits was next examined. New Zealand white rabbits were immunized with the Env SOSIPs along with Quil-A® (InvivoGen Inc.) adjuvant. Four rabbits were taken in each immunized group and three rabbits were taken in placebo group, where they received phosphate-buffered saline (PBS, pH 7.0) + adjuvant. The rabbit immunization was outsourced to a contract research organization (CRO) at Bengaluru, Karnataka with all approvals from animal ethics committee and other regulatory bodies taken before initiating immunizations. All the rabbits received PG80v1.eJ19 SOSIP trimer. Pre-bled sera were obtained from all the rabbits prior to immunizations. Serum samples obtained after boost 2 were assessed for their ability to neutralize autologous and heterologous viruses. The PG80v1.eJ19 SOSIP was found to elicit potent neutralizing antibodies against autologous Env (PG80v1.eJ19) and its contemporaneous Envs obtained at the same time point of the elite neutralizer. Interestingly, the same failed to neutralize Env (PG80v2.eJ38) obtained from the follow-up visit of the same patient and which was also found to be resistant to its contemporaneous serum antibodies. Interestingly, introduction of glycan at the 332 position in the V3 based (Asn332) induced antibodies that were found to neutralize pseudotyped virus expressing autologous PG80v1.eJ19 wild-type Env (that lacks Asn332) better compared to its same version that expressed Asn332, suggesting that neutralizing antibodies have specificity distinct to Asn332. However, presence of Asn332 might have rendered PG80v1.eJ19 relatively less or not susceptible to the autologous neutralizing antibodies, perhaps via glycan-masking. Fine epitope mapping studies of the best neutralizing rabbit sera using the chimeric virus construct showed that the immune response was targeted towards conformational epitopes in C3V4 region. This part of the work was led by Dr. Rajesh Kumar and was supported by the DBT National Bioscience Award research grant awarded to Dr. Jayanta Bhattacharya.

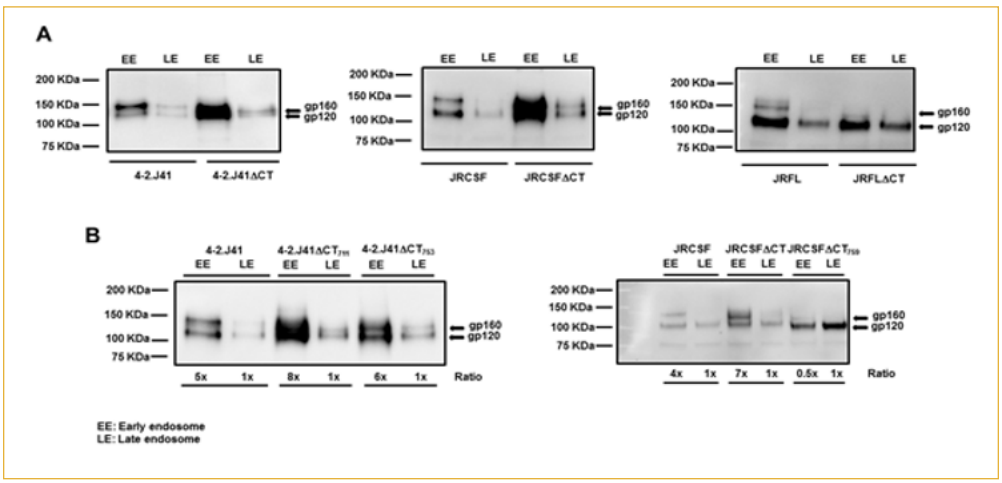


Figure 1.14: Retrograde transport from the early endosome to the late endosome is defective upon C-terminal tail domain deletion of the efficiently cleaved Envs (envelopes) 4.2 J41 and JRCSF (but not JRFL), whose cell surface antigenicity and conformation are also affected. Restoration of the conserved hydrophilic domain in the C-terminal tail of these Envs (4.2 J41ΔCT$_{753}$ and JRCSFΔCT$_{750}$) restores retrograde transport to wild type levels. Therefore, retrograde transport of these Envs corelate with their cell surface antigenicity and conformation suggesting that this transport pathway may play in their maturation into functional Envs.

The team had earlier reported the antigenic, biophysical and biochemical properties of a recombinant HIV-

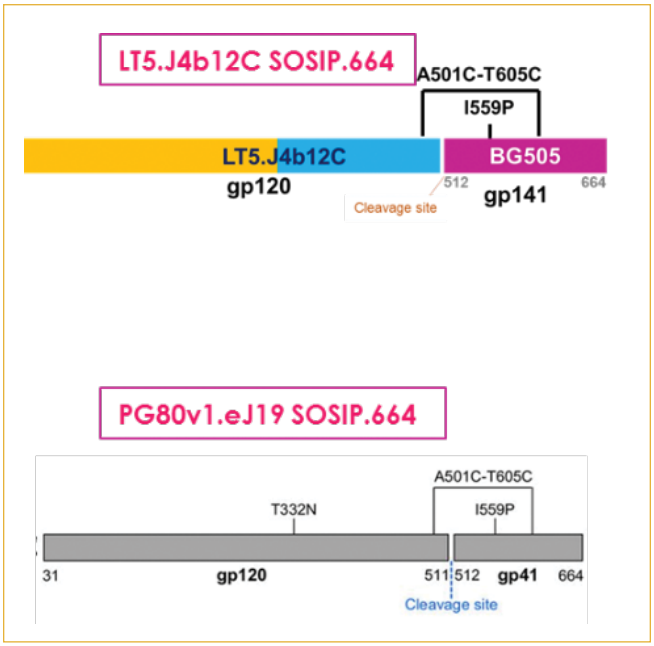


Figure 1.15

1 B/C recombinant Env protein originally obtained from an elite neutralizer (Kumar et al., 2017) that was highly resistant to CD4-induced conformational shift both at the virus and soluble protein levels. However, the trimer fraction of this protein was found to be poorly expressed possibly due to its inherent genetic properties. Since this Env was of interest to examine its immunogenic properties and the team was interested to examine its suitability for use as an antigen bait for single memory B cell sorting in an effort to isolate bnAbs from patient peripheral blood mononuclear cells (PBMCs), they carried out experiments towards suitable modification of this Env construct towards enhanced expression by making gp120/gp41 chimera of LT5.J4b12C and BG505 Envs following the protocol described by Ahmed et al. (2017) of the trimeric form of this Env and without altering its native antigenic and conformational properties (**Figure 1.15**). The modified SOSIP protein version was assessed for its native-like conformation by 2D Negative EM studies in collaboration with the NAC lab at TSRI, California. Interestingly, we also found that depletion of plasma antibodies by this modified soluble trimeric Env, affected the cross-neutralizing ability of some of the plasma antibodies obtained from slow progressors, indicating the modified LT5.J4b12C/BG505 SOSIP could be suitable for use as antigen to sort cross-neutralizing producing memory B cells from these donors. This work was done by Dr. Kumar.

The HIV-1 envelope spike, the sole protein displayed on the viral surface is the primary target for neutralizing antibodies to block infection. Env designed from multiple sources has failed to raise a reasonable breadth and titer of bnAbs in animal immunization studies. The heavy glycan shield around the Env evolved as a protective immune-evasion mechanism for the virus. An alternate approach is to create glycan holes at potentially immunogenic locations which are expected to help in improved induction of the germline B-cells due to better exposure of the immunogenic regions. Subsequent immunization with wild-type (all glycans present) Env will help in affinity maturation of induced B cells to produce bnAbs with reasonable titer and breadth. In the past year, the focus was on designing and characterization of HIV-1 Env trimers with glycan holes at potentially immunogenic locations to understand the impact of deglycosylation on Envs structural and functional integrity and how that influences their antigenicity. The strategy was applied to a previously designed construct in the lab (Ahmed et al., 2017). Three constructs were perceived by removing glycans from V1V2 apex, V3 supersite and CD4 binding sites for screening. Initial data suggests that the deglycosylated variants maintain trimeric native-like conformation with preferential binding to conformation-dependent bnAbs. However, simultaneous removal of glycans from different patches together reduces their ability to maintain native-like conformation. The selected trimeric variants are now in the process of being purified as well-ordered, homogeneous trimer for their structural and functional characterization. So far, using various biochemical and biophysical means they have confirmed that one of the deglycosylated construct (glycan removed at V1V2 apex) appeared as trimer under negative-stain electron microscopy and binds preferentially to bNAbs and not to the non-neutralizing antibodies. This designed

deglycosylated 4-2. J41 Env adds to the pool of potential immunogens for HIV-1 vaccine, particularly for inducing early induction. This work was conceptualized and carried out by Dr. Ahmed. Immunogenicity of one soluble HIV-1 clade C trimeric Env was assessed in the rabbit model as reported above and found to induce potent neutralizing antibodies to tier-2 autologous viruses. The outcome of this study has informed strategies that they plan to employ in redesign and retest in rabbit and other animal models.

Development of a new drug lead with novel pharmacology as a treatment for HIV-1 infection and establishment of proof-of-concept studies in a disease model

Investigators: Dr. Dinesh Mahajan, Dr. Debashis Mitra, NCCS Pune; Dr. Shilpa Jamwal, Dr. Sameena Khan and Dr. Shailendra Asthana from THSTI, Faridabad; Bioneeds India Pvt Ltd, Bengaluru.

This is a BIRAC-CRS funded project for drug lead development led by Dr. Dinesh Mahajan in collaboration with Dr. Debashis Mitra from NCCS Pune and a private company Bioneeds India Pvt Ltd. Dr. Dinesh Mahajan and his team are focused on medicinal chemistry and pharmacology development activities related to pre-clinical drug lead development. This involves the designing and synthesis of molecules to generate Structure-Activity Relationship (SAR) and DMPK analysis of the initial hits to identify a drug like lead, pre-clinical *in vitro* and *in vivo* toxicology for further detailed analysis leading to proof-of-concept studies in an animal model of HIV infection. They identified and developed a small molecule-based new chemical entity which possesses a novel and dual pharmacology involving pathogen and host targets. His team developed a lead molecule and a lead series, which demonstrated potent anti-HIV activity in various *in vitro* models involving cell lines and human peripheral blood mononuclear cells or PBMCs. The lead is evaluated for various *in vitro* as well as *in vivo* pharmacological experiments and currently being pursued in animal efficacy studies. If successful in animal models, this will be a first in class approach for HIV infection involving host-based approach and shall be having potential to treat drug-resistant HIV infection.

Way ahead: A proof-of-concept studies in an animal model of HIV infection are being planned with the developed lead. The team has identified a private company who will do the efficacy studies in humanized mice infected with HIV infection. The study is expected to start on August 2019.

The team has done complete pre-clinical evaluation and characterization of the developed lead and backup molecule. A PCT application has been filed to protect the intellectual property generated for this project. Then, lead and backup molecule has been evaluated for detailed pharmacokinetic analysis and found to be oral bio-available. The lead and backup molecules have been screened for pre-clinical limited toxicology studies in various *in vitro* and *in vivo* models and found safe. The maximum tolerable dose of lead in rats and mice is found to be considerably high than the intended therapeutic dose for animal experiments when evaluated for repeat dose toxicity studies in animals. The lead and backup molecules are found to be safe when evaluated for cardiac toxicity (hERG binding), genotoxicity (Ames test). The lead and backup drug lead are found to have no

interaction with any of the human CYP enzyme or kinase when screened against a panel of 455 human kinase panels. The team has also developed a cost-efficient, high-yield manufacturing process of the identified lead to support animal efficacy studies.

The current regime of HART therapy for HIV infection is under question because of toxicity since a patient has to be on drugs for a lifetime. Integrase inhibitors are a class of molecules introduced for treatment and are very effective compared to other options. There are approved drugs available in the market as well as under clinical development based on inhibition of virus integrase (dolutegravir and elvitegravir). But the issue of resistance due to specific mutations of virus integrase is one of the key drawbacks for integrase inhibitors.

A human study of rapamycin as a drug (known autophagy inducer) on HIV patients who were under treatment for liver transplant (Benedetto et al., 2010) was a strong indication/support for the role of autophagy induction as a potential therapeutic approach for HIV infection. In fact, this human study was one of the reasons for the genesis of this project. Interestingly, the new molecules developed by Dr. Dinesh and his team are found to have both the properties based on *in vitro* analysis (integrase inhibitors as well as potent autophagy inducers). So, it is expected that on successful completion of the proof-of-concept studies in animal model, there will be a strong case to evaluate these molecules in humans as improved form of integrase inhibitors having novel pharmacology of autophagy induction to boost immunity of host cells. Pharmacokinetic and limited toxicity studies were performed in rats and mice. This is a part of pre-clinical evaluation of identified drug leads before going for *in vivo* efficacy experiment in animal model of HIV infection.

Way ahead: The project team is presently focused on the evaluation of the drug leads in animal efficacy studies. These studies are expected to start on August 2019.

Antibody Translational Research - Non-HIV projects

Investigators: Drs. Sweety Samal and Tripti Srivastava

Influenza virus infections pose a major public threat around the world. India remains as vulnerable to influenza viruses; the 2009 H1N1 pandemic resulted in 27,236 cases and 981 deaths in 2009 and 20,604 cases and 1763 deaths during 2010 and since then the virus is found to be spreading rapidly in various parts of the country, and showing diversified epidemiological characteristics. Vaccination against influenza is considered the most effective way to control the spread of disease. Presently, the available licensed influenza vaccines are based on eliciting neutralizing antibodies against Hemagglutinin (HA) protein. However, the immune-dominant HA-based vaccines render selective pressure by strain-specific antibodies, allowing the virus to escape host immune response. And as the epidemics of Influenza A virus continues with constant change in HA protein due to antigenic shift/drift, various approaches have been in consideration to develop vaccines that can induce broadly protective immune responses against influenza

A. One of the approaches is development of next-generation universal influenza A vaccine candidates based on the conserved Matrix 2 ectodomain protein (M2e protein). Influenza Matrix 2 protein an integral structural protein on the surface of viral membrane which serves as a protein-selective ion channel. The protein is a homotetramer of 97 amino acid residues, out of which the Matrix 2 external domain or ectodomain (M2e) of 23 amino acid residues are highly conserved. A total of 17 of 24 amino acids of extracellular domain IV of the M2e protein are 94% identical among different subtypes, making it an ideal candidate vaccine antigen. However, the small size of the protein, as well as its rarity on the virion surface, contributes to the poor immunogenicity of M2e. Hence, one of the challenges in developing M2e-based influenza vaccines is improving immunogenicity. The team has initiated development of M2e-based soluble vaccine subunit by construction of tandem repeats of conserved M2e from Indian H1N1 isolates. The recombinant soluble proteins were biochemically characterized and checked for their immunogenicity in BALB/c mice. The team designed three constructs of which two constructs will express in mammalian expression system and one in bacterial expression system. The expressed proteins were purified and analyzed by SDS-PAGE and Western Blot. The immunogenicity of the expressed proteins is being evaluated in BALB/c mice. Six to eight- week old BALB/c mice were randomly divided into five equal groups of N=5 (where N is the number of individuals per group), except in adjuvant and PBS control (N=3). The blood samples were collected at an interval of two weeks after each vaccination and allowed to clot prior to centrifugation to obtain the serum sample. The preliminary data shows promising results and the team is in the process of development of ant-M2e antibodies from immunized mice spleens by phage display method. This work is led by Dr. Kumar. The commercially available anti-M2e antibodies are expensive and the aim is to produce the antibodies in-house and lower the cost by 10 times of the market value which will be readily available to research community (**Figure 1.16**).

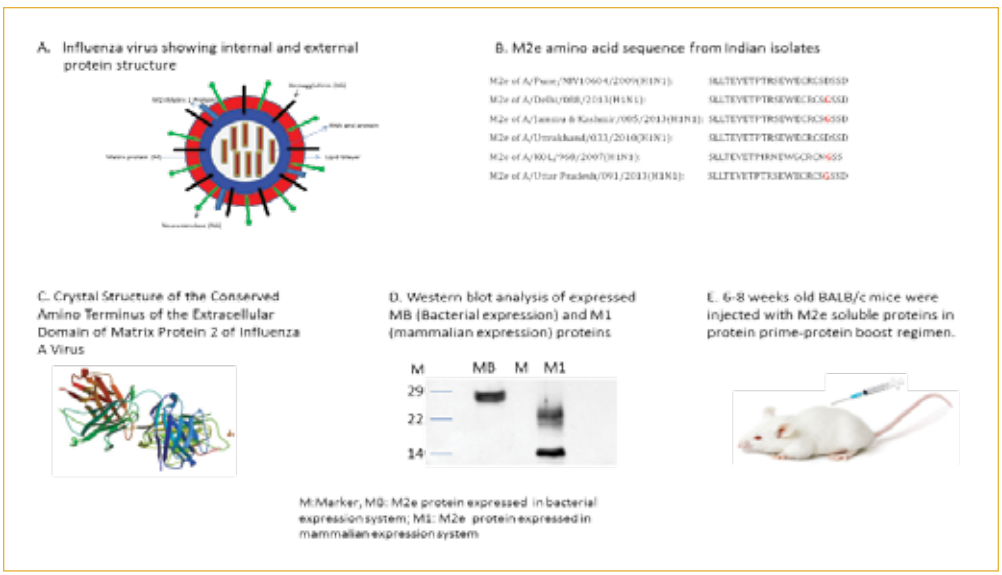


Figure 1.16

The currently available licensed seasonal influenza vaccines need annual update and protection through vaccination heavily rely on elicitation of neutralizing antibodies against Hemagglutinin (HA) protein. Hence, the new generations of influenza vaccines should be based on approaches targeting non-variable regions of antigenic proteins, with the idea of stimulating cross-protective antibodies and thus creating a "universal" influenza vaccine. The present study is based on the concept of validation and assessment of intratrimeric conserved region targeting the prevalent circulating strain of influenza in India using BALB/c mice. This study proposes to design a chimeric monomeric influenza vaccine subunit (cMHA) where the target is to include inter-monomer conserved interface, with inclusion of various circulating globular head from different regions of India/different subtypes.

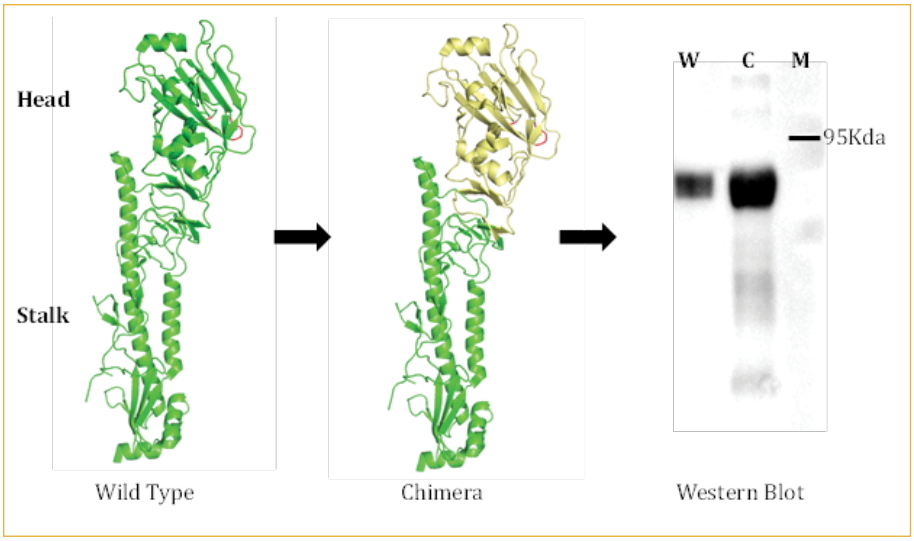


Figure 1.17

So that once used for sequential immunization, varying globular head will generate diverse head-directed antibodies, whereas conserved inter-monomeric conserved region and stalk directed antibodies will be boosted per immunization. The proposed intramonomer sequences are reported to be conserved in at least more than 4468 sequences studied from the HA sequence database. The H1N1 strain prevalently circulating in India was selected, consensus sequence of pandemic 2009 strain prepared, codon optimization of the sequence was done and expressed in mammalian expression system. They further introduced the stabilization mutants, fold-on domain, thrombin cleavage site and leader sequences in recombinant sequences. They replaced globular head sequences from various circulating strains of H3N2 from India to prepare chimeric HA vaccine subunit. The proposed H1N1 protein and chimeric proteins have been expressed in 293T cells and purified through Nickel affinity columns, purified protein further analyzed by SDS-PAGE and Western blots. The resultant proteins have been purified and characterized by conformation stability and other biochemical parameters. Immunogenicity of chimeric globular head containing HA will be used to immunize the mice model (BALB/c, female mice, 6-8 weeks old) sequentially in a protein prime and protein boost regimen either alone or in combination with trimeric protein. The project is underway **(Figure 1.17)**.

Antimicrobial resistance in bacterial pathogens and the human microbiome

Dr. Bhabatosh Das's laboratory is focused to understand the (i) antimicrobial resistance (AMR) traits and mobile genetic elements (MGE) present in the genome of multidrug-resistant (MDR) and extensively drug-resistant (XDR) bacterial pathogens *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Vibrio cholerae* (ii) role of human gut microbiota in the emergence and spread of MDR and XDR bacterial pathogens, (iii) reverse antibiotic resistance in MDR and XDR pathogens by inducing instability of acquired antibiotic resistance genes. For identification of AMR genes, they adopted the pan-genome based sequencing approach as well as functional metagenomics. Based on the DNA sequence signatures, we are identifying the "core" and "flexible" gene pools in the genome of MDR and XDR commensal and pathogenic bacterial species. Extensive genome editing is being undertaken to understand the dynamics of flexible gene pools in the gut microbiota and identify targets that confer stable inheritance of MGEs linked with AMR genes. Inhibiting stability factor(s) may help to eliminate MGEs from the genome of resistant bacteria and resensitize them against available antibiotics.

Resistance phenotypes of enteric bacterial pathogens (N=1097, where N stands for the number of isolates) including *K. pneumoniae*, *P. aeruginosa,* and *V. cholerae*

Name	Year	Pen10	Amp100	Car100	Imp10	Far10	Azt1.6	PolxB50	Tet1.5	Dox3	Chl3	Str100	Spc100	Kan40	Neo30	Ery10	Gen10	Nal Acd4	Cip5	Zeo25	Rif5	Sul160	Trm30
V.ch O96 (IDH06781)	2014	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
V.ch O1 ET (IDH08148)	2015	R	R	R	R	R	R	I	R	R	R	R	R	I	S	R	R	R	R	R	R	R	R
V.ch O139 (IDH08848)	2001	R	S	S	S	S	S	R	S	S	R	R	R	I	S	S	S	R	S	S	S	R	S
V.ch O4 (VCE232)	1960	R	S	S	S	S	S	R	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S
V.ch O1cl (O395)	1960s	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Figure 1.18: Resistance profile and year of isolation of different Vibrio cholerae strains used for whole-genome sequence analysis.

isolated from different parts of India were determined against twenty-two antibiotics representing nine different drug classes. Whole-genome sequences of several isolates of XDR *K. pneumoniae* and *Campylobacter jejuni* were generated to identify AMR genes and MGEs in the genomes of both the species. Antimicrobial resistance in *V. cholerae* has now become a global concern. Dr. Bhabatosh's team conducted whole-genome sequencing and analysis of susceptible, resistant, MDR and XDR *V. cholerae* strains (**Figure 1.18**) isolated over the last 60 years from different parts of India to understand the real-time evolution of cholera pathogen. Their findings indicated that horizontally acquired genetic elements like plasmids, transposons, integrative and conjugative elements (ICEs), pathogenicity islands (PAIs) and gene cassettes are the major players in the antibiotic resistance crisis in enteric pathogens including *V. cholerae, K. pneumoniae,* and *P. aeruginosa*. A single isolate of XDR *V. cholerae* can harbor more than 30 different AMR genes. Most of the resistance genes are constitutively expressed and can neutralize the antimicrobial activity of the respective antibiotic.

They further observed that bacteria could develop resistance to antimicrobials by two simple mechanisms; by altering the target sequence (vertical transmission) or by acquiring resistance-encoding genes from other bacterial species (horizontal gene transfer). The acquired resistance traits can provide resistance by changing membrane permeability, enzymatic degradation, modification of antimicrobial drugs or by modifying the drug targets (**Figure 1.19**). They may also provide an alternative metabolic pathway or actively pump out antimicrobial compounds from the cytosol. The new knowledge generated through this study would help in developing a better understanding of evolution of enteric bacterial pathogens and management of enteric diseases by providing clinical guidance on preferred treatment regimen.

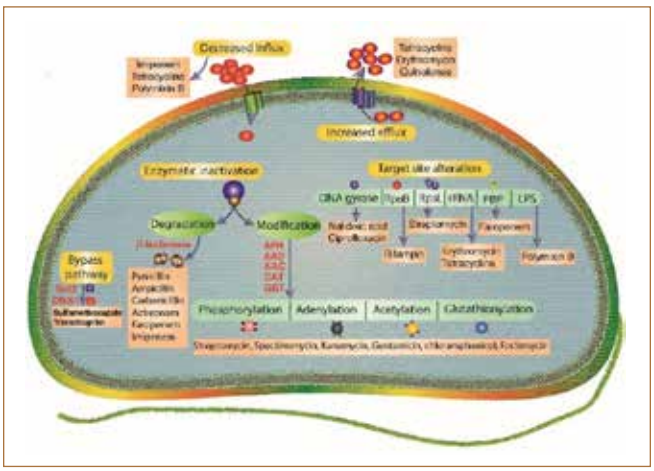


Figure 1.19: Mechanisms of antibiotic resistance in enteric bacterial pathogens.

Way ahead: The team plans to (i) explore the genome of recent isolates of MDR and XDR enteric pathogens to understand the ecology of AMR genes and (ii) engineer the genome of XDR isolates to measure the dynamics of MGEs in the presence and absence of antibiotics.

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MULTIDISCIPLINARY CLINICAL & TRANSLATIONAL RESEARCH

MULTIDISCIPLINARY CLINICAL & TRANSLATIONAL RESEARCH

Accuracy, accessibility, early diagnosis, and affordability form the guiding principles abiding which diagnostics development efforts crusade through the challenges of health care in India. This also demands multidisciplinary efforts to identify unmet needs and develop diagnostics customized to needs in India. The team at THSTI has largely focused on innovation in diagnostics for infectious diseases and over the past one year ventured into the problem of snake bites considering India accounts for half the snake bite-related deaths occurring globally (WHO estimates). The team also focuses on the development of technology platforms for bioprocess improvement.

Diagnostics for blood-borne infections

Dr. Gaurav Batra's team is working on the development of high-sensitivity, multiplex, point-of-care test (POCT) system for detection of blood-borne infections. The performance of commercially available rapid POCTs for HCV and HBV is much inferior compared to central laboratory tests. Moreover, there is no commercially available multiplexed POCT for simultaneous detection of antigen and antibody markers for HIV, HBV, and HCV. There are, however, POCTs available that detect anti-HIV, anti-HCV and anti-HBV antibodies, but not the antigens. A high performance multiplexed POCT for HIV, HBV, and HCV, covering both the antigen and antibody markers, can be of enormous value and may find use in the following health settings:

- Emergency settings (emergency surgeries, emergency deliveries, emergency blood transfusion)
- Blood banks (donated blood screening)
- Sexually Transmitted Infection clinics
- Antenatal screening
- Population screening

Lateral flow assay (LFA) format is the most widely used point-of-care test format because it is easy to use, rapid, affordable and scalable. Nevertheless, traditional LF assays often suffer from many problems, which include poor assay sensitivity, subjectivity in reading the test results and limited multiplexing possibilities. Keeping this in mind, the team is working on a concept where the strengths of traditional LF format are taken. They have replaced the colloidal gold (used for signal generation in traditional LFA) with upconverting phosphor nanoparticles (UCNPs) as a tracer, with optimized flow properties. UCNPs are very stable and provide very high signal amplification that can be easily quantified.

Earlier, they have reported the generation of proof of concept UCNPs based lateral flow assay for the detection of anti-HCV antibody, anti-HIV antibody and hepatitis B surface antigen with initial sensitivity and specificity of >95% for these analytes compared to high-performance central lab immunoassays. They have further optimized the HBsAg assay to make it compatible with whole blood. They have evaluated the assay performance with a low titer panel, seroconversion panels, and

genotype panel. The UCNP based HBsAg LFA detected all the tested genotypes (A-H) of HBV. The UCNP HBsAg-LFA also detected 9 out of 14 low titer samples whereas in parallel performed best performing commercial POCT for HBsAg could not recognize any of the low titer samples.

They have also optimized the UCNP-LFA for anti-HCV antibodies detection. Now the sample can directly be used without any dilution step (practical consideration for POCT assay). Moreover, whole blood sample can also be used in the assay. The performance of the new version of the LFA seems to be very good when a limited number of samples tested on the assay. The HIV p24 assay has been optimized and the initial results suggest analytical sensitivity similar to high performance commercial rapid antigen detection assay for HIV p24 Ag. The above assays will later be incorporated in a multiplexed cassette design for the simultaneous detection of HIV, HCV and HBV infections. Work is underway to further optimize the assays and to perform the evaluation of the tests with large number of clinical samples.

Diagnostics for tropical fevers

Acute Febrile Illness (AFI) is common in the tropics and sub-tropics and can be caused by very diverse pathogens. The availability of reliable point-of-care test (POCT) that can quickly identify a pathogen from a group of pathogens causing similar symptoms is of paramount importance for patient treatment, surveillance and prevention of anti-microbial resistance. Infectious diseases that causes major burden of AFI in tropics and subtropics include Malaria, Dengue, Chikungunya, Typhoid/Paratyphoid, Scrub Typhus, Leptospirosis etc.

Despite the strong need, commercially available POCTs (singleplex) for these infections are of poor quality. Only some of the POCTs for malaria (only Pf) fulfil WHO ASSURED (**A**ffordable, **S**ensitive, **S**pecific, **U**ser-friendly, **R**apid and robust, **E**quipment-free and **D**eliverable to end users) criteria. Even for malaria, better POCTs are required for *Plasmodium falciparum* (low density infections, HRP2 negative strains and elimination settings) and non-Pf malaria especially *P. vivax* (routine diagnosis). Because of the problems in the available singleplex tests, there is strong need to develop high quality POCTs for tropical febrile infections. It should be noted that despite the need no multiplex POCT, which can be used in resource-limited settings for the detection of multiple etiologies of tropical fevers, is available in the market. A two-step approach is required - first, to develop high performance singleplex POCTs for individual infection that can reach the market in relatively less time, and second, to develop multiplexed POCTs using the same diagnostic reagents. The successful development and implementation of singleplex and multiplexed POCTs for AFI will not only help health care providers in choosing appropriate treatment option for the patient but will also help in controlling the problem of antimicrobial resistance through judicious use of antimicrobials.

Dr. Batra's team is working on the development of improved POCTs for different tropical febrile illnesses including Malaria, Dengue, Chikungunya, Scrub Typhus etc.

Developments over the last year are described next:

Generation of an ultra-sensitive point-of-care rapid assay for *P. falciparum* HRP2 antigen: Earlier, they had reported the development of a lateral flow assay (LFA) utilizing unique fluorescent nanoparticle for the detection of plasmodium falciparum HRP2 antigen with ability to detect 1 parasites/µl in spiked whole blood panel (WHO GMP panel). Now, Dr. Batra's team has been able to further improve the rapid test with a detection limit as low as 0.2 parasites/µl, which is a 250-fold

improvement over the currently used rapid tests. The new test is as simple to use as the currently used rapid tests and the results are read after 20 minutes with a simple fluorescence reader. The developed rapid test is stable even at 50°C for more than 15 days. The developed tests will later be incorporated in a multiplexed cassette design with other assays for tropical fevers.

Toward the development of a high sensitivity point of care test for the *Plasmodium* lactate dehydrogenase (pLDH) antigen: To develop high-sensitivity, point-of-care test for the detection of all the species of *Plasmodium* (PF, Pv, Po, Pm, and Pk) and the differentiation of falciparum and vivax malaria, Dr. Batra's team is working on the generation of monoclonal antibodies against pLDH that can recognize pan epitopes and species-specific epitopes. For this work, they first created recombinant clones expressing LDH from five *Plasmodium* species. Five recombinant LDH were purified to homogeneity with very high specific activity and in intact tetrameric form. These antigens are being used for the generation of monoclonal antibodies (mAbs) against pLDHs. Antibody screening work is ongoing.

Second generation dengue NS1 antigen detection assays for routine diagnosis and surveillance: Dr. Batra's team earlier generated large repertoire of synthetic human recombinant monoclonal antibodies and mouse monoclonal antibodies, against the dengue virus NS1 antigen, with wide specificity profile e.g. serotypes specific, pan-dengue, pan-flavi NS1 etc. These antibodies have been extensively characterized for their binding properties. His team is now optimizing the assay for the serotype-specific NS1 detection. They are building a well characterized panel of samples positive for different tropical febrile illnesses. This panel will help the diagnostic development programmeto large extent.

Population: Children and adult with appropriate clinical and laboratory confirmation for different febrile illnesses causing pathogens.

Comparison: Control group with alternate diagnosis.

Objective: To develop a panel of clinical specimens and to test the performance new diagnostic assays for different acute febrile illnesses.

Their long-term plan is to first develop high performance point-of-care assays for the detection of major tropical fevers and then develop a multiplexed point-of-care test for simultaneous detection of major tropical fevers. In the next year, they will generate high-affinity antibodies against pLDH and develop point-of-care assay for dengue NS1.

Diagnostics of pulmonary and extrapulmonary TB

India continues to grapple with the burden of TB, one of the main reasons for which is the lack of a proper diagnostic. For meeting this exigency of an affordable and reliable TB (both pulmonary and meningitis) diagnostic, **Dr. Tarun Sharma's** group in collaboration with Prof. Jaya S. Tyagi (AIIMS, Delhi) is developing aptamer-based diagnostics assay for TB (**Figure 2.1**).

TB meningitis (TBM)

Dr. Sharma's group has developed a high affinity ssDNA aptamer diagnostic reagent (H63 SL-2 M6, referred as M6) against HspX, an *M. tuberculosis* antigen biomarker for TBM using the Systematic Evolution of Ligands by EXponential enrichment (SELEX) approach. In an Aptamer Linked Immobilized Sorbent Assay

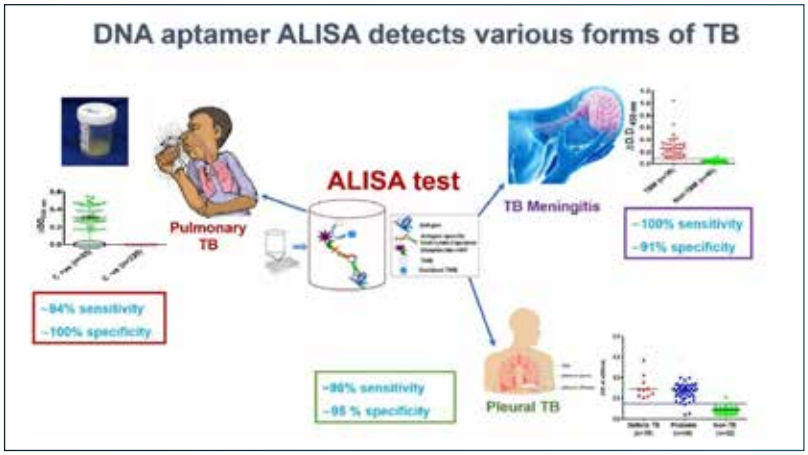

Figure 2.1: Showing diagnostic performance of aptamer-based assay for pulmonary TB, TB meningitis and Pleural TB.

(ALISA) performed in a 96-well microplate, applied to archived CSF specimens, the M6 aptamer significantly differentiated between pediatric TBM and non-TBM subjects (n=87, ***p < 0.0001) with ~100% sensitivity and ~91% specificity using composite reference standard as the gold standard.

Pleural TB

To further evaluate the clinical utility of this aptamer for other sensing platforms of EPTB, the M6 aptamer was evaluated in a blinded manner in pleural fluid samples (N=96) for its ability to detect HspX. A composite reference standard was used as gold standard. The sensitivity of M6 ALISA was ~96% in comparison to ~27% of antibody ELISA (p-value ≤0.0001). For the same samples, a DNA-based assay, devR qPCR exhibited a higher sensitivity of 50% quite higher than ~22% of Xpert (p-value ≤0.01). This novel aptamer-based test aces the sensitivity criterion and closely matches the specificity requirement, albeit in a limited number of samples, that are spelt out in the 'Target product profile' for extrapulmonary tuberculosis samples by WHO (required sensitivity ≥80%, specificity 98%). Taken together, the M6 aptamer-based assay has shown superior performance in pleural fluid specimens in comparison to ELISA, qPCR and Xpert.

Pulmonary TB

The performance of M6 aptamer-based assay was also assessed on sputum samples for diagnosing pulmonary TB. In a blinded study on 314 sputum specimens, the M6 ALISA displayed a high sensitivity of 94.1% (95% CI: 86.8 – 98%) as compared to 68.2% sensitivity (95% CI: 57.2 – 77.9%) of Antibody ELISA (p-value < 0.05), using culture as the reference standard. The specificity for both ALISA and ELISA were 100%. Out of nine smear-negative culture-positive samples, six were positive for Aptamer ALISA, while only two were detected by Antibody ELISA. Importantly, ALISA detected 80 of 85 culture-positive TB as positive when compared to 57 of 81 diagnosed as TB by X-ray (p-value < 0.0001). These findings demonstrate the superiority of the aptamer-based test over smear microscopy, antibody-based ELISA, and chest X-ray for TB detection (p-value < 0.0001 for all) and illustrate the potential of the M6 ALISA as a screening tool for pulmonary TB.

TABLE 2.1. Performance of aptamer-based test against proposed sensitivity and specificity for new test as defined by WHO.

TPPs (sensitivity and specificity) as per WHO guidelines	Proposed sensitivity and specificity	Performance of our tests
For TBM	≥80% sensitivity and ~98% specificity	≥95% sensitivity and~97% specificity
For Triage test	≥90% sensitivity and ~70% specificity	≥94% sensitivity and~100% specificity
For Pleural TB	≥80% sensitivity and ~98% specificity	≥96% sensitivity and~95% specificity
For Abdominal TB	≥80% sensitivity and ~98% specificity	≥91% sensitivity and~98% specificity

A pilot study was performed on clinical specimens and showed an encouraging result. Now they are moving to validate this technology at multiple centers across India.

Way ahead: The early stage validation has been successfully concluded. They now propose to go for multisite validation followed by product (diagnostic kit) development in partnership with a suitable industry.

Detection of snake bite

Due to lack of diagnostics for differentiating between poisonous and dry bites and ascertaining the venomous species, polyvalent antiserum is administered which are reported to illicit severe immune responses. Dr. Sharma's lab is working to develop point-of-care (POC) diagnostics for differentiating the venomous bites from dry bites and ascertaining the biting species, so that monoclonal antisera could be administered to reduce the immune responses. Need of such diagnostics can be easily gauged by the fact that India reports 45,900 annual snakebite deaths.

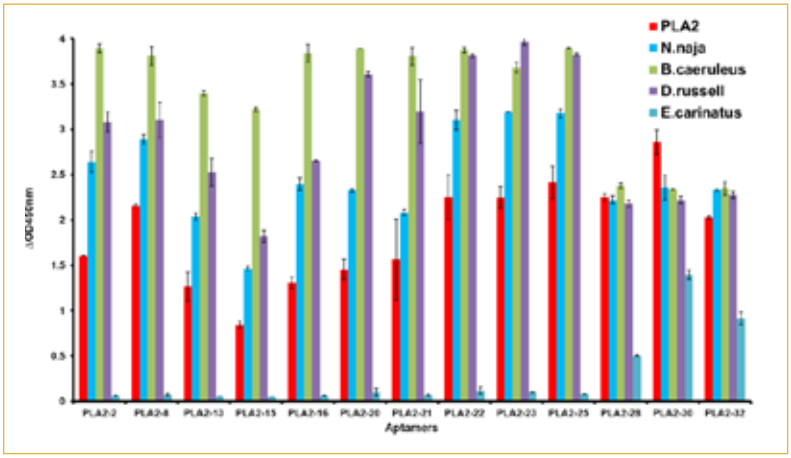

Figure 2.2: Evaluation of binding affinity of selected aptamers against crude venoms of Big four venomous species of India. The selected aptamer candidates displayed differential but decent binding against all the crude venoms, an indicative of universal application of the selected aptamers.

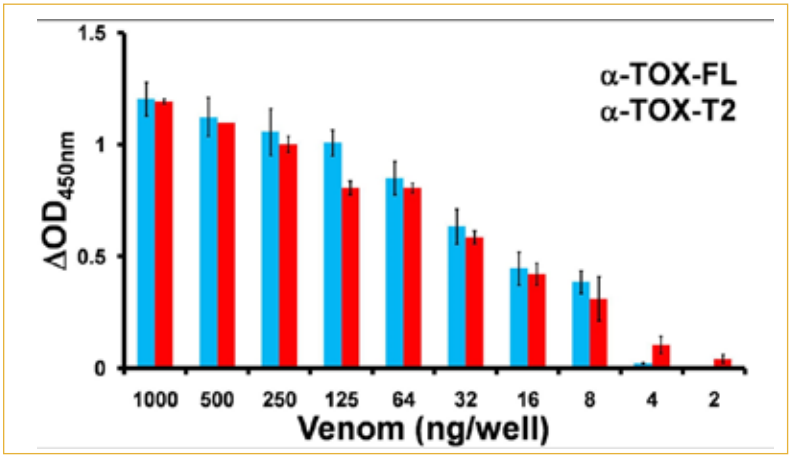

Figure 2.3: Low end detection limit of α-Tox-FLand α-Tox-T2 aptamers. α-Tox-T2 represents more sensitive detection of B. caeruleus venom in comparison to its parent counterpart.

Phospholipase A2 (PLA2), is a shared snake venom protein present in all venomous snakes. As Dr. Sharma's group espoused to develop diagnostic for differentiating venomous bites from dry bites they aimed to develop aptamers against PLA 2 itself. They performed standard SELEX to select high affinity aptamers against PLA2. Their preliminary studies with thus developed aptamers have shown encouraging results. As espoused, the selected aptamers displayed binding with PLA2 as well as with crude venoms of Big Four venomous species of India. However, the binding affinity was different, which can be attributed to heterogeneous venom composition (**Figure 2.2**).

Simultaneously the group has empirically reduced the length of a reported aptamer to develop aptamers with highly augmented sensitivity compared to their parent aptamers (**Figure 2.3**) to recognize α-toxin component of the venom (a neurotoxin present in the venom of Indian Krait species).

These truncated aptamers were able to selectively recognize *Bungarus caeruleus* when tested against venoms of other snake species namely *Naja naja, N. kaouthia, N. oxiana, Daboia russelii, B. fascinatus, B. niger* and *Echis carinatus* (**Figure 2.4A and B**).

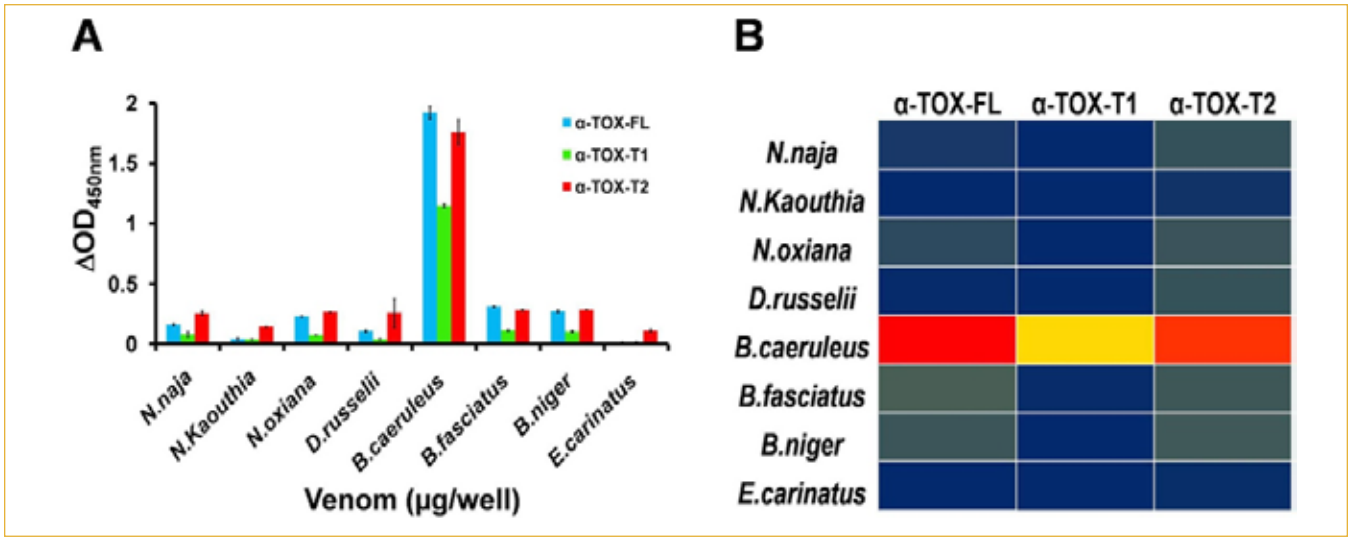

Figure 2.4: A) Showing ELAA results representing selectivity of α-Tox-FL, α-Tox-T1 and α-Tox-T2 aptamers to B. caeruleus venom. B) Selectivity of α-Tox-FL, α-Tox-T1 and α-Tox-T2 aptamers to B. caeruleus venom is represented as three-colour gradient heat map (a heat-map representation of ELAA response). Red colour indicates highest binding while blue represent the lowest binding.

Way ahead: Clinical validation of the envisaged aptamer will commence after completion of in vitro validations in near future. For this serum, swab from bite site, blister fluid and urine samples will be collected and diagnostic utility of these aptamer-based test will be established in the aforementioned sample types in near future. After the ongoing *in vitro* validation, they aim to develop electrochemical sensors as POC diagnostics. The envisaged POC diagnostic will be evaluated against body fluids for detection of snake bites.

Biosensing for environmental pollutants

Dr. Sharma's lab is working on developing cheap, rapid and highly sensitive sensors for pesticide detection. His group is currently developing sensors for organophosphate pesticides, a class of pesticide which is widely used in India. Contamination of organophosphates in food and water poses grave health concerns.

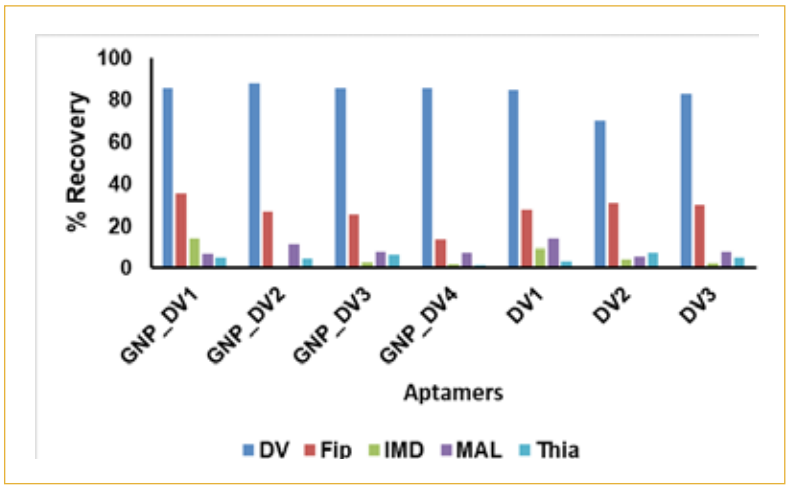

Figure 2.5: Recovery percentage of peroxidase activity after addition of pesticides (DV-Dichlorvos, Fip- Fipronil, IMD- Imidacloprid, MAL- Malathion, Thia- Thiamethoxam) to GNP and aptamer complex. The binding of aptamers on GNPs surface blocks their peroxidase activity. The aptamers upon binding to the pesticides leaves the GNP surface resulting in the recovery of peroxidase activity. However, as the aptamers are highly specific for DV, with addition of DV maximum quantum of recovery in peroxidase activity is observed.

Dr. Sharma's lab has developed a novel SELEX strategy to enhance the sensitivity of aptamer-based nanozyme assays. They have developed a panel of high affinity aptamers for dichlorvos (an organophosphate pesticide) using their devised SELEX approach. The developed aptamers were then subjected to nanozyme assay for evaluating the sensing ability of the aptamers. The nanozyme assay confirmed the selectivity of the developed sensing assay for its cognate target dichlorovos (**Figure 2.5**).

Further, the aptamers have been characterized with circular dichroism (CD) and isothermal titration calorimetry (ITC) for their binding and conformational characterizations. Currently the group is pursuing truncation of aptamers to improve the sensitivity of the selected aptamers.

Way ahead: Dr. Sharma's group aspires to adapt and evaluate the utility of these aptamers on various sensing platforms for facile, instrument-free but highly sensitive detection of pesticide contamination in food and water.

Antimicrobial Resistance (AMR) Diagnostics

Antimicrobial Resistance (AMR) has become one of the major escalating threats to global public health and underlines the urgent need for action as resistant-infections not only causes need for expensive treatments, prolonged hospital stays and increased healthcare costs but also loss of productivity. Currently ~0.7 million people die of drug resistance in illnesses due to bacterial infections globally every year with an estimated increase to ~10 million deaths per year by 2050 including 4.1 million deaths from Africa and 4.7 million from Asia, at a cost of $100 trillion.

Unnecessary/inappropriate use of antibiotics has been a significant factor contributing to emergence/spread of Antimicrobial Resistance (AMR). In India, ≥67% of patients are given antibiotics unnecessarily and making the country the largest antibiotic consumer (~11 units/person) worldwide. This is primarily because of unavailability of rapid diagnostics for establishing pathogen identity and antimicrobial-susceptibility-testing (AST). Currently, culture-based diagnostics are the 'gold standard', although several culture-independent molecular techniques (PCR, DNA-microarrays, mass-spectrometry, next-generation sequencing) are also available. The implication of these diagnostics in public health setup is still insufficient due to high turn-around-time (~18hrs), resource requirement and/or ultimately high cost. Whereas, in case of serious infections, such as sepsis, percentage of saving patients gets reduced (by ~7%) every hour if a patient is not treated with appropriate antibiotics. Hence, need for rapid test for pathogen identification and AST profiling still remains unfilled.

The teams led by **Drs. Susmita Chaudhuri** and **Niraj Kumar** are currently working to develop process & products that could enable pathogen identification and antimicrobial susceptibility testing within 90 minutes of time (**Figure 2.6**). For this, they have been focusing on

Development of rapid and cost-effective methods for pathogen capture and culture from clinical samples

Identification of pathogen-specific targets (proteins and genes) and development of diagnostic-grade binders against them

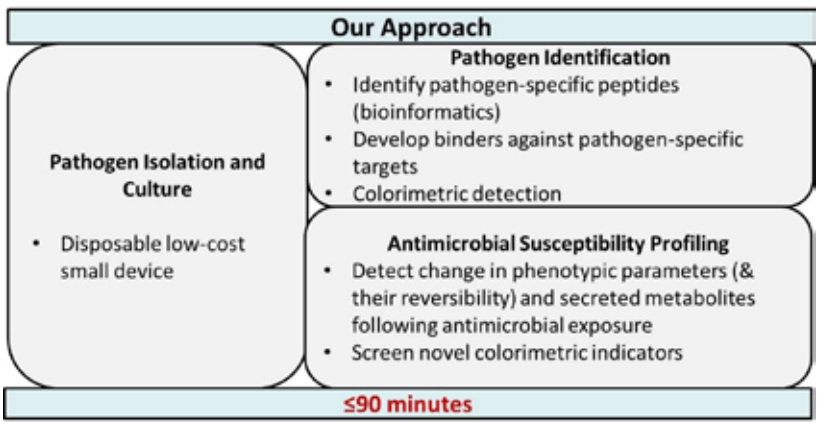

Figure 2.6: Overview of the research plan

Development of rapid protocols for antimicrobial susceptibility testing

They have,

- Evaluated the growth kinetics of selected blood-stream pathogens seeded at different cell density (including low density) in multiple growth media
- Identified *Staphylococcus aureus*-specific genes/proteins which would now be utilized for developing S. aureus-specific diagnostic-grade binder(s)
- Developed an early-metabolite marker-based method and initial proof-of-concept for a phenotypic anti-microbial susceptibility profiling assay in nano-volume multi-array culture format along with a flow-cytometry based protocol for the same.

Rapid pathogen identification and their antimicrobial susceptibility profile is an established unmet clinical need. The proposed study would enable the development of process and/or products for rapid and cost-effective pathogen culture, identification, and antimicrobial susceptibility profiling. This will ultimately reduce, empirical use of antibiotics for treating patients, risk of emergence and spread of antimicrobial resistance among pathogens, cost, and duration of treatment and morbidity and mortality by the disease.

To date, the teams have tested the performance of the in-house developed assays using laboratory-based non-pathogenic *Escherichia coli* or only a few blood-stream bacterial pathogens spiked on different culture media. They are now aiming to evaluate the performance of these developed assays using broader-spectrum of pathogens isolated from clinical samples.

Based on the knowledge generated, they aim to develop a diagnostic product that includes panel of antibiotics to be tested and follow the Clinical and Laboratory Standard Institute (CLSI) standard plus the present line of treatment for sepsis and Urinary tract Infections (UTI). The readout of the assays would be combined with an in-house developed algorithm to correlate the rapid result with usable information on antimicrobial susceptibility profile of a clinical sample.

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L-R: First row – Vibha Taneja, Anjali Anand, Soon Jyoti Das; Second Row – Dr. Bandhan Chatterjee, Dr. Tarun Sharma, Raj Kumar

L-R: Dr. Niraj Kumar and Shrikant Kumar

MATERNAL AND CHILD HEALTH

Bridging classical clinical research and modern sciences like multi-omics technology and data science to find solutions for diseases of public health importance

Prof. Shinjini Bhatnagar leads a group of multidisciplinary researchers including physician-scientists and biologists who aim to develop accessible, affordable and affable knowledge-based innovative solutions for maternal and childhood diseases. The mandate of the group is to *develop programs around great opportunities that are identified through effective filtration methods and triggered by the burden of disease and public health relevance.*

The maternal child health domain in THSTI includes large inter-institutional interdisciplinary research programs that form the core (i) **GARBH-Ini** (*interdisciplinary **G**roup on **A**dvanced **R**esearch on **B**irt**H** outcome-DBT **IN**dia **I**nitiative*) and (ii) Innovative solutions for sepsis in neonates and young infants.

Domain i: GARBH-Ini (interdisciplinary Group on Advanced Research on BirtH outcome-DBT INdia Initiative) (Dr. Shinjini Bhatnagar, Collaborators and Co-PIs as mentioned below)

Preterm birth (PTB) is a major public health problem globally. In India, 3.6 million of the 27 million babies born annually are preterm. Preterm birth is a complex syndrome with multiple etiologies that include interacting biological, psychosocial, and environmental factors.

Most available evidence in this domain has come from traditional approaches that analyzed data from a single time point, usually immediately after childbirth. The collection of multidimensional data over the entire period of pregnancy will provide a deeper understanding and better estimates of risk factors that lead to PTB, enabling robust prediction and prevention of PTB. The ultimate aim is to have an effective risk stratification that will facilitate timely referral and develop into a critical health system intervention, particularly for our settings.

In order to fulfil the objectives, a cohort (GARBH-Ini Cohort) of almost 6500 pregnant women has been established at the Gurugram Civil Hospital with a repository of well-characterized clinical phenotypes with 700,000 biospecimens and 400,000 ultrasound images at THSTI. This flagship program of THSTI is an example of how large interdisciplinary translational programs can be initiated around important public health issues. It has recently been recognized as one of the five *Atal JaiAnusandhan Biotech Missions* of Department of Biotechnology, Ministry of Science and Technology, Government of India and is envisaged to become a global resource to answer critical questions in the maternal and child health domain.

Accounting for a cohort design to detect epidemiological risk factors and nested case-control design for identifying biomarkers, an *a priori* sample size of 8000 was estimated. The program started in May 2015 and among a total of 19,901 women screened till March 2019 nearly 6500 (1921 between April 2018 and March 2019)

were enrolled after ultrasound documentation of uterine pregnancy of <20 weeks period of gestation (POG) and confirmation of all eligibility criteria. Nearly 60% of the participants in the cohort were enrolled within 14 weeks POG. The current attrition rate is 10%. The enrolled women are followed up 4-5 time-points across 3 trimesters of pregnancy to document extensive clinical and epidemiological information, varied maternal and neonatal biospecimens and perform serial ultrasonographic examination.

Early findings

The ultimate deliverable of this program is the development of a dynamic multi-dimensional predictive model to provide a decision algorithm for early prediction and timing of intervention of PTB. This model is built around specific questions as depicted in **Figure 3.1**.

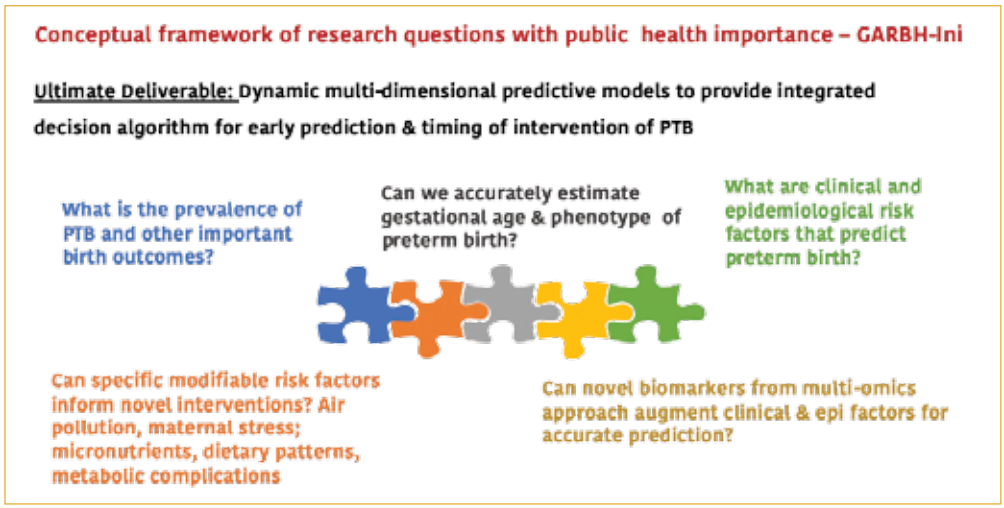


Figure 3.1. Conceptual framework of research questions with public health importance

A. Prevalence of preterm birth and other important pregnancy outcomes - Initial results

A high proportion of 13.4% preterm birth is remarkable as these figures are higher than those reported from economically developed (8.6%) or the low-income countries in Northern (7.3%) or Sub-Saharan Africa (12.3%). The cohort has provided opportunities to evaluate other pregnancy outcomes; a stillbirth rate of 2% is important given its crucial public health implications. There is a prevalence of 12% small for gestational age as assessed by the ultrasound at 30-32 weeks of gestation but interestingly this increases to nearly 38% at birth, indicating that late fetal growth restriction (FGR) is three folds more prevalent than early FGR in our population. This finding has clinical relevance because interventions targeted in the late trimester may prove to be more cost-effective.

B. Accurate estimation of gestational age and phenotypes of preterm birth

Accurate estimation of preterm birth rate and the proportions of different phenotypes of preterm birth has been a challenge.

(i) Developing population-specific models for accurate dating of pregnancy (Dr. Shinjini Bhatnagar, Dr. Ramachandran T., and Dr. Bapu Koundinya Desiraju from THSTI with Dr. Himanshu Sinha, Dr. Raghunathan Rengasamy from IIT-Madras)

The current methods of estimation of gestational age were derived from the western population. They have discrepancies varying from +/- 3 days for 1st trimester dating to +/- 2 weeks for 3rd-trimester dating. This translates to the inaccuracy of +/- 2% in preterm birth rate. For antenatal dating, data from the study was used to develop models for 1st and 2nd trimester. While the 1st-trimester model didn't improve the accuracy beyond the currently used Hadlock formula, there is a 40% improvement for the 2nd -trimester.

Way ahead: Similar approaches will be applied to develop dating models for the 3rd trimester. Also, the raw USG images will be used by image processing methods in addition to biometry to improve the accuracy further.

(ii) Validation of an already developed dried neonatal blood spot derived diagnostic analyte panel to correctly classify gestational age at birth (Dr. Shinjini Bhatnagar, Dr. Pallavi Kshetrapal and Dr. Yashwant Kumar from THSTI, Dr. Siddharth Ramji and Dr. Seema Kapoor from MAMC, Dr. Nansi Bhogossian from University of South Carolina, USA)

The prevalence of unbooked pregnancies (pregnancies with no antenatal care prior to delivery) varies from 1.5% in developed countries to 30-45% in LMICs. These pregnant women do not have reliable antenatal dating of pregnancy with clinical decisions of postnatal preterm care depending on the postnatal dating of pregnancy using Ballard's or Dubowitz scores that have high inter-observer variability and translates to the inaccuracy of +/- 3-4 weeks. Ultrasound evaluation in early pregnancy predicts gestational age with +/- 7 days of accuracy but only 24% of pregnant women undergo ultrasound evaluation in India during pregnancy. Alternative measures need to be developed for gestational assessment. Metabolomic and biochemical signatures are being identified from 1198 maternal and baby (cord blood and neonatal heel prick) dyads comprising of 1097 term and 101 preterm babies using high-resolution mass spectrometry.

Way ahead: Tandem mass spectrometry will be performed on the dried blood spot samples to analyze the low molecular weight molecules (metabolites). A targeted approach will be utilized to validate the test panel of 10 analytes developed in high-income countries for the accuracy of its prediction model in the GARBH-Ini setting. An untargeted analysis of the metabolites acquired would help to develop algorithms that could predict gestational age in a population-specific manner for poor resource settings. These predictive population-specific associations can then be validated and tested on separate samples in the ongoing cohort and other cohorts. The ultimate aim is to use a validated panel of metabolites to predict gestational age that can be developed into a simple diagnostic tool.

The next major challenge in the development of a prediction model for PTB is the ambiguity around PTB phenotype leading to inadequacies in the prediction and management of PTB. The current methods of phenotyping have used, just the conventional clinical characteristics such as clinical severity based on gestational age at delivery or the mode of onset of labor. The novel approach from Villar et al 2014 was adapted to describe phenotypes of birth outcomes using the events occurring around parturition process. The team could identify four distinct birth phenotypes. This exemplifies the applicability of such methods to the study population and similar approach will be used to

phenotype preterm birth. The advantage is that the risk prediction models may work better for some specific phenotypes making clinical management more optimal.

C. Clinical and epidemiological predictors for preterm birth

To identify risk factors that predict preterm birth, they used classical epidemiological analytical methods, and identified risk factors that included previously known risk factors such as prior preterm birth, short interpregnancy interval, multiple gestations, short cervix, and antenatal vaginal bleeding. They also identified some emerging risk factors such as the use of biomass fuel for cooking and exposure to passive smoking. However, combining all these factors in a multivariable prediction model could explain the PTB risk by just 10%. This has identified the need for advanced data-driven approaches to identify novel risk factors; these analytical methods will be used to develop prediction models in the next few years.

D. Specific modifiable risk factors associated with pregnancy outcomes inform novel interventions

This program gives an opportunity to study unexplored/emerging risk factors not just for their predictive ability, but to develop specific interventions to prevent preterm birth.

i. ***Ambient and household air pollution (Dr. Ramachandran T, Prof. Shinjini Bhatnagar)***

Global burden of diseases study 2015 lists ambient and household air pollution as the second largest risk factor cluster contributing to the global DALYs (Disability Adjusted Life Years) lost (103.1M and 85.6M respectively). Spatially, air pollution can be outdoor (ambient) and indoor (household) air pollution and there seems to be a seamless contribution of one component towards the other. PM2.5 exposure from multiple sources like ambient air pollution, household air pollution, second-hand smoke exposure, and active smoking was found to be the fifth-ranking mortality risk factor in 2015. Nearly 9.5% of all PTB could be attributed to exposure to indoor air pollution (assessed at enrolment by history of use of biomass fuel for cooking and exposure to second-hand tobacco smoke) in the GARBH-Ini cohort. This result indicates that provision of clean fuel and avoidance of exposure to second-hand tobacco smoke could be an important public health intervention and needs to be studied further. However, quantitative estimates of risk of adverse pregnancy outcomes across different ranges of exposure are still unclear.

Way ahead: The MCH team is collaborating with the Department of Chemical Engineering, Indian Institute of Technology, Madras to evaluate associations between air pollution and poor outcomes, more specifically PTB and FGR in terms of (i) dose-response relationship (ii) windows of exposure during the antenatal period, where there is higher risk and (iii) possible sources of air pollution.

The research team has developed a novel integrated exposure assessment system which includes data from satellite monitoring systems, mobile low-cost multi-pollutant monitors and household microenvironment monitors. The knowledge of the dose-response relationship will help identify the

threshold of PM2.5 exposure and the window of vulnerability to which novel interventions can be designed. Stratification of at-risk women will help design preventive interventions targeted towards those vulnerable subsets. Profiling of sources of air pollutants based on risk of adverse outcomes will inform policy interventions targeted toward source mitigation and help formulate evidence-based health advisory.

Maternal nutritional status

(i) Maternal dietary patterns (Prof. Shinjini Bhatnagar and Ms. Neera Parmar)

In the GARBH-Ini cohort, women starting their pregnancy with BMI<18.5% have a higher risk of PTB and based on this data interventions to improve maternal pre-/early pregnancy weight can reduce the PTB rates by 7.5%. Limited evidence is available in the Indian settings on the changing nutrient intakes and dietary patterns across pregnancy and how these may be associated with length of gestation and fetal growth. Dietary patterns become important because they ascertain the nutrient intakes in the context of socio-cultural setting. One of the important objectives of the study is to ascertain if the risk of PTB and/or FGR increases with (i) low dietary intakes (ii) and/or poor quality of dietary proteins taken in the antenatal period increase.

Way ahead: Food frequency questionnaire, as an assessment tool for dietary patterns and nutritional intake, has been developed using standard methods and is being validated. This study will provide trimester-specific dietary information that can be used to generate hypotheses for subsequent intervention studies to optimize length of gestation and fetal growth.

(ii) Micronutrient deficiency (Prof. Shinjini Bhatnagar, Dr. Uma Chandra Mouli Natchu, St. John's research institute, Bangalore; Dr. Partha Majumder, NIBMG, Kalyani)

The effects of nutritional influences are likely to be gene-environment interactions. We decided to explore multiple micronutrients such as selenium, zinc, vitamin-D, B12 and folic acid whose deficiencies have a high population prevalence in India. This allows us to examine associations between:

1. nutrient deficiencies and pregnancy outcomes.
2. genes involved in nutrients and the possibility of epigenomic changes that may explain or begin to explain the effects on pregnancy outcomes.

Such analyses might provide possibilities for interventional trials of nutrient supplementation (single or a combination of a few) in pregnancy to reduce poor pregnancy outcomes (all-cause or specific-cause).

Way ahead: The biological estimation is being completed on Inductively Coupled Plasma Mass Spectrometry. Genome-wide association studies to identify genetic susceptibility of the adverse pregnancy outcomes due to these micronutrient deficiencies are being performed on the same set of samples.

Maternal stress

(Dr. Nitya Wadhwa and Prof. Shinjini Bhatnagar, THSTI; Dr. Tushar Maiti, RCB; Dr. Arindam Maitra, NIBMG, Kalyani)

The prevalence of prenatal stress in India is 20-25%. Data on how prenatal stress could be associated with gestational age and/or birth weight is ambiguous as it available only from cross-sectional studies with small sample sizes. Shortening of telomere length, epigenomic and proteomic alterations, hair cortisol levels could be putative correlates of stress and can be evaluated as potential biomarkers for predicting PTB and FGR. A cohort of 1,869 pregnant women has been identified within the larger GARBH-Ini cohort to assess prenatal psychosocial stress using a validated clinical instrument. They are being followed longitudinally till the end of pregnancy to document the gestational age and birth weight. 1,279 of the 1,869 women enrolled have had a birth outcome. The initial analysis has shown that 407 had low (range 0-7), 328 moderate (range 23-37) and 242 high (range 55-125) stress scores respectively. The telomere length, plasma protein and hair cortisol estimation of biospecimens collected from these women at 18-20 and 26-28 weeks is ongoing.

Way ahead: Once the laboratory testing is complete, an integrated time series analysis is proposed to identify clinical and/or biological markers of stress that can be used to predict women who will go on to have PTB or fetal growth restriction.

Gestational diabetes mellitus

(Dr. Pallavi Kshetrapal, Dr. Ramachandran T, Prof. Shinjini Bhatnagar, from THSTI; Dr. Nikhil Tandon and Dr. Yashdeep Gupta from AIIMS, New Delhi)

Gestational diabetes mellitus (GDM) is an important pregnancy complication associated with adverse outcomes. The incidence of GDM of nearly 13% in the GARBH-Ini study population is notable. Oral glucose tolerance test (OGTT) commonly used for the diagnosis of GDM has poor acceptability among the patients as it is cumbersome and time-consuming. Simpler methods that can predict GDM in the 1st trimester of rule-out GDM during 2nd trimester can supplement OGTT for efficient diagnosis of GDM.

Initial analysis has shown that simpler point-of-care HbA1C estimation tested in GARBH-Ini cohort has shown higher specificity (85%) but poor sensitivity (15%) both for prediction and diagnosis of GDM. Models developed using machine learning methods with relevant clinical markers in addition to HbA1C were able to improve the sensitivity for prediction of GDM during 1st trimester marginally to 32%.

Way ahead: Development of multivariable models for diagnosis of GDM using point-of-care HbA1C and clinical parameters collected at 26-28 weeks of gestation are being attempted.

Fetal growth restriction (FGR)

(Prof. Shinjini Bhatnagar, Dr. Shailaja Sopory, Dr. Ramachandran T. and Dr. Koundinya Desiraju)

GARBH-Ini study provides a unique opportunity to study gestational weight gain and fetal growth longitudinally. Such longitudinal data is virtually absent in India and infrequent in LMICs.

1. ***Gestational weight gain as a marker for fetal growth:*** Gestational weight gain (GWG) is defined as the weight gained by a pregnant woman from conception to just prior to parturition and can be used as a surrogate marker of fetal growth. It has gained public health importance as it is a potentially modifiable risk factor. The longitudinal distribution of gestational weight gain in an unselected population has been described for the first time in India in the GARBH-Ini cohort. A longitudinal comparison of GWG showed that 75% of our women had inadequate weight gain according to the Institute of Medicine's recommendations. The comparison between the GARBH-Ini study population and the standard (currently INTERGROWTH-21st) population showed that the women were leaner during early pregnancy (50th centile difference at 20 weeks: 0.9 kg) and the difference increased as the pregnancy progressed (50th centile difference at 40 weeks: 3.1 kg). At 40 weeks of gestation GWG in our participants in 50th centile as low as that of 10th centile in INTERGROWTH-21st global standards (**Figure 3.2**).

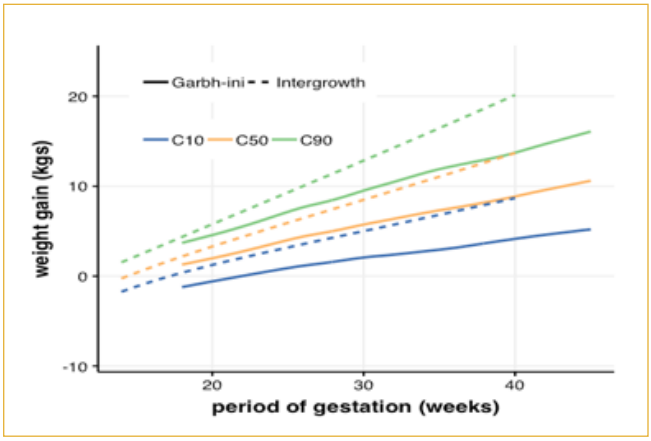


Figure 3.2: Comparison of gestational weight gain patterns of GARBH-Ini and INTERGROWTH-21st population

Way ahead: Regular fetal growth monitoring can help early diagnosis of fetal growth restriction and prevent associated adverse outcomes. The current mode of fetal growth assessment using ultrasonography is costlier and inaccessible to a large subset of the population. Gestational weight gain patterns in GARBH-Ini cohort have shown strong correlation with birth weight of the baby. Statistical models to estimate fetal weight using maternal anthropometric measures such as weight, height, abdominal circumference, and symphysis-fundal height will be generated using the serial measurements in GARBH-Ini participants. Such models after validation in participants enrolled prospectively in the cohort will be useful tools to enable better monitoring of fetal growth and early detection of FGR

2. FGR is captured as (i) small for gestational age based on birth weight or (ii) fetal weight estimated by ultrasonography in the third trimester. There is limited data on fetal growth from low and middle-income countries and lack of consensus on the choice of reference standards that can be used to detect fetal growth restriction. The hypothesis is that the fetuses in Indian women have distinct growth trajectories and the risk factors of early and late fetal growth restriction are different in Indian settings as compared to the developed countries. We compared fetal growth in our study population with INTERGROWTH-21st standards and found that fetuses in our participants had lesser weight at 30-32

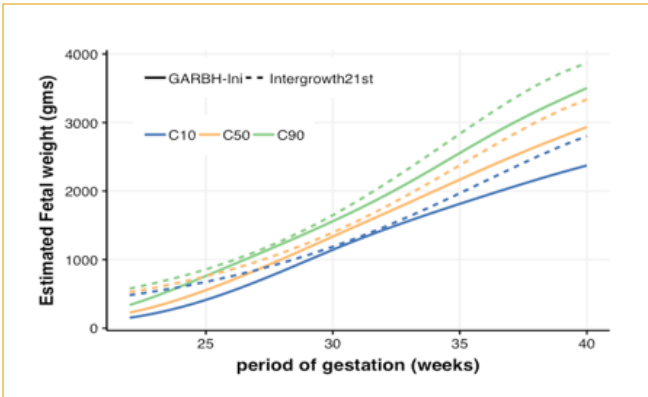


Figure 3.3: Comparison of gestational weight gain patterns of GARBH-Ini and INTERGROWTH-21st population

weeks than IG21st standards which more than doubled at birth (Figure 3).

Way ahead: Our next objectives are to develop region-specific growth trends from a larger number of prospective enrollments in the GARBH-Ini cohort and use these trends to determine risk factors of early fetal growth restriction which is defined as an estimated fetal weight <10th centile for gestational age before 32 weeks of gestation and late fetal growth restriction, which sets in after 32 weeks of gestation.

Multi-dimensional Omics for predictors of PTB

(Prof. Shinjini Bhatnagar, Dr. Pallavi Kshetrapal, Dr. Yashwant Kumar and Dr. Bhabhatosh Das, THSTI; Dr. Tushar Maiti, RCB; Dr. Arindam Maitra and Dr. Partha Majumder, NIBMG)

In order to develop predictive biomarkers for PTB, it is important to study biological changes that occur during a normal pregnancy. PTB may be a result of static (genomic) predispositions and dynamic (epigenomic, proteomic) modulations at different stages of pregnancy. Genomic, epigenomic and proteomic molecular profiles in addition to the microbiome are being looked at in mothers who have delivered preterm and term matched for appropriate covariates (parity, month of conception).

Epigenomics

Profiling of the temporal variations in DNA methylation and other epigenomic landscapes in maternal peripheral blood that occur during pregnancy is being carried out; these variations are being correlated with maternal environmental factors. The preliminary analyses have revealed variations in the promoter methylation patterns at least at 100 different loci in the preterm delivering maternal blood as compared to term pregnancy.

Proteomics

The temporal expression of proteins in different stages of pregnancy in healthy term birth (N=34) evaluated using high throughput mass spectrometry-based proteomics has shown that 30 distinct proteins ('the hub proteins') are modulated across pregnancy in maternal saliva and the vagina. Protein-protein network analysis suggests that immune modulation, metabolism, and host defenses are possible major pathways that could drive a successful pregnancy. As a next step the temporal expression of proteins is being compared between PTB and term birth across different stages of pregnancy in order to develop protein biomarkers for early birth.

Microbiome

The longitudinal follow-up of women has provided a platform to evaluate vaginal microbial diversity across pregnancy in Indian women and study novel associations between vaginal microbial community dynamics and PTB (and other adverse birth outcomes) with the hypothesis that these associations will form the basis for targeted interventions to reduce PTB. The microbes isolated from serial high vaginal swabs of mothers (N=40) are being screened and characterized to explore their functional repertoire by decoding their genome sequence. Initial analysis has shown that the (i) vaginal microbiota of reproductive age Indian women is mostly dominated by *L. iners* and *L. crispatus* (unique as no such data available till now in India), (ii) the genome of the *Lactobacilli* is enriched with mobile genetic elements like GIs (CRISPR-Cas), phages and insertion sequences, and (iii) among the several novel Lactobacillus species/subspecies observed in the vaginal ecosystem of Indian women more than 60 different bacterial species (N=363) have been identified. Further associations are being studied between vaginal infections (detected by clinical scoring, Nugent scoring, clue cells, and bacterial vaginosis) and with the microbial diversity in order to develop simple clinical markers of infections

Metabolomics

With the hypothesis that distinct metabolites in maternal sera early in pregnancy are associated with preterm birth, the maternal sera collected at the late first trimester was analyzed for low molecular weight molecules. These signatures related to maternal metabolic dysfunction have been identified using high-resolution mass-spectrometry based metabolomics and machine learning approaches. Principal component analysis of data acquired for the metabolites reveals presence of signature metabolites associated with PTB. Validation of these signature metabolites on a larger set of clinical samples (N=50 term and 50 preterms) would be initiated to identify the metabolites that could be utilized for

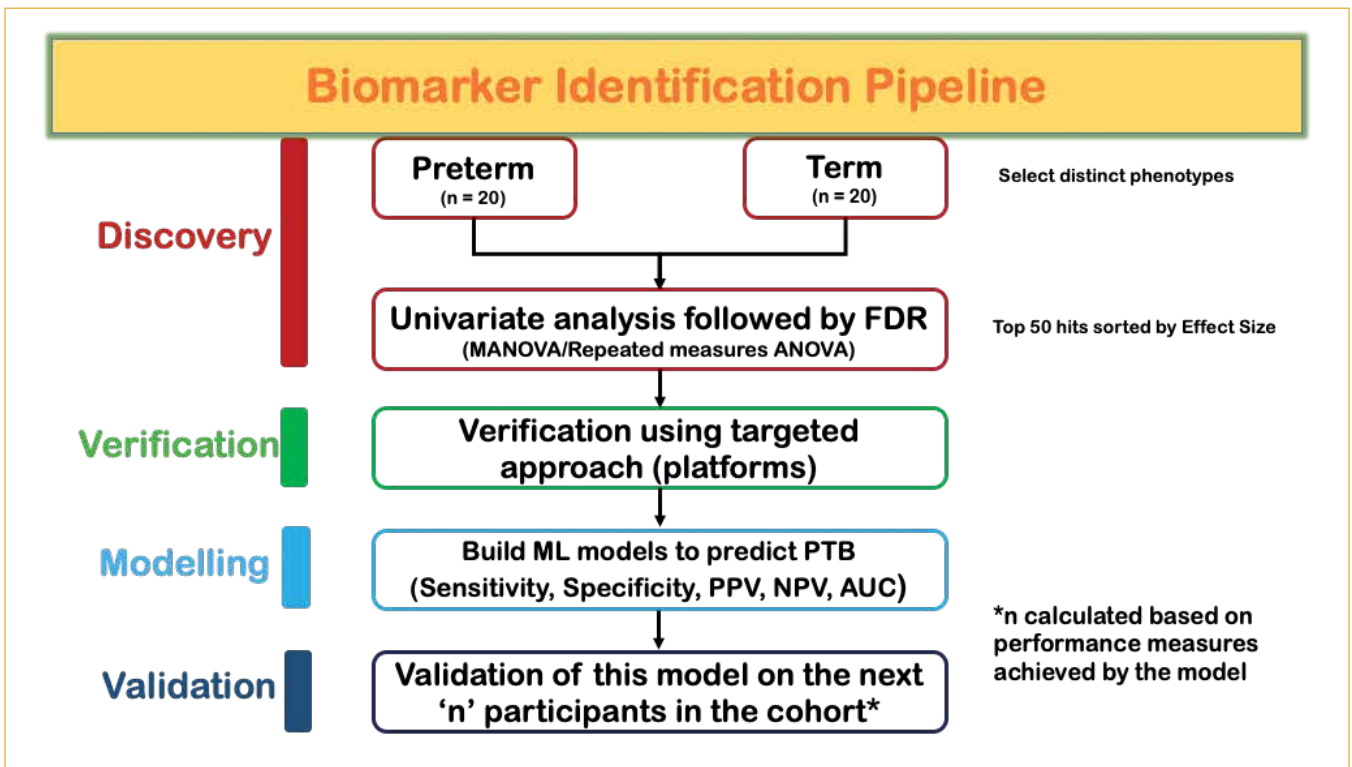


Figure 3.4: A conceptual framework of the biomarker discovery from the multi-omics analysis and translational pathway

development of biomarker panels for prediction of PTB. Proper validation of such a metabolome is a crucial step in identifying biomarkers for early prediction of PTB and for timely interventions. The conceptual framework of the biomarker discovery from the multi-omics analysis and translational pathway is described in **Figure 3.4**.

Way ahead:

Epigenomics

Initial results of genome-wide CpG methylation sites (>850,000) assayed on 20 preterm and 20 term mothers, matched using apriori selection criteria at four time points across pregnancy revealed methylation patterns of late preterms similar to term births. Based on these results, signatures identified in the discovery cohorts need to be validated on 100 pairs of matched preterm and term births sub-classified into categories of early, moderate and late preterm phenotypes. In addition, transcriptomic signatures between term and preterm pregnancies will further help in defining the transcriptomic clock of pregnancy.

Proteomics

Results on GARBH-Ini cohort population demonstrates variations in protein profiles with the progression of pregnancy in maternal bodily fluids. These candidate proteins from the discovery study will be validated (N=50 term and 50 preterms) using targeted MS (MRM-MS) to confirm the signatures as predictive biomarkers for PTB.

Microbiome

Several novel *Lactobacillus* species/subspecies have been identified in the vaginal ecosystem of women enrolled in the GARBH-Ini cohort. Sixty different bacterial species have been isolated and identified in the vaginal fluid (N=416). The plan is to isolate all the *Lactobacillus* species and decode their genome sequences. Further characterization of all Lactobacillus species carrying potential anti-inflammatory and antimicrobial functions will help develop quick diagnostic tools for the detection of bacterial species in women associated with the higher risk of PTB. The ultimate aim will be to develop rapid diagnostics for predicting PTB phenotypes and microbial therapeutics as interventions to reduce PTB risk.

Metabolomics

Differential metabolic changes were found to be associated with healthy and PTB in maternal sera collected at the first trimester. Initial analysis of the sera acquired metabolites from the GARBH-Ini cohort participants reveals ~20 metabolites differential in the TB and PTB. Longitudinal metabolic flux in the circulating blood of the mothers with term (N=50) and preterm (N=50) delivery will be acquired. Using the vast data generated on sera metabolites, the team plans to develop biomarker panels to help stratify PTB phenotypes and the best time for prediction and intervention along the pregnancy. The integration of data from the metabolites and proteomic platforms will also help understand the essential signaling pathway regulatory differences between the term and PTB.

Data generated on the multi-omics platform is being carried out on phenotypically well-described samples of preterm and term births. Integrated analysis of this vast data will help identify multidimensional tools and development of models to predict preterm birth.

Future direction for the GARBH-Ini Cohort:

The GARBH-Ini cohort is a platform for facilitating evidence-based solutions that have clinical and public health relevance. Some important leads have emerged from the initial analysis and these have been briefly summarized above which are being developed and carried forward. And will form the basis for future translational outcomes in the following years.

In addition, future directions will involve continuing the pregnancy cohort into a birth cohort, function of the human placenta and use of artificial intelligence-driven tools as mentioned below:

Multi-Omic Signatures of Human Placenta: Real-time assessment of underlying mechanisms for prediction of birth outcomes

(Dr. Pallavi Kshetrapal and Dr. Yashwant Kumar THSTI; Dr. Tushar Maiti, RCB; Dr. Arindam Maitra and Dr. Souvik Mukherjee, NIBMG, Kalyani)

Dr Pallavi's lab focuses on studying the molecular pathways that are clinically relevant in adverse pregnancy. Using a range of different omics approaches, the lab is focused towards identification of biomarkers from human circulating blood and from human placenta that can be utilized for development of translational modalities against adverse pregnancies.

The proper development of the growing fetus depends on the nourishment it receives during the gestational period from the placenta. Parturition takes place due to changes occurring in the mother and fetus through the fetal hypothalamic pituitary adrenal axis (HPA) activation and by endocrine and paracrine mediators secreted by the placenta. The placental signatures of these signals and the precise mechanism that helps in maintaining the pregnancy and then initiating the parturition process are still unclear.

The GARBH-Ini platform has facilitated the development of placental-derived exosomes as a possible tool for monitoring the placental functions in real-time. The MCH team collaborating with the National Institute of Biomedical Genomics (NIBMG) and Regional Center for Biotechnology plans to

i) Identify the placental specific markers in the extracellular vesicles secreted by the placenta at the level of transcripts/ proteins and metabolites.

(ii) Identify the placental cell-specific signatures using single-cell omics and to identify the differential signatures as cell-free RNA in circulating maternal blood.

(iii) Study the post-delivery placental proteome, metabolome, and microbiome it supports to help healthy term pregnancy outcomes.

Maternal infections/inflammation and adverse pregnancy outcomes (PTB and FGR)

(Dr. Shailaja Sopory, Ms. Khushboo Kaushal, Dr. Ramachandran T., Dr. Shinjini Bhatnagar)

Pregnancy is a dynamic state where the balance of Th1 and Th2 immune responses change along the different stages of pregnancy to maintain either a pro- or anti-inflammatory status. Inflammation or infectious exposure during pregnancy can disrupt this balance and lead to adverse pregnancy outcomes like FGR and PTB. Though infections can be recognized by clinical symptoms, subclinical infection or inflammation can only be recognized by measuring biomarkers of inflammation in the blood. Though there are cross-sectional and case-control studies from India that evaluated the effect of specific infections on pregnancy outcomes, there is limited data that carefully documents local or systemic infections longitudinally across pregnancy and their implications on birth outcomes. Further, there is a paucity of evidence of how inflammation with or without infection would be associated with poor birth outcomes. Preliminary analysis of data from self-reported cases of illnesses in the GARBH-Ini cohort shows a 45% greater risk of PTB in women with at least one episode of gastroenteritis (diarrhea lasting ≥ 2 days) during the antenatal period. The objective is to identify biomarkers of maternal infection and inflammation that will be useful in identifying pregnant women who are at risk of preterm birth and fetal growth restriction.

Way ahead: An important future objective is to document every episode of clinical infection across pregnancy, and validate it with laboratory evidence in the next 4000 participants enrolled in the cohort. The longitudinal profile of pro-and anti-inflammatory cytokines during different trimesters of pregnancy will be evaluated to establish critical windows during which infections and/or inflammation may be associated with PTB and the growth of the fetus. Once the inflammatory profile in normal healthy pregnancy has been established, associations between inflammation and FGR and or PTB will be studied using nested case-control designs within the ongoing cohort.

The study will also test the hypothesis that associations between antenatal infection/inflammation is associated with an increased incidence of postnatal infections, and with growth and neurodevelopmental outcomes in the early years of life. These insights will help develop interventions that boost (specific) pathogen-specific immunity or particular components of the immune system (alter certain cytokines or T–cell subsets).

Artificial intelligence-driven tools for predicting adverse pregnancy outcomes

(Prof. Shinjini Bhatnagar, Dr. Koundinya Desiraju from THSTI; Dr. Allison Noble, University of Oxford and Dr. Reva Tripathi, Jamia Hamdard University)

Detection and triage of pregnant women at risk of adverse outcomes is currently possible by ultrasonographic (USG) evaluation of the fetal position, number, lie,

biometry and position of placenta. This also requires highly trained operators and costly equipment which is lacking in resource-poor settings. Nearly 150,000 images of biometry and fetal well-being from serial ultrasound examinations in GARBH-Ini cohort are being used to train machine learning algorithms to discriminate a pregnancy at risk of adverse outcomes from a normal pregnancy. More advanced methods such as video sweeps are being added in the imaging of the prospectively enrolled GARBH-Ini cohort participants to improve the predictive efficiency of these algorithms. These trained algorithms can then be used as a software tool that gives traffic-signal based output for medical personnel with minimal or no USG training in resource-poor settings.

In recent time, USG images are being evaluated as predictive biomarkers for adverse birth outcomes since it is non-invasive, generates images with rich information on the fetus, placenta, cervix, uterine and umbilical arteries. A universal screening tool (with high sensitivity and specificity) is being developed using Convolutional Neural Networks (CNN) and USG images, taken during mid-trimester of pregnancy, to predict preterm birth. Although building these algorithms requires advanced computation, once developed they can be distributed to hospitals as simple software tools to predict preterm birth early in pregnancy.

Influence of birth phenotypes on childhood health, growth and development evaluated in a prospective birth cohort

(GARBH-Ini team)

High PTB (13.5%) and FGR (27%) rates have been documented in the GARBH-Ini study. It is well known that babies born with these adverse birth outcomes are at a higher risk of poor growth, delayed development and major morbidities and mortality during infancy and early childhood. But, the risk of adverse childhood outcomes is not uniform for all the babies born preterm and/or with fetal growth restriction. Some of the main reasons for this heterogeneity are that (i) adverse outcomes do not occur completely independent of each other; e.g. 24% of the babies born preterm in our cohort were also growth restricted, (ii) PTB and FGR are not single homogenous entities; multiple phenotypes of PTB have been proposed based on the clinical severity (gestational age at birth); maternal, placental and fetal characteristics; type of delivery and evidences of initiation of parturition. Similarly, FGR has been categorized into early and late phenotypes based on time of occurrence, severity of restriction and the proportions of various fetal biometry parameters. These heterogeneities contribute to the variations in their childhood growth, development, and morbidities.

The hypothesis is that babies born with different phenotypes of PTB and FGR have different childhood growth trajectories and development. The exposures during antenatal period (such as maternal dietary patterns, environmental exposures, and metabolic status), and infancy (such as feeding practices and environmental exposures) would have an effect modifying role to childhood growth and development, further influenced by the birth phenotypes. Identification and characterization of the determinants of stunting and developmental delay will aid design of novel predictive algorithms and innovative interventions to reduce early infancy, childhood morbidity and mortality attributed to these birth phenotypes.

Timelines on deliverables over the next 5 years:

The timelines for the deliverables from GARBH-Ini program over the next 5 years are depicted next.

Collaborations:

Members of GARBH-Ini

The unique characteristic of GARBH-Ini program has been the rich multi-disciplinary collaborations with institutes of national and international repute. Our collaborations have been strongly focused on capacity building of young researchers of THSTI and acceleration of the translational outcomes.

MEMBERS OF GARBH-Ini:

Translational Health Science and Technology Institute, NCR Biotech Science Cluster, Faridabad, Delhi NCR, India *(Shinjini Bhatnagar, Vineeta Bal, Bhabatosh Das, Bapu Koundinya Desiraju, Pallavi Kshetrapal, Sumit Misra, Balakrish G. Nair, Uma Chandra Mouli Natchu, Satyajit Rath, Kanika Sachdeva, Shailaja Sopory, Amanpreet Singh, Dharmendra Sharma, Ramachandran Thiruvengadam, Nitya Wadhwa);*

National Institute of Biomedical Genomics, Kalyani, West Bengal, India *(Arindam Maitra, Partha P. Majumder);*

Regional Centre for Biotechnology, NCR Biotech Science Cluster, Faridabad, Delhi NCR, India *(Tushar K. Maiti, Dinakar M. Salunke);*

Clinical Development Services Agency, Translational Health Science and Technology Institute, NCR Biotech Cluster, Faridabad, Delhi NCR, India *(Shubhra Bansal, Monika Bahl);*

Gurugram Civil Hospital, Haryana, India *(Sunita Sharma, Umesh Mehta, Brahmdeep Sindhu);*

Safdarjung Hospital, New Delhi, India *(Sugandha Arya, Rekha Bharti, Harish Chellani, Pratima Mittal);*

Maulana Azad Medical College, New Delhi, India *(Siddarth Ramji, Reva Tripathi, Anju Garg);*

The Ultrasound Lab, Defence Colony, New Delhi, India *(Ashok Khurana);*

Hamdard Institute of Medical Sciences and Research, Jamia Hamdard University, New Delhi, India *(Reva Tripathi);*

All India Institute of Medical Sciences, New Delhi, India *(Smriti Hari, Yashdeep Gupta, Nikhil Tandon);*

Government of Haryana, India *(Rakesh Gupta);*

International Centre for Genetic Engineering and Biotechnology, New Delhi, India *(Dinakar M. Salunke); and*

Indian Institute of Science Education and Research, Pune, Maharashtra, India *(Vineeta Bal).*

More recently we have established unique collaborations between physicians, biologists and data scientists to develop solutions for maternal and child health using innovative Artificial Intelligence and machine learning methods from multidimensional data acquired through well-designed observational and interventional studies (**Figure 3.5**).

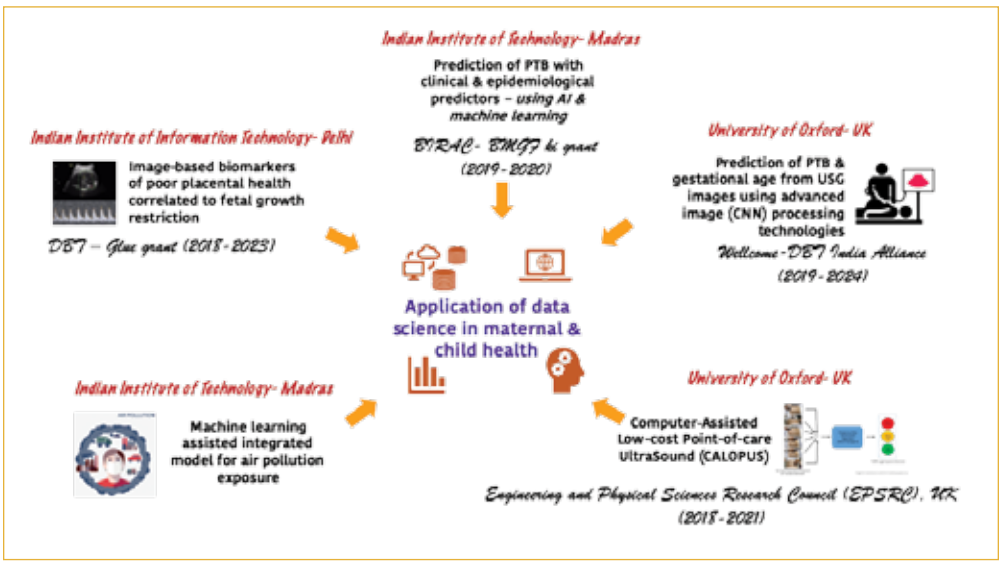


Figure 3.5

Domain ii. Finding innovative solutions for sepsis in neonates and young infants.

The maternal and child health program works as a multidisciplinary team where each scientist/ researcher complements the expertise of the other. This approach is aimed to produce solutions that single disciplines could not have wholesomely conceived. Working around the concept of bench to bedside and

back to the bench, the team has been trying to tease out the development and maturation of the neonatal immune system and what happens to the immune system during sepsis. This effort, to have a sound rigorous understanding of underlying biology on which intervention strategy is built is a multidisciplinary one. **Dr. Nitya Wadhwa leads a program that targets zinc as an intervention to reduce early life morbidity and mortality and Dr. Shailaja Sopory is leading the effort to understand how zinc works under normal and under different disease conditions and the pathways that are responsive to zinc supplementation that determines clinical outcomes.** This multidisciplinary research program will provide mechanistic insights into how immune cells respond to changes in zinc levels. Understanding the targets that are modulated by zinc supplementation is crucial to understand the subgroup effects of zinc and for further tailoring zinc supplementation trials.

Maternal and child health with a focus on early life morbidity and mortality and host immune response

(Investigators: Dr. Nitya Wadhwa and collaborators as mentioned below and the clinical coordinators: Dr. Debjani Purakayastha, Dr. Ritu Kashyap, Dr. Romila Rawat, Dr. Kabita Barua, and Dr. Komal Wadhwa)

The framework of sustainable development goals (SDG) targets to end preventable deaths in the under 5 age group and reduce under 5 mortality (U5M) to 25 per 1000 live births (LB) by 2030. Currently the U5M in India is 50 per 1000 live births. Globally, neonatal deaths contribute to 45% of U5 deaths and 24% of global neonatal deaths take place in India. The neonatal mortality rate (NMR) in India is 28 per 1000 LB and serious systemic infections or sepsis constitute an important cause of neonatal death.

An important focus area for the MCH program of THSTI is to find solutions to prevent or treat early-life infections. While appropriate antibiotics are available in many hospitals in low and middle-income countries, second-line antibiotics are unavailable or are prohibitively expensive in peripheral health facilities. It is important to develop inexpensive, effective, and accessible interventions that can be added to standard therapy for sepsis to improve treatment outcomes and reduce case fatality.

A previous study has shown the beneficial effect of zinc supplementation in improving patient outcomes by reducing the risk of treatment failure which has meaningful implications to the costs and health benefits from the intervention. If the results of the ongoing study are consistent with this earlier trial, then this study would contribute critical evidence towards revising treatment recommendations for low resource settings in South Asia and elsewhere.

Dr. Wadhwa is leading a large collaborative program between THSTI, University of Bergen, Norway and Tribhuvan University, Nepal to evaluate the role of oral zinc as an adjunct to standard antibiotic therapy for clinical sepsis, in reducing mortality and treatment failure in young infants aged 3 to 59 days. This program coordinated by THSTI is being done as a large multi-country (India and Nepal), multi-center individually randomized double-blind placebo-controlled parallel-

group clinical trial. It is being funded through a Global Health and Vaccination Research (GLOBVAC) grant from the Research Council of Norway (RCN) and the Centre for Intervention Science in Maternal and Child Health (CISMAC), Norway.

This study is being executed as a bilateral collaborative project under the 'Program of Cooperation' between the Department of Biotechnology, Indian Institutions and the Norwegian Institutions under the agreement in Science and Technology between the Government of the Republic of India and the Government of Kingdom of Norway.

This academic clinical trial is being conducted at two sites in Kathmandu, Nepal and four in Delhi, India. Ethics approval for the conduct of the study was obtained from 10 ethics committees including the IEC at THSTI and each of the study hospitals.

***Study population*:** Infants 3-59 days old with s/o clinical severe infection (CSI) as defined by WHO, IMCI presenting to the emergencies of the hospital sites; estimated sample size is 4200 young infants with clinical severe infection. Half of the enrolled infants will get 5mg elemental zinc dispersible tablets orally twice a day for 14 days and the other half will get dispersible placebo tablets in the same twice a day, dose schedule. The primary outcome is death during hospitalization for clinical severe infection and death anytime in the 12-week study period. Enrolled infants are administered intervention for first 14 days and followed-up till recovery and discharge and further for 12 weeks from day of enrolment. The multisite trial was initiated in a phased manner in February 2017. As of March 2019, the study enrolled a total of 1,758 young infants across 6 sites. About one-fourth of the infants enrolled with clinical sepsis had associated diarrhea. 59 infants died during initial hospitalization for the sepsis and about 4% died in the 12-week follow-up period after recovering from the initial episode of clinical sepsis. The study flow is given in Figure 3.5 The treatment failure rate in enrolled infants is 14% and our loss to 12-week follow-up is contained at 2.3%. The study is ongoing and the group proposes to complete the enrolments by August 2020.

Way ahead: Dr. Wadhwa is collaborating with experts in economic evaluation at the University of Bergen and has applied for funding to evaluate cost-effectiveness, equity impact, and financial risk protection gains of the adjunct zinc intervention for young infant sepsis. In the face of immense resource scarcity, countries such as India struggle with several competing priorities and alternatives both within and outside the health sector. Hence, documenting efficacy and safety of interventions is no longer adequate to justify inclusion of new health interventions in national health programs. Health policymakers are increasingly demanding evaluation of investment returns from new health interventions rather than solely focusing on efficacy. The estimated sample size for this sub-study within the clinical trial is 2000.

List of collaborators:

Zincsevinf squad:

PI: *Dr. Nitya Wadhwa*

Co-PI: *Dr. Shinjini Bhatnagar (THSTI); Dr. Sudha Basnet (Tribhuvan University, Nepal), Dr. Tor Strand (University of Bergen, Norway)*

Co-Investigators:

THSTI: Drs. Uma Chandra Mouli Natchu, Shailaja Sopory

Vardhman Mahavir Medical College and Safdarjung Hospital: Drs. Meenakshi Bhatt, Harish Chellani, KC Aggarwal, Rani Gera, Ajay Kumar, Sugandha Arya, Kanika Kapoor, Bhavna Anand, SS Meena

Maulana Azad Medical College and associated hospitals: Drs. NB Mathur, Siddharth Ramji, Sangeeta Yadav, Ajay Kumar, Urmila Jhamb, Raghvendra Singh

Chacha Nehru Bal Chikitsalaya: Drs. Mamta Jajoo, Manish Kumar, Kirtisudha Mishra, Medha Mittal, Karnika Saigal

Kasturba Hospital: Drs. Anuradha Govil, Sunita Bhatia

University of Bergen, Norway: Dr. Halvor Sommerfelt

Institute of Medicine, Nepal: Dr. Laxman Shrestha

Understanding the biological mechanisms for clinical effects of zinc supplementation

(Investigators: Dr. Shailaja Sopory, Ms. Yamini Goswami, Ms. Khusbhoo Kaushal)

Dr. Sopory's group is looking at host immune responses in infants with sepsis. Sepsis is a systemic condition, which could be bacterial, viral or fungal in origin. Sepsis is a major clinical problem with no definitive etiological agent identified in most cases. Neonates have underdeveloped innate immune responses that include decreased cytokine production, and reduced neutrophil and DC functions. It is now being recognized that the compensatory anti-inflammatory responses in children may be one of the key contributors to the pathobiology of pediatric sepsis. Therefore, understanding the molecular details behind the altered balance between inflammatory and anti-inflammatory response in neonatal sepsis is essential to improve clinical outcomes.

This study is embedded in a randomized double-blind placebo-controlled trial as mentioned in the clinical trial that has been described above. A blood sample is being collected at enrolment (v1), 48-72 hours post-Zn supplementation (v2) and at discharge (v3). The distribution of zinc is being examined in different immune subsets of patients at admission and at recovery for comparisons between the supplemented and placebo group using surface markers to identify the different cell populations and look for intracellular zinc levels by flow cytometry. The samples have been collected and processed for neutrophil (mature and immature), eosinophil counts and intracellular zinc levels at v1 (N=92), v2 (N=75) and v3 (N=45). In a cross-sectional analysis, there are significantly higher neutrophil counts at enrolment, which come down by 48-72 hours and stabilize and there does not seem to be a significant difference in the intracellular zinc levels in neutrophils at different time points.

Way ahead: It is hypothesized that since the beneficial effects of zinc supplementation on infections like diarrhea and sepsis are seen within a few days of starting zinc, a major mechanism could be through its action on innate immunity. The follow-up effect of zinc supplementation may well be because of its effect on adaptive immunity. Nevertheless, it is apparent that zinc supplementation has not resulted in beneficial outcomes in all clinical situations. Besides the dosage issues, these studies indicate that either the pathways affected by zinc supplementation are irrelevant for certain clinical conditions or the effect is specific to aetiological agents involved in sepsis. Therefore, understanding the targets that are modulated by zinc supplementation is crucial for further tailoring zinc supplementation trials.

It is still not clear if the effect of zinc is through the correction of Zn deficiency or direct immunomodulation and whether the effects differ depending on the etiology of infection (broadly, viral vs. bacterial). As a large number of neonates with sepsis turn out to be culture-negative, high throughput sequencing will be used to look at the etiology of sepsis and correlate the same with success of zinc supplementation. Whole blood gene expression studies will help distinguish bacterial and viral etiologies depending on the host

List of collaborators:

- Zincsevinf squad
- Dr. Guruprasad Medigeshi, THSTI

gene expression pattern. The gene expression profiles at enrolment will also be used to diagnose and predict the outcome of sepsis.

Maternal Nutrients and Child health

Investigators: Dr. Suchitra Gopinath and Mr. Jayesh Sevak

Dr. Suchitra Gopinath's research is focused on identifying molecular mechanisms mediating lean muscle mass proportions *in utero* and the maternal factors that influence infant body composition. She is particularly interested in lean muscle development due to micronutrient deprivation in mothers.

Her team has demonstrated that the deficiency of one specific micronutrient, vitamin D, is in itself sufficient to initiate skeletal muscle atrophy even during development in a molecular mechanism involving phosphorylated Stat3 and Myostatin. The hypothesis is that maternal vitamin D deficiency will have a measurable impact on fetal muscle development through its activity on multipotent stem cells that might lead to skewed body composition in the infant. To identify associations between maternal vitamin D levels and myogenic potential within the fetus, they have enrolled women at delivery at the Gurugram Civil Hospital, Gurugram and estimated vitamin D levels in maternal sera. Simultaneously, mesenchymal stem cell (MSC) lines were established from umbilical cord tissue-derived from these women after informed consent as a means to recapitulate fetal myogenesis. It was observed that MSC lines obtained from umbilical cord tissue display robust growth, possess higher yield and senesce at a much slower rate in culture compared to MSCs derived from umbilical cord blood in all samples. So far, 12 such lines have been established from enrolled women who, without exception display profound deficiency in vitamin D levels ranging from 10 nmoles/litre to 40 nmoles/litre (Sufficiency range is 75-200 nmoles/litre). Additionally, 95% of the women also displayed deficiency in vitamin B12, while 50% showed a reduction in folate levels. Almost all women except for one displayed iron sufficiency. To identify the mechanisms by which one single micronutrient might impact myogenesis in multipotent stem cells, RNA-sequencing analysis has been performed on MSCs treated with vitamin D and differentiated into the muscle. Compared to the untreated MSCs, vitamin D pre-treated MSCs upregulated genes associated with undifferentiated myogenic cells but downregulated several genes associated with terminal differentiation into the myogenic lineage. These results, it seems, to suggest that vitamin D may be responsible for maintaining a pool of committed myogenic precursor cells in an undifferentiated state. This would be essential at later stages for achieving the appropriate level of muscle mass. On the other hand, in vitamin D-deficient women, this pool of precursor cells might be exhausted due to premature myogenic differentiation resulting in loss of muscle mass in the fetus.

Way ahead: The group intends to assess myogenic differentiation potential and proliferation characteristics of the 12 MSC lines. They are also trying to increase their sample size to obtain enough numbers for stratification and subsequent comparisons. Amongst the genes that are upregulated in vitamin D-treated MSCs, they intend to focus on the interaction between vitamin D receptor (VDR) and chromatin modulators in these genes to define the epigenomic landscape

List of collaborators:

- Dr. Aneeshkumar Arimbassery (NII)
- Dr. Niraj Kumar (THSTI)

during vitamin D signaling. The group wants to address whether women with severe vitamin D deficiency have altered epigenomic responses that perturb myogenic progression in the fetus. To correlate molecular data with clinical parameters, this system will be coupled with neonatal anthropometric data such as Mid upper arm circumference (MUAC), skinfold thickness, and arm muscle area (AMA) in the infants to identify molecular mechanisms that influence lean muscle proportions in the infant.

Young Investigator Program

With the goal of strengthening and expanding the pool of physician-scientists in the country, and nurturing outstanding young physicians who have a strong aptitude towards research and a desire to grow into independent principal investigators a young investigator program was started in the GARBH-Ini cohort. This young investigator program aims to provide rigorous, high-quality training for doing hypothesis-driven research to the investigators. Dr. Ramachandran Thiruvengadam, a qualified pediatrician with post-graduate training in medical biochemistry joined as a young investigator in July 2016. With the guidance of a mentor panel, he has developed a research project to study the association between exposure to air pollution and pregnancy outcomes and preliminary data collection is underway. The details of the project are provided under maternal and child health program.

L-R: Dr. T. Ramachandran, Dr. Suchitra Gopinath, Dr. Shailaja Sopory, Prof. Shinjini Bhatnagar, Dr. Ruchi Tandon, Dr. Pallavi Kshetrapal, Dr. Koundinya Desiraju, Dr. Nitya Wadhwa

L-R: Dinesh Chauhan, Ramesh Kumar, Sagar Singh, Mamta Rai, Sachin Biloni, Rakesh Kumar, Satish, Ashu Sharma, Ritesh Ranjan, Shrichand Pandey, Manish Sethi, Mukesh Juyal, Rahul Sharma, Amanpreet Singh, S. S. Suresh, Nitya Wadhwa, Veenu Kumar Mani, Debjani Purakayastha, Rajkumar Tanwar, Shilpa Chopra, T. Ramachandran, Sandeep, Priyanka Sharma

L-R: Varsha Dwivedi, Shilpi Sehgal, Pragya Tailor, Neha Yadav, Amitab Bachan, Dr. Ramasamy Thirunavukkarasu, Archana Rao, Dr. Savita Singh, Dr. Pallavi Kshetrapal

L-R: Ashish Tyagi, Yamini Goswami, Raj Kumar Tanwar, Khushboo Kaushal, Anubhuti Gupta, Saimah Raza, Shailaja Sopory, Deepa Nair, Anita Chaudhary, Gaurav Singh, Manoj Mahato

NON-COMMUNICABLE DISEASES

Autoimmune Disorders

The interplay among immune cells in autoimmune disorders:

Dr. Amit Awasthi led immunobiology laboratory at THSTI is trying to comprehend the interplay between effector and regulatory T cells in inflammatory conditions such as Inflammatory Bowel Disease (IBD), psoriasis and multiple sclerosis. The focus is to decipher the molecular pathways that define the generation and functions of effector and regulatory T cells in various autoimmune disease conditions. His laboratory is primarily aiming to understand the functions of Th9 and Th17 cells in IBD, asthma and cancer immunity. The lab is also establishing an experimental model of a tumor to decipher anti-tumor functions of T cells and the role of checkpoint inhibitors in cancer immunotherapy.

One of the projects entails the development of an understanding of molecular pathways that lead to the generation and functions of Th9 cells. CD4+ T cells can differentiate into Th1, Th2, and Th17 cells. Nutrients, water, and oxygen are the fundamental constituents that are required for all the living cells, even more so for the cells of the immune system, which are metabolically hyperactive during immunological reactions. In general, the energy requirement of resting naïve T cells is fulfilled by aerobic metabolism of glucose via oxidative phosphorylation. Metabolic checkpoints were identified as one of the key regulators of T cell responses. Classically, the activity of metabolic enzyme or concentration of a specific metabolite was suggested to be an important checkpoint in immune response in infection and inflammation in autoimmunity. Activated CD4+ T cells proliferate and acquire distinct effector phenotypes such as Th1, Th2, Th9 and Th17 cells, which contribute to specialized functions in eliminating intra and extracellular pathogens as well as inducing tissue inflammation in autoimmunity and allergic inflammation. On the contrary, regulatory subsets of CD4+ T cells, which include Foxp3+ regulatory T cells (Tregs) and type 1 regulatory T (Tr1) cells, suppress effector T cell functions and contribute to resolution of tissue inflammation in autoimmune diseases (**Figure 4.1**).

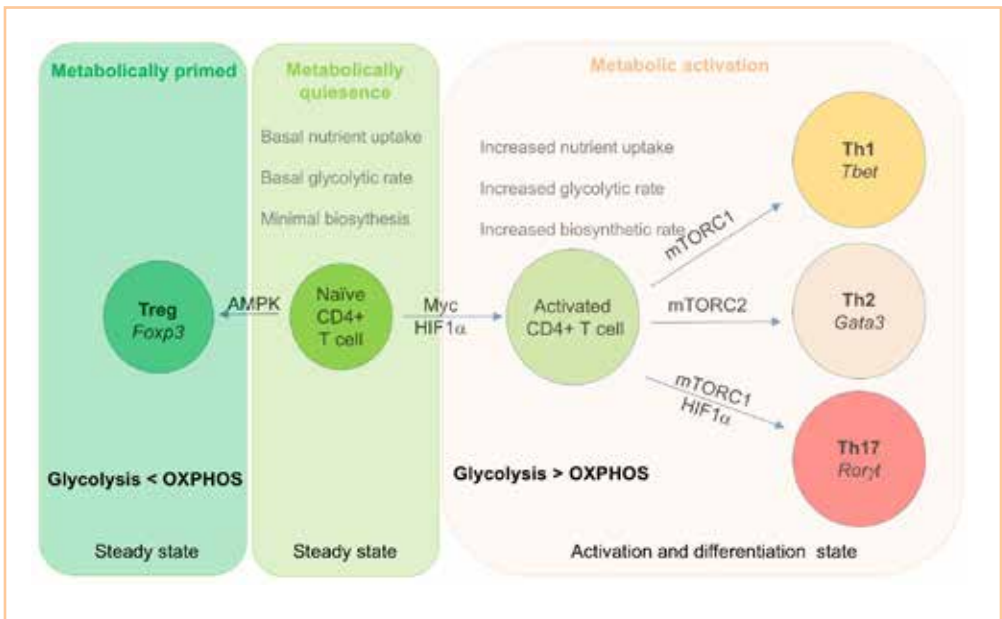


Figure 4.1: Role of metabolic checkpoints transcription factor in T cells activation and differentiation. Metabolism plays a key role in the activation and differentiation of effector subset of T cells. This diagram depicts key transcription factors that regulate the differentiation of effector cells. Naïve T cells are metabolically quiescent and rely on OXPHOS for their energy requirement. Both Myc and HIF1a play a crucial role in activation of T cells upon antigen encounter.

Interleukin 9 (IL-9)-producing helper T (Th9) cells have a crucial effector function in inducing allergic inflammation, autoimmunity, immunity to extracellular pathogens

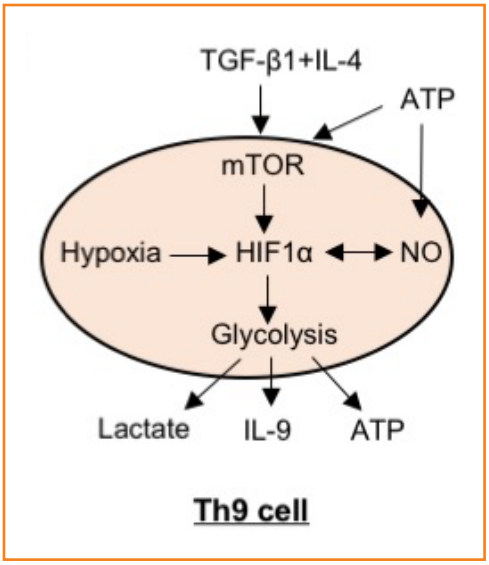


Figure 4.2: Schematic model of human Th9 cells

and anti-tumor immune responses. Although the cytokines that lead to the differentiation of human Th9 cells have been identified, other factors that support the differentiation of Th9 cells have not been identified yet. Dr. Awasthi's team was the first to identify that the extracellular ATP (eATP) induces the differentiation of human Th9 cells. They further showed that eATP induces the production of nitric oxide (NO), which creates a feed-forward loop in the differentiation of human Th9 cells, as inhibition of purinergic receptor signaling suppressed the generation of human Th9 cells while exogenous NO could rescue a generation of Th9 cells even upon inhibition of purinergic receptor signaling. Moreover, they identified that ATP-induces transcription factors, mTOR and HIF-1a, which are essential for the induction of human Th9 cells. Their findings, thus, identify that ATP-induced nitric oxide potentiates HIF1α-mediated metabolic pathway that leads to IL-9 induction in Th9 cells. *Here they identified that the ATP-NO-mTOR-HIF1a axis is essential for the generation of human Th9 cells and modulation of this axis may lead to therapeutic intervention of Th9-associated disease conditions* (**Figure 4.2**).

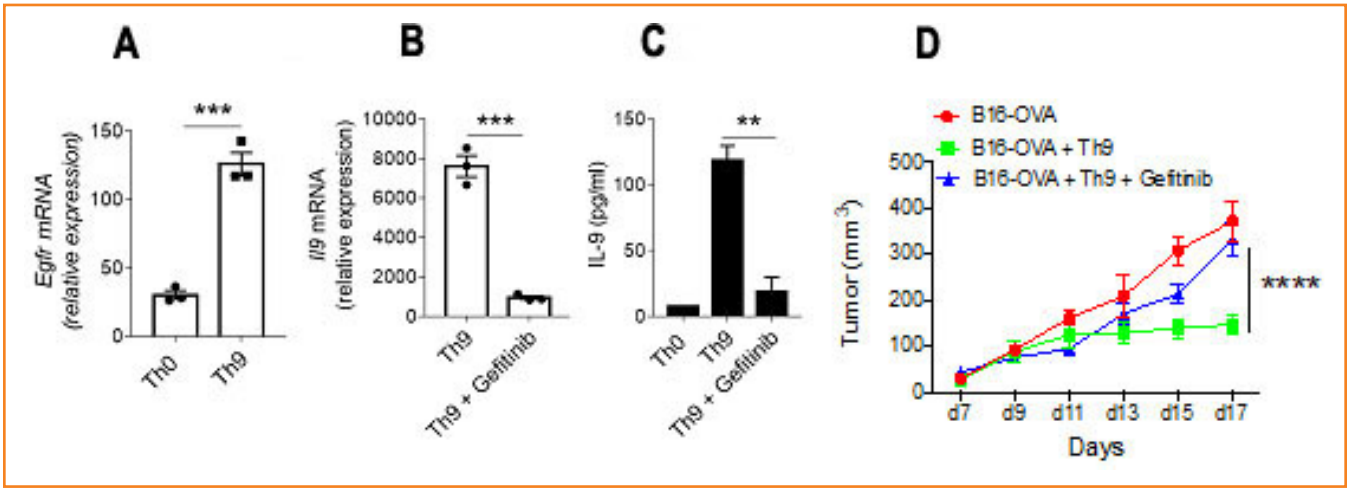


Figure 4.3: RNAseq-based Pathway analysis of Th9 cells. EGFR pathway is highly enriched in Th9 cells.

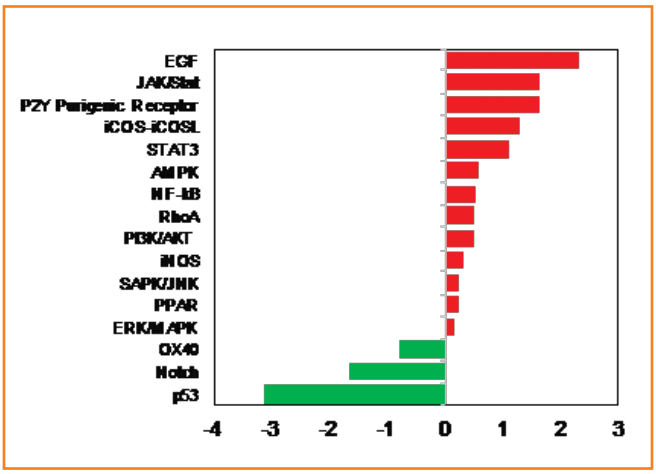


Figure 4.4(A-D): EGFR expression in Th9 cells. Inhibition of EGFR suppressed IL-9 in Th9 cells and suppressed anti-tumor functions of Th9 cells in vivo.

Transcriptional regulation of Th9 cells:

Given the importance of Interleukin 9 (IL-9) and Th9 cells in the immune system, Dr. Awasthi and co. wanted to identify the molecular pathway that leads to the differentiation of Th9 cells. To accomplish this, they employed the whole genome RNA sequencing (RNAseq) to define the Th9 cells differentiation pathway at the molecular level. Global gene expression profiling analysis coupled with pathway analysis has identified that differentiation of naïve T cells in the presence of Transforming Growth Factor beta 1 (TGF-β1) and IL-4 to induce Th9 cells show the upregulation of Epidermal Growth Factor Receptor (EGFR) **(Figure 4.3)**, suggesting the role of EGFR in the development of Th9 cells. The team further confirmed their findings through a quantitative PCR (qPCR) analysis which

showed that the expression of EGFR is enriched on Th9 cells as compared to Th0 **(Figure 4.4A)**. They further tested the functional role of EGFR in Th9 cells by blocking EGFR by gefitinib, an EGFR specific inhibitor, and found that gefitinib could significantly suppress the development of Th9 cells **(Figure 4.4B)**. The *in vivo* functions of EGFR in Th9 cells in tumor model was evaluated, as Th9 cells are known to induce anti-tumor functions.

To do this, they generated an ova-specific B16F10 tumor model in which B16F10 melanoma cells express ova antigen; they used OT-II TCR transgenic T cells and differentiated them into Th9 cells in the presence or absence of gefitinib, a selective inhibitor of EGFR to block tyrosine kinase-dependent signaling. They found that Th9 cells regress tumor growth while gefitinib treated Th9 cells failed to control tumor growth, implicating that EGFR signaling is crucial in inducing anti-tumor functions of Th9 cells **(Figure 4.4D)**. In conclusion, they have identified a new pathway that is essential for the *in vivo* functions of Th9 cells.

Non-alcoholic Fatty Liver Disease/Non-alcoholic Steatohepatitis:

(Team Leader: Dr. Madhu Dikshit, THSTI)

Non-alcoholic Steatohepatitis (NASH) is one of the major manifestations of metabolic disorders and is a high-risk factor for chronic hepatic fibrosis and hepatocarcinoma. NASH is a serious form of non-alcoholic fatty liver disease (NAFLD) in which liver has lipotoxicity, inflammation and liver injury. NASH is an unmet medical need and currently the leading cause of liver transplants globally. With no FDA-approved drugs, NASH has emerged as a disease in focus for pharma, biotech companies and research institutes alike. At THSTI, multidisciplinary approaches are being used to address NASH as a chronic metabolic disease. The work amalgamates collective efforts of a multidisciplinary team of scientists with different expertise and complementary skills to achieve defined objectives. Two broad aims of the program are:

- Drug lead development with proof-of-concept (POC) studies in animal models
- Identification and validation of novel targets and molecular signatures for NASH

Identification and development of new drug leads:

Current efforts around the globe to design effective therapy for non-alcoholic fatty liver disease (NAFLD) are delimited by the complicated nature of the condition that ranges from simple steatosis to non-alcoholic steatohepatitis (NASH) and finally progressing to liver fibrosis and hepatocarcinomas.

Dr. Dinesh Mahajan's group at THSTI is focused on the identification and development of new drug leads based on small molecules as well as phytopharmaceutical extracts of Indian herbs known in traditional literature. This work is being pursued in collaboration with his colleagues from the non-communicable research group at THSTI. The major focus of Dr. Mahajan and his team in this therapeutic domain is development of therapeutic drug leads either based on small molecule or plant extract for fatty liver diseases.

During their efforts of developing small molecules as possible drug leads for fatty liver diseases, exploiting novel approaches, the team identified a series of small molecules with the ability to induce autophagy. These molecules are now being examined further through *in vitro* and *in vivo* pharmacological characterizations. The approach of his medicinal chemistry group is to address key issues such as pharmacophore understanding (based on structure-activity relationship); liabilities associated with ligand structure (PK/ADME optimization); off-target based potential safety issues when striving for high potency during designing or synthesis of molecules. This medicinal chemistry approach ensures high success rate in lead discovery efforts.

Dr. Mahajan and his team identified an initial hit molecule and a hit series for further evaluation for fatty liver based on autophagy induction. The newly identified hits not only demonstrated a very potent lipid-lowering effect but also restricted the triglyceride load and improved the overall cellular parameters. The project team has evaluated the initial leads for detailed pharmacokinetic analysis in rats and mice models. One of the leads was also found to be orally bio-available in rats when evaluated for detailed pharmacokinetic study. A dose tolerance study was performed to determine the highest safe dose of the identified lead to plan a proof-of-concept study in animal model of disease with Dr. Sanjay Banerjee. The identified initial lead was found to be safe and dosing concentration was decided based on dose tolerance study. The team has also developed a new synthetic route to make gram scale quantity of the identified lead to support animal experiments.

The chemistry team is also working on the generation of more analogs around the initial hit to understand the structure-activity relationship (SAR) and to determine the active pharmacophore. This is a work in progress. The successful outcome of these efforts will help in identification of an improved lead with drug like properties possessing desirable DMPK profile to pursue proof-of-concept studies in animal model of NAFL/NASH.

There are relevant clinical studies that suggest that the induction of autophagy does have pharmacological implications in non-alcoholic fatty liver and non-alcoholic steatohepatitis. The beneficial effect of caffeine for liver impairment is attributed to the autophagy inducing capability of caffeine in human study. Also, in the observatory clinical study, it was found that patients affected with fatty liver do have considerably low cellular autophagy levels.

Dr. Mahajan has also considerable interest in the development of Indian herbs extract as drugs for fatty liver. He has a major research project from DBT to develop two Indian herbs from north-east for metabolic disorders. The development of phytopharmaceutical-based drugs is also being facilitated by an active collaboration of THSTI and Dabur India.

Way ahead: The project team is presently focused on the evaluation of proof-of-concept studies in animal models of NAFLD. To support this goal, the chemistry team is working on process development to provide sufficient material to perform animal experiments (acute/chronic toxicity and efficacy). Also, efforts are being invested to understand the mode of action and pharmacology of the newly identified molecules. They are also working to generate more analogs around identified hits to create a SAR and drug lead development. The SAR study will eventually lead to the identification of a more potent drug lead along with pharmacophore understanding and broaden the patent space for intellectual protection.

Identification and validation of novel targets and molecular signatures for NASH:

Dr. Amit Yadav's team started with text-mining information on disease-gene databases to find out NASH and NAFLD related genes and processes, single nucleotide polymorphisms (SNPs) and variants. These resources were compiled and 499 genes associated with NASH and NAFLD were mined. A pathway-level data mining strategy was employed to highlight the important common and discordant pathways in the two related diseases based on their pathway-level connectivity. The pathways were compared based on the ratio of reactions covered by the mapped genes, after multiple corrections (<1% FDR), which depicted how these genes occurred in a pathway and network context. The importance of PTMs and immunomodulatory pathways (cytokines, PPAR-α, interleukins) was observed. Several associated pathways related to lipid metabolism, transport and assimilation, β-oxidation, transcription factors, and kinase signaling were also observed. These are the top priority pathways for omics level studies to find better drug targets and biomarkers. Fatty acid metabolism and signaling molecules are more important in initial stages of liver disorders (NAFL) while, cell death and late immune responses, necroptosis, prostaglandins, secondary metabolism, RIPK-1 signaling, ubiquitination, etc. become more important in NASH.

Later, lipidation-specific modifications were searched in the human database (neXtProt) but no extensive studies have been carried out so far. S-palmitoylation has the highest number of annotated lipid sites across 13 proteins, followed by myristoylation on 4 proteins involved in NAFL. Prostaglandins and farnesylation are enriched in NASH. Upon an in-depth study on interactors of lapidated proteins with first neighbor propagation found mostly apolipoproteins, kinases and cytokines involved in lipid transport, AMPK signaling, PPAR signaling, fat digestion and absorption, renin secretion etc., corroborating with earlier pathway-level data and hinting towards lipoprotein metabolism in insulin resistance, one of the hallmarks of liver disorders. These pathways and interactions suggest that fibrotic markers from matrix remodeling during the disease may also be predicted early by omics level data integration.

Way ahead: A joint proposal with AIIMS and fellow scientists of the NCD program has been planned to explore NASH progression and establish proteomics and lipidomic biomarkers through mass spectrometry in animal model and clinical samples.

The NCD team has decided to work on two protein targets - apoptosis signal-regulating kinase 1 (ASK1) and farnesoid X receptor (FXR). Inhibition of ASK1 and FXR has been demonstrated as a therapeutic option for the treatment of NASH. **Dr. Sameena Khan's** team aims to develop potent ASK-1 inhibitor and FXR agonist, as a drug lead with PoC in animal model, which can be progressed for Investigational New Drug (IND) studies for patients affected with NASH. One of the objectives is to discover ASK1 inhibitor and FXR agonist, which can reduce the NASH symptoms. Currently, there are no approved effective pharmacological therapies for NASH. In line with this, Dr. Khan's group is working to establish the target protein and lead molecule relationship in order to validate the predicted targets.

The work done so far is described here:

- Cloning and expression of ASK1 in the His tag (pETM-11) and GST tag (pETM-30) vector

- His-tagged ASK1 (649-978aa) protein expressed in B834 expression strain and purified by affinity, anion exchange, and gel filtration chromatography. In solution, ASK1 exists as a dimer.
- Standardization of the ASK1 kinase activity assay. The compounds will be further tested for the ASK1 inhibition.
- Screening and binding assay performed with selonsertib (a selective inhibitor of ASK1) and about 8 different compounds by Surface Plasmon Resonance (SPR).
- FXR (244-476 aa) gene cloned into pETM-11 (His-tagged) and pETM- 30 (GST + His tagged) and expressed in B834 expression strain.

Way ahead: The aim is to develop a drug lead having the potential of either to stop the progression of fibrosis or reverse the fibrosis from stage 3 to 2 or a lower stage. Inhibition of ASK-1, a serine/threonine kinase, has demonstrated improvement in inflammation and fibrosis during phase II clinical trial. The clinical trial findings suggest that selonsertib (ASK-1 inhibitor) reduces liver fibrosis in patients with NASH and stage 2-3 fibrosis. Considering these findings from other studies, initiation of a drug discovery project based on "me too" approach is planned. The pre-clinical, as well as clinical finding of existing phase III drug candidate (selonsertib), will be exploited to develop a best in class novel ASK-1 inhibitor with new IP space at THSTI with established proof-of-concept studies in animal model of the disease. This approach will not only be having a higher chance of success but can reduce the time to pre-clinical development, since the pharmacological validation of the ASK-1 as a drug target is established in a shorter phase II trial.

Broadly, the workflow will entail, the novel hit identification based on bioinformatics and medicinal chemistry approach. This will involve ligand and structure-based pharmacophore modeling and medicinal chemistry understanding of the existing drug lead and other close analogs as well as protein target. The existing information on known ligands and ASK-1 protein will be used to develop a novel hit and lead molecule with new IP space. The new chemical hit identified so will be further evaluated on different pharmacological assays to develop all the way till identification of a druggable lead having PoC studies in animal models. The NCD area has experts from the domain of bioinformatics, structural biology, medicinal chemistry, *in vitro* and *in vivo* NASH disease biology and pharmacology to facilitate drug discovery and development efforts. Two different NASH models in rats have been established, which can be used for this discovery project. Strategic clinical connections have been established at AIIMS and scientific discussions have been initiated with clinicians to get clinical insights on NASH.

Biomarker identification in NAFLD:

The existing gold standard for assessment of hepatic steatosis and fibrosis is a liver biopsy, an invasive procedure with complications of pain, bleeding, and even a small risk of death (0.01%). Due to slow progression, there is no stage-specific diagnostic test, except repeated liver biopsy, which also has about 30% variability for the confirmed diagnosis. It is also not feasible to perform repeated biopsies to assess for changes in steatosis and fibrosis on follow-up. Presently, there are no biomarkers (apart from liver biopsy) available to differentiate patients with and without NASH. Keeping in mind the huge burden of NAFLD, there is an unmet need of stage-specific biomarkers to facilitate accurate diagnosis, and also for the assessment of disease progression or regression.

Model for End-Stage Liver Disease (MELD) score is a marker of disease severity and mortality in individuals with chronic alcoholic liver disease. **Dr. Renu Goel's** team is working to find out a diagnostic biomarker for disease severity along with the Model for End-Stage Liver Disease (MELD) score which can be used as a predictor of short-term mortality in individuals with alcoholic hepatitis. Her team employed sequential window acquisition of all theoretical mass spectra (SWATH-MS) to seek crucial proteins involved in disease progression. Quantitative proteomics of bone marrow plasma with low and high MELD scores was compared with normal bone marrow plasma from non-cirrhotic portal hypertensive patients whose liver function test was found normal using a SWATH-MS strategy. In total, 232 proteins were differentially expressed in all groups. Seventeen proteins were downregulated and 81 upregulated in patients with MELD score < 15 with control. Moreover, 37 proteins are downregulated and 59 upregulated when comparing patients with a MELD score > 15 with control subjects. The differentially regulated proteins were enriched for activation of acute-phase signaling and Liver X Receptor-Retinoid X Receptor (LXR/RXR) activation pathways. The inhibition of coagulation, complement, and intrinsic prothrombin pathways are revealed by functional analysis. Humoral immune response, immune cell trafficking, and inflammation pathways were found to be enriched under physiological system development. Proteins preliminarily discovered in this study may be associated with dysregulation bone marrow microenvironment during disease progression. *To the best of our knowledge, this study presents the most complete view of bone marrow plasma in low and high MELD score, identifying hundreds of differentially expressed proteins, which together form a rich resource for novel drug targets or diagnostic biomarker discovery.*

Considering there is no marker available to distinguish patients with NASH from those without NASH at present, **Dr. Yashwant Kumar** is using serum metabolomics and lipidomics studies to understand early processes of NASH disease development and establish a robust biomarker for non-invasive diagnosis of disease. In collaboration with clinicians from AIIMS, Dr. Yashwant's group will be working well-characterized human serum samples from NASH and non-NASH subjects to discover non-invasive metabolic biomarkers.

Way ahead: They plan to analyze a large number of clinically well-characterized human samples from control and NAFLD subjects for the development of translatable biomarkers. They aim to develop a non-invasive biomarker to distinguish NASH from non-NASH subjects and for different stages of fibrosis in NASH.

Cardiometabolic Syndrome:

Therapeutic screening in cardio-metabolic disease animal models:

Dr. Sanjay Banerjee examines plant-based and nutritional products to screen them in different cardio-metabolic disease animal models. His team is working to find the role of vitamin D deficiency in developing cardio-metabolic complications. They recently showed that lower 25(OH)D3 and 1,25(OH)2D3 levels are associated

with type 2 diabetes mellitus and type 2 diabetes mellitus with coronary artery disease, among Indian patients respectively. To confirm the cause and effect relationship, animal experimentation was done. The animal data further showed that rats fed with either a vitamin D deficient diet or high-fat/high-fructose diet caused cardiac dysfunction along with insulin resistance. They demonstrated that vitamin D deficiency in rats resulted in cardiac contractile dysfunction and was linked to myocardial insulin resistance, a recognized predecessor of heart failure. The findings in vitamin D deficient diet-fed animals were compared to a high-fat/ high-fructose-fed group of rats (**Figure 4.5**). Vitamin D deficiency in rats mimics high-fat/high-fructose-induced metabolic syndrome and cardiac dysfunction. *This study conclusively demonstrates that vitamin D deficiency is an independent risk factor for heart failure, at least in part, through induction of myocardial insulin resistance.*

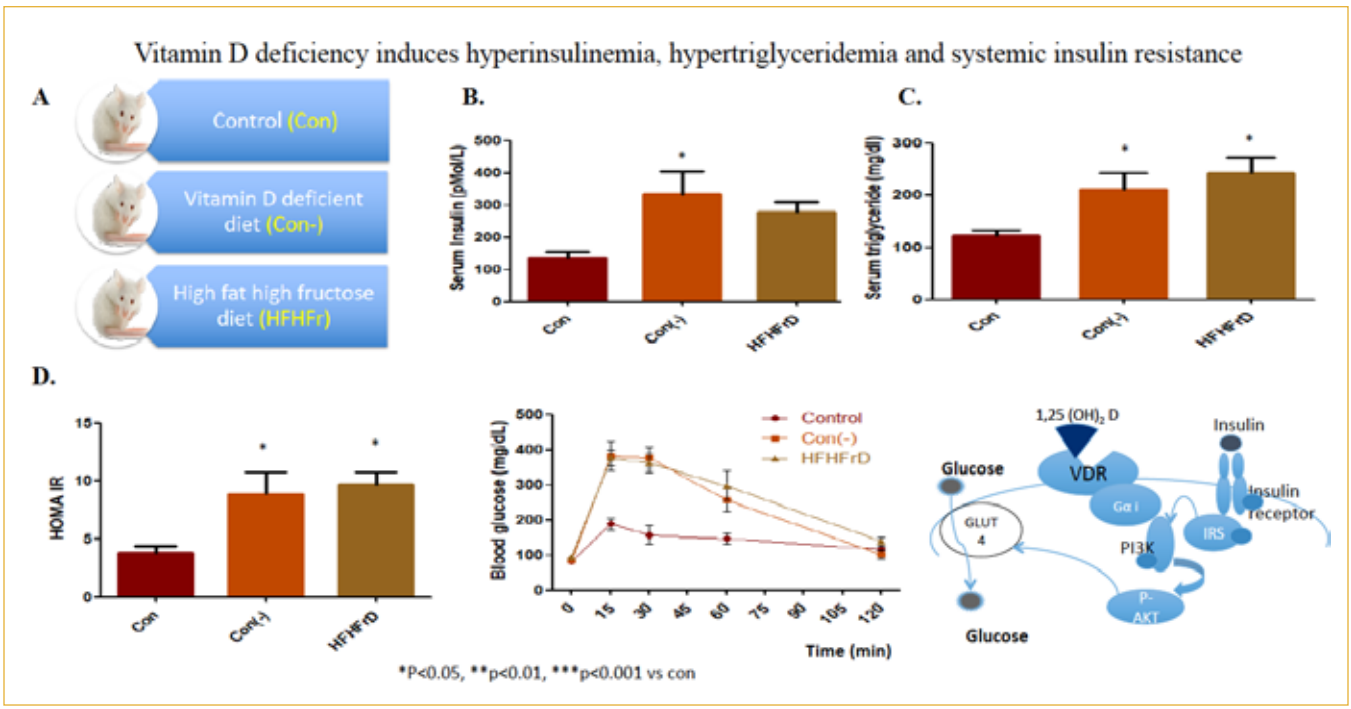


Figure 4.5(A-D): Vitamin D deficiency induces hyperinsulinemia, hypertriglyceridemia and systemic insulin resistance.

Data from this lab also indicates that less activation of vitamin D receptor (VDR) due to vitamin D deficiency may cause the cardiometabolic syndrome. The team will continue their efforts to find the effect of VDR activator in a rat model of metabolic syndrome. They are expecting that activation of VDR is essential to increase insulin sensitivity and enhance cardiac function in a model of metabolic syndrome.

Dr. Banerjee with other scientists in collaboration with the Institute of Advanced Study in Science and Technology (IASST), Guwahati assessed five north-east plant extracts - *Premna herbacea*, *Allium hookeri, Lysimachia candida, L. flosreginae* and *Dilleina indica* in both preventive and curative animal models of metabolic syndrome. They fed a high-fat/high-fructose diet in rats to induce obesity, insulin resistance, hyperlipidemia, and systemic inflammation. In the preventive model, three out of the five plant extracts (*P. herbacea*, *A. hookeri* and *L. candida*) reversed insulin resistance, high fasting blood glucose levels and body weight gain. On examining liver-specific changes, an improvement of hepatic triglyceride levels was

observed. Similarly, the curative model showed that two out of five plant extracts (*Premna herbacea* and *Lysimachia candida*) reversed insulin resistance, high fasting blood glucose levels and body weight gain. Serum and hepatic triglyceride levels decreased after the administration of both plant extracts stated above. Two best plant extracts will be further tested for their efficacy in human subjects.

Dr. Banerjee's team also tested *Musa balbisiana* fruit pulp powder in a rat model of isoproterenol (Iso) induced cardiac hypertrophy by subcutaneous administration of isoproterenol (5mg/kg/day) for 14 days through ALZET® mini pump. An ultra-high-pressure liquid chromatography-mass spectrometer (UPLC-MS/MS) analytical method was used to characterize the chemical composition of *M. balbisiana* (MB) fruit pulp powder. Cardiac hypertrophy was confirmed with increased heart weight/tail length ratio as well as assessment of hypertrophic markers in heart. Oral administration of MB significantly ($p<0.05$) decreased heart weight/tail length ANP, BNP, β-MHC and collagen gene expression. Likewise, MB further increased antioxidative enzyme activity while malondialdehyde (MDA) level was reduced. Findings from this study strongly suggested that supplementation of dried *M. balbisiana* fruit pulp can be useful for the prevention of cardiac hypertrophy and inflammation.

Designing in vitro platforms to screen herbal extracts / NCEs for treating metabolic diseases:

Dr. Ajay Kumar and his group screen traditional herbal extracts/new chemical entities (NCEs) to evaluate their efficacy in the treatment of metabolic disorders. using *in vitro* cell-based assay platforms developed in-house. They use techniques like fluorescent microscopy, multi-mode spectrometry, fluorescence-activated cell sorting (FACS), etc. to study disease phenotypes like lipid accumulation, cell health, and cell cycle distribution in human hepatic as well as Kupffer cell lines.

Dr. Ajay is examining traditional botanical formulations from north-east India to investigate their possible role in the treatment of lifestyle-induced metabolic disease. Five herbal formulations from the region were administered in a rat model along with high-fat/high-fructose (HFHF) diet in preventive as well as curative experimental models. The team successfully completed expression analysis for samples from both preventive as well as curative experimental models. Genes representing key cellular metabolic pathways, including fat metabolism

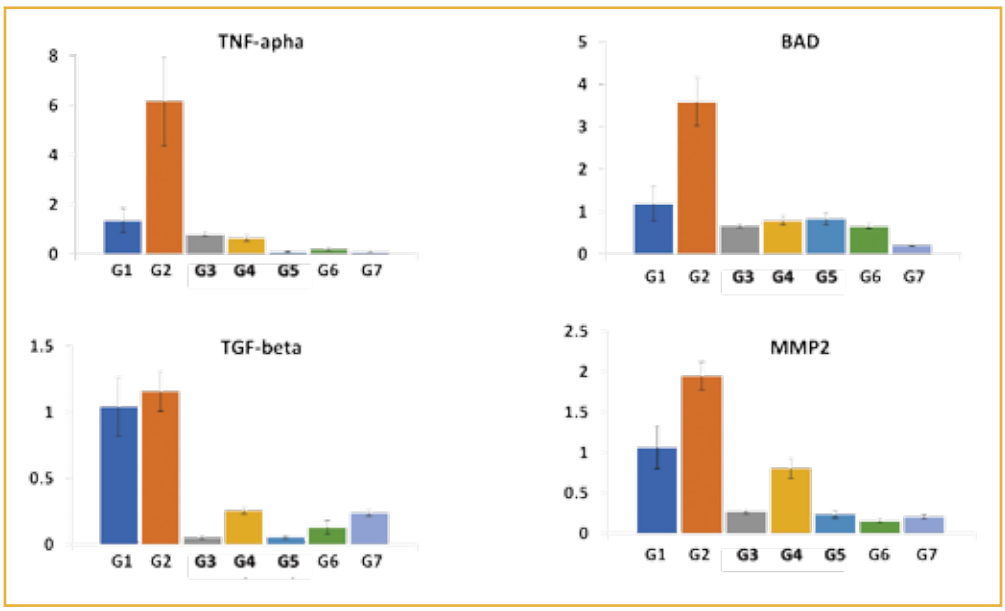


Figure 4.6: A representative bar-graph depicting fold change in gene expression of some key genes implicated in metabolic diseases in rat liver samples. Rats were fed on HFHF diet and subsequently treated with different herbal formulation over a period of 20 weeks. Each bar is an outcome of four independent replicates. Here group G1 – Control rat fed on normal diet; group G2 – rat fed on HFHF diet (disease model); groups G3-G7 – HFHF fed rats treated with five different herbal formulations.

(CS, SLC25A1, ACLY, ACACA, ACACB, HMGCR), apoptosis (BAX, BCL2, CASP3), inflammation (TNFA, IL12R, IL4R, IL1B, IL10) and fibrosis (TGFB1, ASK1, COL1A1, ACTA1, ACTB) were targeted (**Figure 4.6**). Primers specific to target genes were designed and respective amplification conditions were standardized in the lab.

Way ahead: Future plans include investigating circulating levels of different cytokines (IL10, IL1B, IL4, IL6, TNFα, VEGF, MCP1) and metabolic hormones (amylin, GLP1, ghrelin, GIP-total, C-peptide-2, insulin, leptin, pancreatic polypeptide, PYY, glucagon, GIP) in blood of rat liver samples from curative as well as preventive experimental models.

The *in vitro* model for lipid accumulation in HepG2 cell line was standardized (**Figure 4.7**) and is currently in use to screen herbal extracts for their role in hepato-protection.

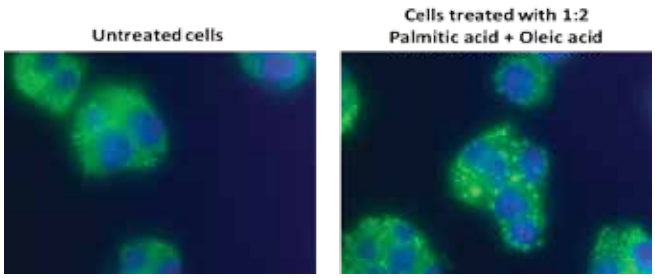


Figure 4.7: Nile red/DAPI merged images of HepG2 cells in presence and absence of lipid induction with palmitic and oleic acid in 1:2 ratio. Green colour represents the cellular lipids while nuclei are depicted in blue colour.

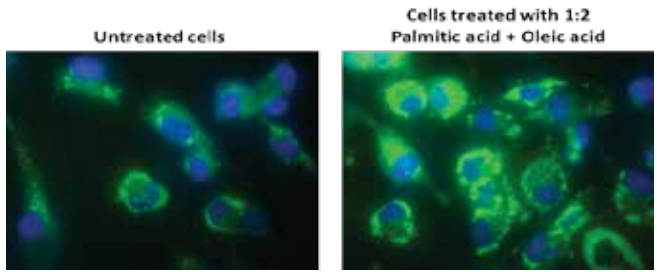


Figure 4.8: Nile red/DAPI merged images of THP1 cells in presence and absence of lipid induction with palmitic and oleic acid in 1:2 ratio. Green colour represents the cellular lipids while nuclei are depicted in blue colour.

Likewise, macrophage cell line THP1 was used to establish a model for both lipid accumulation as well as inflammation (**Figure 4.8**). Cellular lipids were stained in presence of Nile Red dye to quantitate lipids. Cell viability was determined using MTT assay while cell cycle distribution was studied by propidium iodide staining using FACS.

Way ahead: To screen herbal extracts as well as new chemical molecules or small molecules for their efficacy in NAFL or NASH using established *in vitro* models. To establish an *in vitro* cell-based model of fibrosis using LX2 cell line.

Large-scale metabolomics profiling for biomarker discovery in NCD/metabolic syndrome:

Understanding metabolic changes during diseased condition is a complex process. To decipher these complexities, metabolomics and lipidomics study can be used which provides a better understanding of such dynamic processes. Dr. Yashwant's work focuses on the discovery of translatable biomarkers in metabolic syndromes such as diabetes, cardiovascular disease, and NAFLD.

Lipid dysregulation has already been reported in metabolic syndromes. Traditional lipid markers such as cholesterol, LDL, and HDL are routinely used to assess the risk of cardiovascular events. However, these markers do not reflect the complex alterations of lipid metabolism in diabetes or cardiovascular disease. Also, the current established pharmacological therapy in T2D and cardiovascular disease are mainly available for very late stages of diseases. Early detection of diabetes and cardiovascular disease using lipid biomarker signature will increase life expectancy.

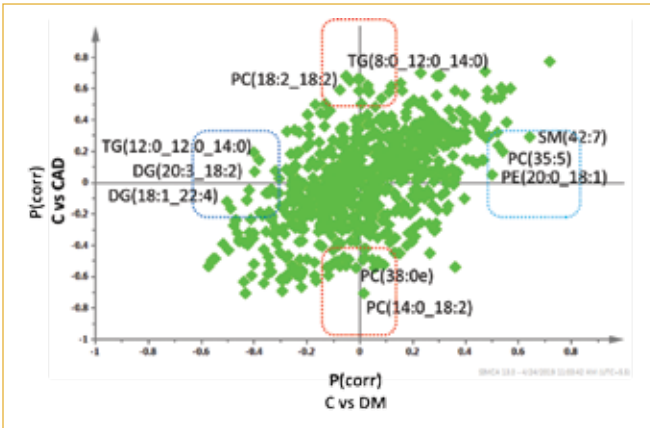


Figure 4.9: Orthogonal projections to latent structures discriminant analysis (OPLS-DA) of (A)control vs Diabetes millets (DM) and (B) control vs Coronary artery disease (CAD) shows altered lipidome in DM and CAD condition as compared to control subject.

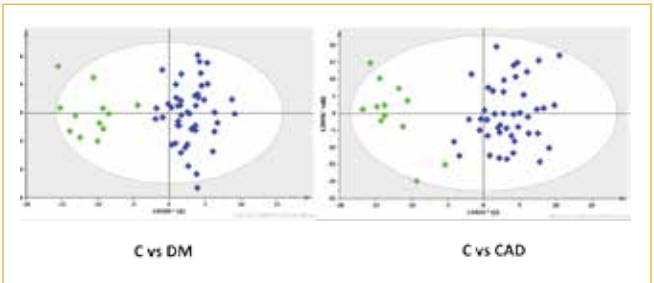


Figure 4.10: Shared and unique structure (SUS) plot of correlation coefficient (p(corr)) of C vs DM and C VS CAD shows the unique lipids in control vs Diabetes millets (blue dotted box) and control vs Coronary artery disease (red dotted box).

Dr. Yashwant measured the total lipids in subjects with Coronary Artery Disease (CAD) and Diabetes Mellitus (DM) along with their controls to distinguish the lipid signature specific to the disease conditions. Untargeted lipidomics and multivariate data analysis have shown an altered lipidome in the diseased condition (**Figure 4.9**). Phosphatidylethanolamines (PE), sphingomyelin (SM), triacylglycerol (TG) and diacylglycerol (DG) lipid ions are differentially expressed in subjects with diabetes mellitus as compared to controls. Similarly, different lipid ions of phosphatidylcholines (PC) and triacylglycerol (TG) are exclusive to subjects with cardiovascular complications (**Figure 4.10**). Identification of these lipid ions in the early stage of disease progression can improve the prediction of cardiovascular disease outcomes in patients with diabetes mellitus.

Molecular modeling studies for molecular mechanistic understanding at the structure-dynamics-function level of associated target proteins

Dr. Asthana's team is working to establish a computational platform for small molecule discovery by targeting individual proteins and by identifying the interface sites from protein-protein interactions utilizing his expertise in computational biophysics, structural bioinformatics and computer-aided drug discovery.

- Identification of a common tag nucleotide variant in Matrix Metalloproteinase – 7 (MMP7) promoter that increases risk for hypertension via enhanced interactions with cAMP response element-binding (CREB) transcription factor (in collaboration with Prof. N Mahapatra, IIT Chennai)

  MMP7 (Matrilysin), a potent extracellular matrix-degrading enzyme, is emerging as a new regulator of cardiovascular diseases. However, potential contributions of MMP7 genetic variations to hypertension remain unknown. Dr. Asthana's study probed the association of a tag single nucleotide polymorphism (tag SNP) in the MMP7 promoter (-181A/G; rs11568818) with hypertension in an urban south Indian population (N=1517). The heterozygous, AG genotype significantly increased risk of hypertension as compared to the wild-type AA genotype (OR=1.641, 95% CI: 1.276 - 2.109; p-value=0.0001). AG genotype carriers also displayed significantly higher diastolic blood pressure and mean arterial pressure than AA genotype subjects. The study was also replicated in a north Indian population (N=977) (OR=1.520, 95% CI: 1.106 - 2.090; p-value=0.01). Transient transfection experiments using MMP7 promoter-luciferase reporter constructs revealed that the variant -181G allele conferred greater promoter activity than the -181A allele. Computational prediction and structure-based conformational and molecular dynamics simulation studies suggested higher binding affinity for the transcription factor CREB to the -181G promoter. The predicted thermodynamic computational studies predicted a stable binding free

energy in -181G than -181A promoter which corroborates with over-expression/ down-regulation of CREB and chromatin immunoprecipitation experiments that provided convincing evidence for stronger binding of CREB with the -181G promoter. In conclusion, the MMP7 A-181G promoter polymorphism increased expression of MMP7 under pathophysiological conditions (hypoxic stress and catecholamine excess) via increased interactions with CREB and enhanced the risk of hypertension in its carriers.

- Impaired interactions between NKX2.5 and GATA4 in individuals carrying a novel pathogenic D16N NKX2.5 mutation (in collaboration with Dr. Banerjee, THSTI)

  NK2 Homeobox 5 (NKX2.5), a homeobox-containing gene, plays an important role in embryonic heart development and associated mutations are linked with various cardiac abnormalities. The team sequenced the NKX2.5 gene in 100 congenital heart disease (CHD) patients and 200 control individuals. The analysis revealed a total of 7 mutations; 3 intronic, 3 coding and 1 in the 3′ untranslated region (UTR). One of these mutations was found to be associated with tetralogy of fallot (TOF) and two (rs2277923 and a novel mutation, D16N) were strongly associated with ventricular septal defect (VSD). A novel missense mutation, D16N (p-value=0.009744), located in the tinman (TN) region and associated with VSD, is the most significant finding of this study. Computational analysis revealed that D16N mutation is pathogenic in nature. Through molecular modeling, docking and molecular dynamics simulation studies, they identified the location of mutant D16N in NKX2.5 and its interaction map with other partners at the atomic level. They found NKX2.5-GATA4 complex to be stable. However, in case of the mutation, significant conformational changes and loss of key polar interactions observed might be a cause of pathogenic behavior. This study underscores the structural basis of D16N pathogenic mutation in regulation of NKX2.5 and how this mutation renders the structural-functional divergence that possibly causes a diseased state.

Metabolic Disorder: Diabetes

Four hundred twenty-two million adults were living with diabetes in 2014 and 1.5 million deaths occurred in 2012 (WHO, 2016). Diabetes is associated with high plasma glucose level (PGL) and is the fifth leading cause of death worldwide. In case of a healthy subject, fasting glucose level should be below 6.1 mmol/l (110 mg/dl). If the level is between 6.1 to 7 mmol/l (110-126 mg/dl), the subject is pre-diabetic and is considered diabetic if fasting PGL exceeds 7 mmol/l (126 mg/dl).

Gut microbiome of Prediabetic subjects

Trillions of microbes living in the gastrointestinal tract (GIT) thrive in a homeostatic equilibrium in the GIT ecosystem and encode key functionalities that are crucial to host metabolic functions, synthesis of macro- and micronutrients, xenobiotics metabolism, development of innate and adaptive immune systems, tissue and organ developments and resistance against invasion of enteric pathogens. Recent studies have indicated an association of gut microbiota and microbial metabolites

with type 2 diabetes mellitus (T2D). However, large-scale investigation of the gut microbiota of prediabetic (PD) subjects has not been reported. *Identifying robust gut microbiome signatures characterizing early prediabetic stages assumes importance both from an etiological and preventive diagnostic perspective.*

Dr. Das and his collaborators have adopted metagenomic approaches to profile and compare gut microbiota of prediabetic individuals (N=262) and those with normoglycaemia (N=275) from two cohorts, one each in India and Denmark. After correcting for strong country-specific cohort-effect, 15 operational taxonomic units (OTUs) including members from the genera *Phascolarctobacterium, Barnesiella, Flavonifractor, Tyzzerella_4, Bacteroides, Faecalibacterium* and *Agathobacter* were identified to be enriched in normoglycaemic subjects with respect to prediabetic subjects. On the other hand, they could identify 140 OTUs suppress in the prediabetic subjects, which included members from the genera *Megasphaera, Streptococcus, Prevotella 9, Alistipes, Mitsuokella, Escherichia/Shigella, Prevotella 2, Vibrio, Lactobacillus, Alloprevotella, Rhodococcus* and *Klebsiella*. Comparative analyses of relative abundance revealed that the *Streptococcus*, *Escherichia/Shigella*, *Prevotella 2*, *Vibrio* and *Alloprevotella* OTUs exhibited more than four-fold enrichment in the prediabetic gut microbiota. The gut microbiome signature of prediabetes was more prominent when subjects from the two geographic locations were evaluated separately. The study reports a probable association of *Megasphaera* OTU(s) with impaired glucose tolerance, which is significantly pronounced in Indian subjects. The results present trans-ethnic gut microbiome signatures associated with prediabetes, in Indian and Danish populations. The identified associations may be further explored as potential early indicators for individuals at risk of dysglycemia.

Diet, artificial sweeteners, gut microbiome and Type 2 Diabetes:

Dr. Prabhanshu Tripathi is studying the effects of diet and artificial sweeteners on the gut microbiome and their consequences on type 2 diabetes (T2D). Consumption of sugary drinks is associated with increased risk of obesity and T2D. Due to this setback, artificial sweeteners (AS) consumption became increasingly popular and was introduced largely in our diet in order to reduce calorie intake and normalize blood glucose levels without altering our taste for "sweetness". However, present literature on intake of AS as a risk factor of type 2 diabetes is inconsistent. The aim of his work is to determine the effect of diet, artificial sweeteners, and medications on gut microbiota and consequences on host biology with emphasis on T2D and establishment of possible therapies.

Dr. Tripathi's lab pursues *in vitro* studies to explore the role of artificial or non-caloric sweeteners on Caco 2 cells (human epithelial colorectal adenocarcinoma cells). These studies are aimed to find the direct effect of these compounds on the gut epithelium. They treated monolayer of these cells with different concentrations of commonly used FDA approved artificial sweeteners (Aspartame, Saccharin, Acesulfame) for different time points and found that some of these artificial sweeteners can reduce transepithelial resistance and produce leaky guts that can increase access of the gut bacteria to inner layers of the epithelial lining and can have a detrimental effect. They also found significant changes in different proteins like glucose transporters and PYY (also known as peptide tyrosine tyrosine or pancreatic peptide YY) at protein as well as mRNA levels in artificial sweetener treated cells. In the animal studies, gut microbiome profile analysis using fecal

sample collected before and after treatment showed distinct gut microbiome profiles in different artificial sweeteners treatments at genera level. Genera profiles in aspartame and sucralose treatment are very different from rest of the samples which can be further correlated with their metabolic profile.

Way ahead: They will delineate the change in microbiota to good bacteria versus bad bacteria and probiotic bacteria or bacterial components with therapeutic or prophylactic potential, which will be further evaluated through clinical research.

The cardiovascular and hepatic complication in diabetes:

A team led by Dr. Banerjee is actively involved in understanding the mechanisms underlying cardiac and hepatic complications in diabetes and finding the novel targets and serum protein signatures for therapeutic interventions. The focus is on studying the alteration of tissue-specific metabolic perturbation and mitochondrial function. The team is assessing how function of several crucial proteins is altered through post-translational modification especially via acetylation.

The role of acetylated protein in disease biology is still in its infancy and its role in malfunctioning diabetic heart is poorly understood. Expression and activities of sirtuins, enzymes responsible for protein deacetylation, were characterized in the diabetic heart. Dr. Banerjee and his group found that the acetylation of mitochondrial proteins plays a key role in progression of cardiomyopathies in rats fed with chronic high-fructose diet. They demonstrated, for the first time, that SIRT-1 activation in the nucleus enhanced SIRT3 activity and caused deacetylation of mitochondrial transcription factor, TFAM in mitochondria. The work indicates that SIRT1 could be a promising target to improve cardiac function in diabetes mellitus. Data also indicates that SIRT1 regulates SIRT3 in mitochondria. To confirm this finding, diabetic rats will be screened with SIRT3 activator and assess the efficacy in reducing insulin resistance and cardiac complication. A combination of both SIRT1 and SIRT3 activator will be tested in diabetic rats and compared with either SIRT1 or SIRT3 activator. They expect that both SIRT1 and SIRT3 activator together may show synergistic effect to reduce insulin resistance and other related complications.

Early prediction of Coronary Artery Disease (CAD) risk in patients with Type 2 Diabetes (T2D):

CAD is the leading cause of morbidity and mortality in patients with T2D. Dr. Banerjee and his group assessed 46 serum protein markers including cytokines/chemokines, metabolic hormones, adipokines and apolipoproteins to differentiate Indian CAD patients with and without T2D. Dr. Banerjee's group has identified three distinct sets of serum markers for diabetes, CAD and diabetes associated with CAD using a nonparametric-based machine learning approach. The Random Forest algorithm classified T2DM based on the abundance patterns of nine markers i.e., IL-1β, GM-CSF, glucagon, PAI-I, rantes, IP-10, resistin, GIP and Apo-B; CAD by 14 markers i.e., resistin, PDGF-BB, PAI-1, lipocalin-2, leptin, IL-13, eotaxin, GM-CSF, Apo-E, ghrelin, adipsin, GIP, Apo-CII and IP-10; and T2DM with CAD by 12 markers i.e., insulin, resistin, PAI-1, adiponectin, lipocalin-2, GM-CSF, adipsin, leptin, Apo-AII, rantes, IL-6 and ghrelin. These multiple marker classifiers may be useful for monitoring progression from a healthy to T2D stage and T2D to T2D with CAD stage (**Figure 4.11**).

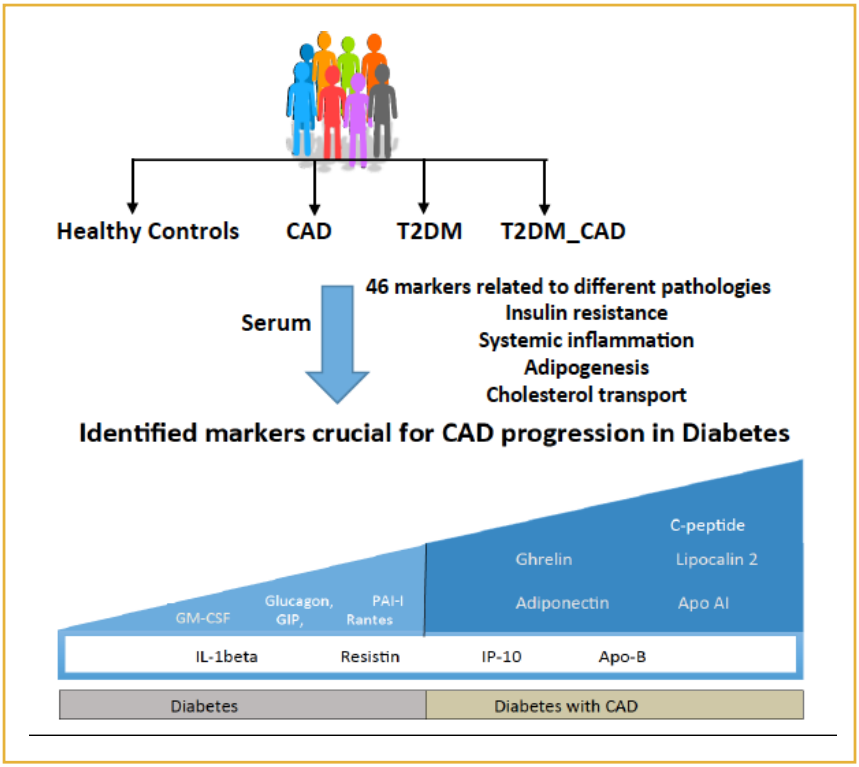


Figure 4.11: Identification of distinct sets of serum markers for diabetes and diabetes associated with CAD using a nonparametric-based machine learning approach.

Biomarker discovery for diabetes:

Dr. Goel's team uses proteomics to examine dysregulated pathways associated with T2D progression. They carried out quantitative proteomics analysis of livers from each of the three groups – G1 fed with maintenance diet, G2 fed *ad libitum* high-fat/high-fructose diet for 20 weeks and G3 fed high-fat/high-fructose diet with a formulation of herbal compounds; (i) to determine the panel of proteins responsible for dysregulated pathways from the control groups and (ii) to study how these dysregulated proteins trends come to normal after administration of a herbal formulation to HF/HFr diet-fed rats. They employed label-free quantification based on precursor signal intensity SWATH-MS because of its fast and low-cost measurement for the quantification of proteins associated with insulin resistance that causes diabetes. Peptides were extracted from rat liver tissues from all the groups and injected separately in data-dependent acquisition (DDA) mode to build the spectral library. The spectral library built from the DDA runs was then used by PeakView and MarkerView to extract the peptide and the quantification information in each of the SWATH runs. Data analysis was done by Spectronaut™. The sampling of chromatographic peaks is crucial to precise quantification (**Figure 4.12**).

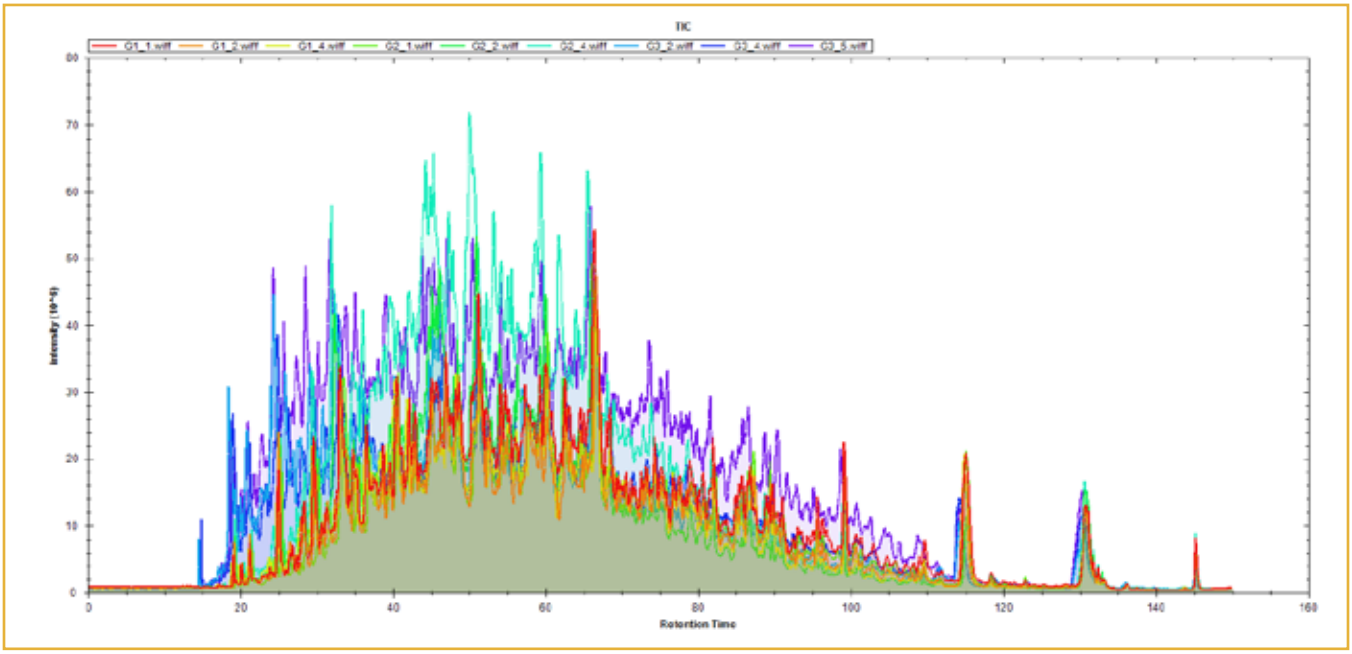


Figure 4.12: Overlay of total ion chromatogram of G3 group

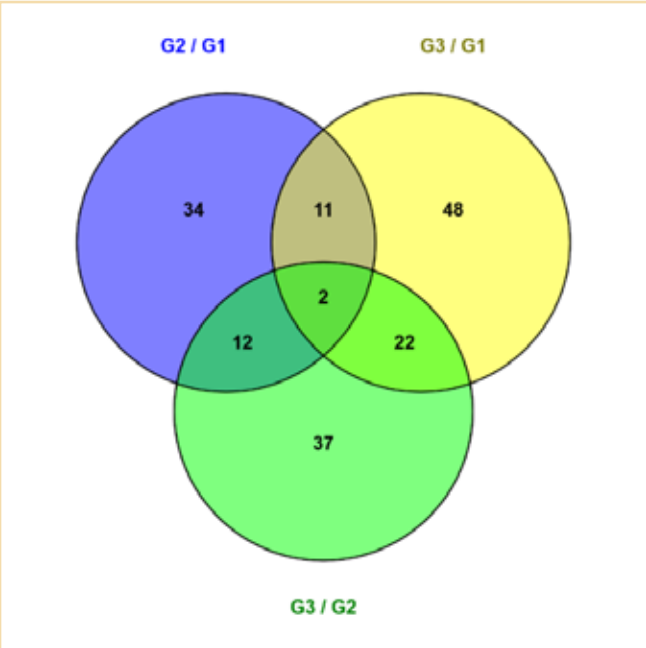


Figure 4.13: Common and unique proteins among the group of G3.

Stringent criteria of <10% coefficient of variation (CV) and <1% false discovery rate (FDR) was adopted to obtain the differential list of proteins. Some of the differential regulated proteins are carboxylesterase 1D, hydroxyacid oxidase 1, acyl-CoA binding protein, isocitrate dehydrogenase, mitochondrial, ATP synthase subunit D, mitochondrial and microsomal triglyceride transfer protein. A comparison among all groups revealed only two proteins common to all the groups (**Figure 4.13**).

They also generated a heat map to see how the different groups cluster according to their differential regulation of the protein. The same group of animals clustered together in the hierarchical clustering algorithm as shown in **Figure 4.14**.

Pathway analysis showed metabolic and carbon metabolism pathways were enriched in both G3/G2 and G3/G1 groups **(Figure 4.15)**. Glyoxylate and dicarboxylate metabolism and citrate cycle were enriched in G3/G2 groups.

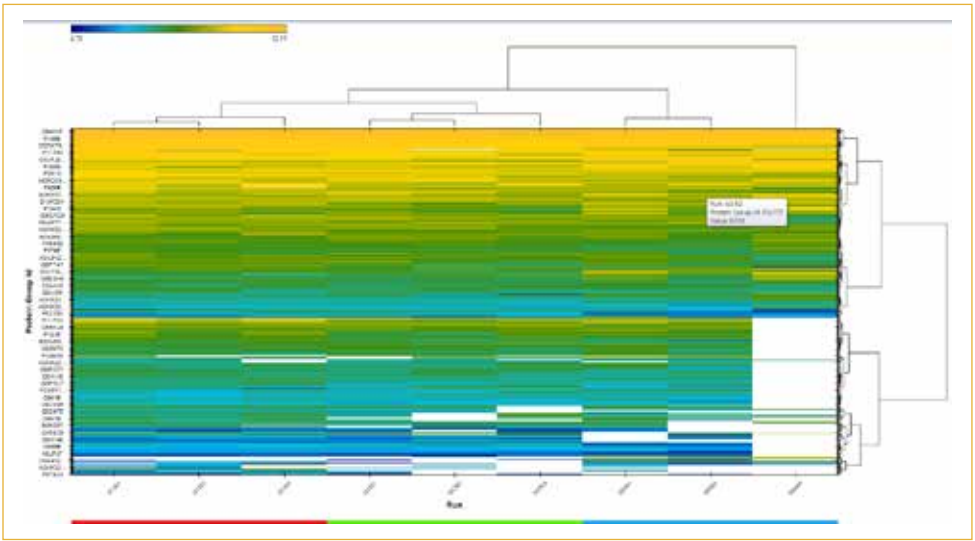


Figure 4.14: Hierarchical clustering of all the groups of the G3

They found few interested proteins exhibiting differential regulation in HF/HFr diet rats, which were at a normal level on the administration of herbal formulation to rats for 18 weeks. These findings will be instrumental in understanding dysregulated pathways associated with progression of T2D.

Understanding the role of calcium in glucose-insulin dynamics in pancreatic β-cell under diabetic condition:

Dr. Samrat Chatterjee heads the 'complex analysis group' focused on solving biological problems using mathematical and computational tools. They use the clustering algorithm, graph theory, flux balance analysis, etc. to study large-scale data and capture important genes/proteins/metabolites responsible for the process being studied. They build small-scale models based on differential equations to study underlying mechanisms of biological processes.

Type 2 diabetes (T2D) is associated with abnormalities in the functioning of pancreatic β-cell, the primary cause of T2D. Dr. Chatterjee's team is studying the calcium-regulated insulin secretion dynamics of β-cell and its interaction with the plasma glucose, especially under diabetic condition. In the cytoplasm of β-cells,

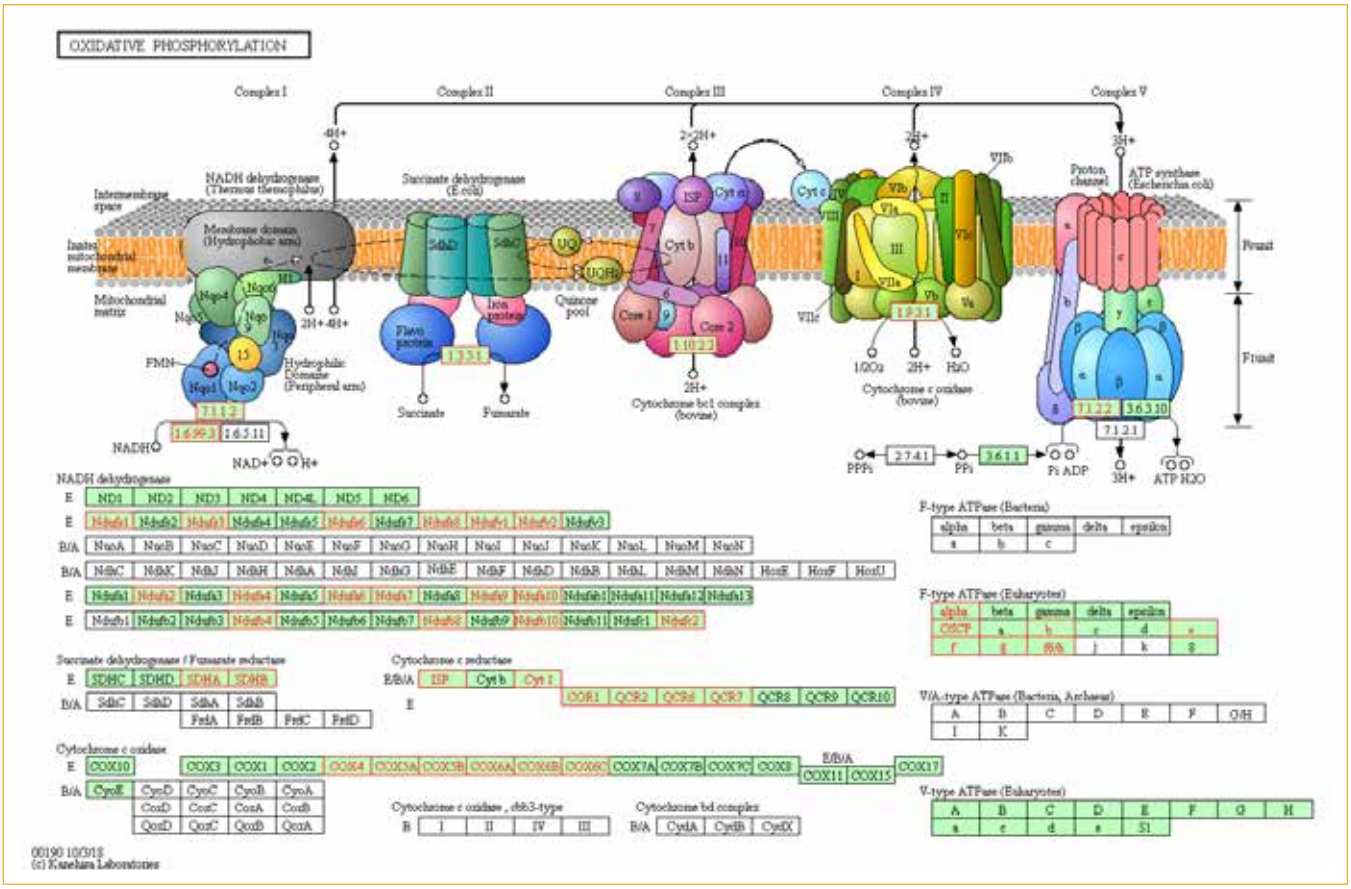


Figure 4.15: Oxidative phosphorylation was enriched in the G3 /G2 groups.

calcium ions (Ca^{2+}) waves trigger the fusion of insulin-containing granules with the plasma membrane aiding insulin's entry in the extracellular space, causing an increment of insulin concentration in the blood plasma. The team studied the dynamic interaction of glucose-induced insulin secretion mechanisms through glucose metabolism and ATP-dependent calcium influx. They constructed a mathematical model for T2D consisting of four variables to represent glucose and insulin concentrations in the blood plasma and concentrations of ATP and calcium within the β-cell. For simplicity, intermediate biophysical mechanisms between ATP-dependent potassium channel closure, subsequent depolarization, and voltage-dependent calcium influx through L-type channel into β-cell were not considered, but the time required for accomplishing these processes is important and was taken into consideration for making a realistic model for glucose-insulin interaction. Thus, a time delay in the process of ATP-dependent calcium influx was introduced and a four-dimensional delay differential equations (DDE) model was considered to capture the broader dynamics of glucose-insulin interaction (**Figure 4.16**).

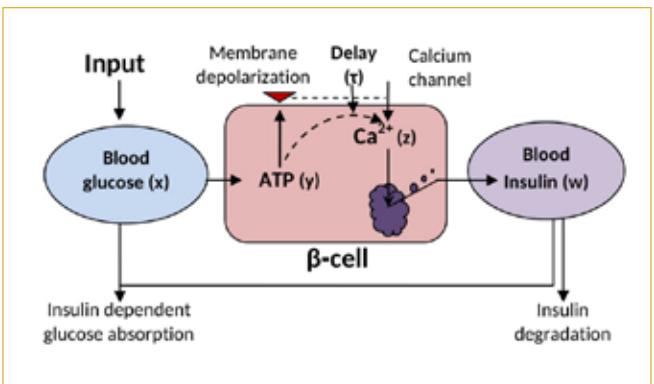


Figure 4.16: The schematic diagram describing the model for the glucose-calcium-insulin.

The aim of the study was to observe the role of ATP-dependent calcium input rate of β-cell in insulin secretion, sensitivity of insulin secretion to the glucose input rate in blood plasma and glucose uptake rate of β-cells. The role of time interval between ATP generation and calcium influx in maintaining normal

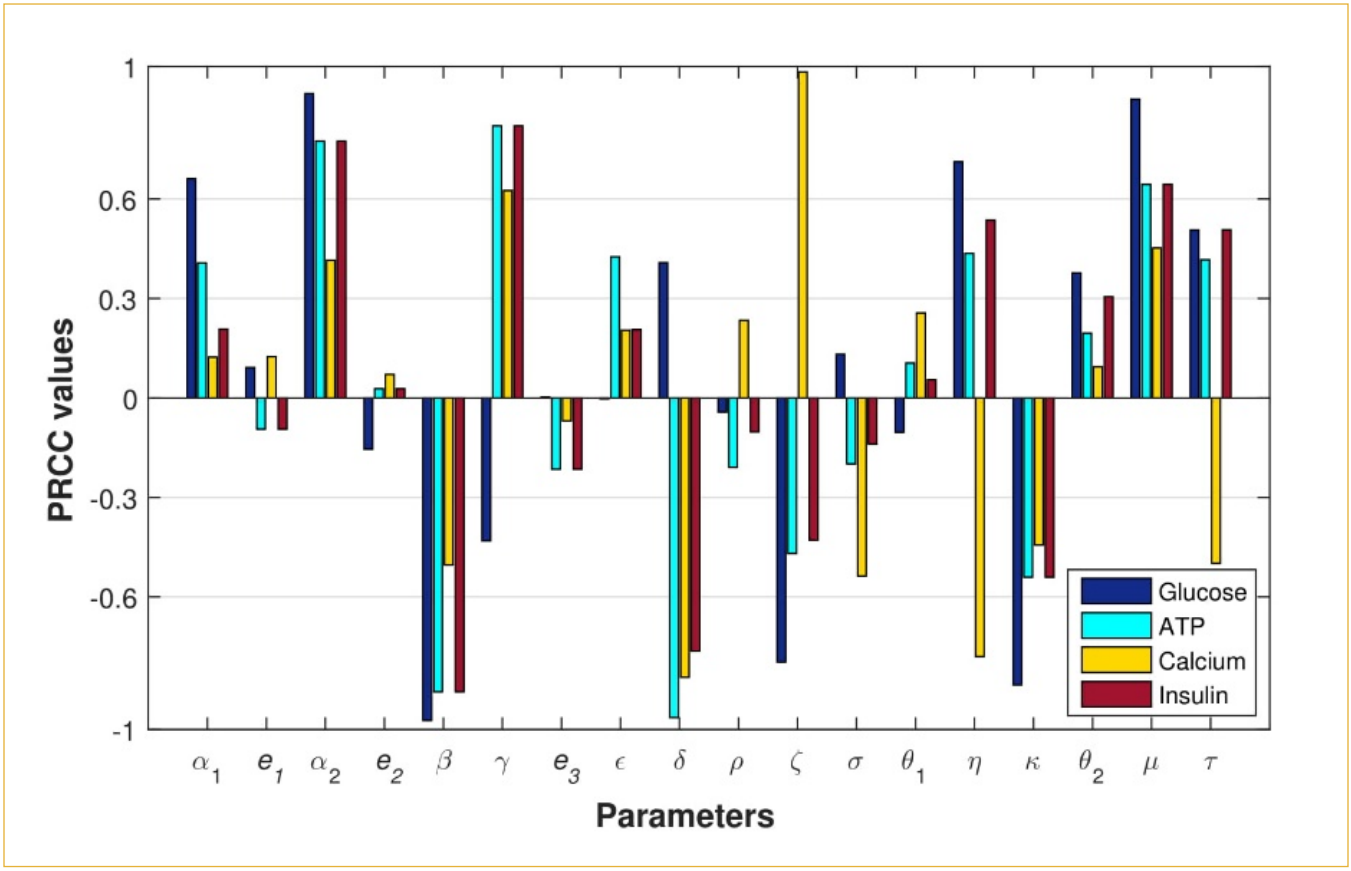


Figure 4.17: Global sensitivity analysis using Latin Hypercube Sampling (LHS) and Partial Ranked Correlation Coefficient (PRCC) technique. The sensitivity of each parameter is represented by a bar for each state variable and is measured by its length.

insulin secretion is critically explored. They first identified the significant parameters using global sensitivity analysis (**Figure 4.17**) and then, through parameter recalibration exercise, explored possible strategies to maintain normal plasma glucose level due to dysfunction of different system components under diabetic condition. A comparative study was also conducted between mono-therapeutic and multi-therapeutic strategies for diabetes.

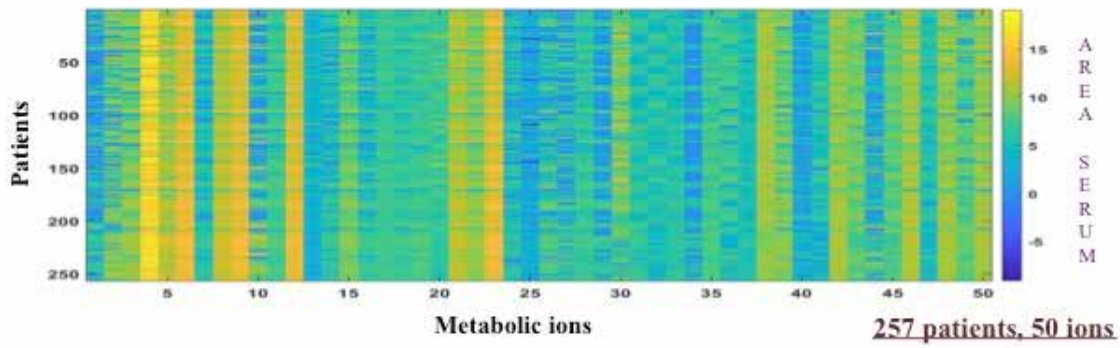
The main finding here was that calcium accumulation and fluctuation for the large delay between ATP production and calcium inflow might lead to a pathological progression toward diabetes. The recalibration exercise showed that elevated plasma glucose level (PGL) might be controlled and brought back to its normal oscillatory range by modulating different system parameters. In such a case, calcium-dependent insulin release rate is found to be an effective strategy in controlling PGL. The plasma insulin degradation rate is another efficient controller of hyperglycemia irrespective of whether it is caused due to reduced absorption of glucose by different cells or due to a longer delay in calcium inflow. Other control mechanisms of hyperglycemia have also been demonstrated through parameter perturbations both individually (mono-therapeutic strategy) and in combinations (multi-therapeutic strategy).

Way ahead: The study will be extended further in pancreatic β-cells and cardiomyocytes. The team will study the role of calcium in other cellular functions and relate them to diabetic conditions using different models. They are teaming up with biologists and clinicians for validating these results.

Model using a machine-learning algorithm for predicting susceptibility to type 2 diabetes (T2D):

Dr. Chatterjee is also working to develop methods to pre-diagnose the susceptibility of an individual to type 2 diabetes (T2D). Serum samples were obtained from Dr. Nikhil Tandon, a clinician with AIIMS, Delhi who conducted longitudinal studies on T2D occurrence in cohorts. Samples were collected from individuals in the cohorts for two years and fasting plasma glucose (FPG) levels were measured as an indicator of T2D incidence. Blood samples for 257 individuals were collected at two time points. Sera of the clinical blood samples were extracted and processed in a mass spectrometer to analyze the metabolites. The raw data generated by the mass spectrometer was then aligned to reference spectra to reduce noise. After aligning all the data, metabolite profiles of 257 individuals were obtained with approximately 30,000 metabolites. A model was eventually built to predict future diabetes susceptibility using machine learning (ML) algorithm. The data was first split into two parts—one for training and the other for testing. Different ML algorithms were analyzed and the best performing ML algorithm selected for their model. After analysis, 50 metabolites were obtained and was enough for prediction with an accuracy of around 80%, see **Figure 4.18**.

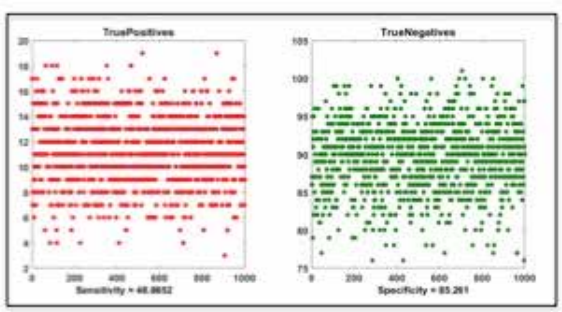
A



B

Percent of whole data used for testing	50	40	30	20	10
Percent of misclassification(with standard deviation)	22 (03)	19 (03)	19 (04)	16 (05)	13 (07)

C



D

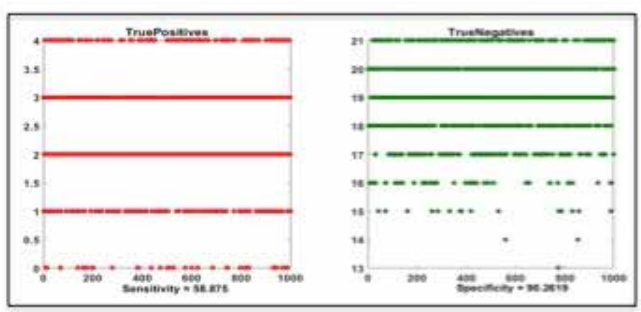


Figure 4.18(A-D): Final model (with 50 metabolites) performance. (A) Data for all the samples with selected 50 metabolites. (B) Performance of final model (built with 50 metabolites) with different sizes of training dataset. The table showing misclassification on total accuracy for different test sizes. (C) sensitivity and specificity of final model with 50:50 partition of the data. (D) Sensitivity and specificity of final model with 90:10 partition of the data.

Way ahead: Dr. Chatterjee's team is now working on the identification of these metabolites and will compare them with available literature. They plan to increase the sample size to increase the accuracy of the model. They performed an analysis to relate sample size with the accuracy of the model and observed that there is only a 2% decrease in the misclassification error (from 22% to 20%) for 600 samples (493 future normals and 107 future diabetics). This analysis showed that it is difficult to increase the accuracy with this data structure. One possible reason is the low sample size for diabetic patients in comparison to non-diabetics.

Tools & technologies developed:

Novel chemical technologies to aid drug development

The chemistry lab at THSTI develops new chemical technology to facilitate drug development efforts. Dr. Mahajan and his team developed few proprietary synthetic methodologies (PCT application filed) for chemical transformation in cost-effective and environment friendly ways. They are in progress of demonstrating the utility of these synthetic methodologies for cost-effective and cleaner synthesis of existing approved drugs, new APIs or other molecules of commercial importance.

The team developed a new chemical technology to exploit CO_2 gas as a chemical feedstock or reagent for the manufacturing process leading to various commercial molecules also including approved drug molecules. The technology has been secured as PCT patent application. The team has also established an industrial collaboration with a pharma company, Penam Laboratories, to co-develop a manufacturing process of an oncology drug exploiting this proprietary technology.

Integrating multiple proteomics search algorithms in a unified framework

Dr. Yadav's team has completed the pipelines for OMSSA, X!Tandem, MSGF+ and MassWiz search tools. They have incorporated separate target-decoy searches in one pipeline and it can run multiple files in a batch process. Using publicly available standard mix datasets, the robustness and accuracy of the pipelines and parsers are being evaluated using the MIX3 test data. They are also developing the file format operability for various tools in the same pipeline. Based on these analyses, a unified framework will be devised to integrate them into a single pipeline (next year's objectives). After the pipeline is benchmarked, the framework will be optimized for specific steps in a database search. His team developed codes and wrote the automated pipelines for the four databases search engines i.e. X!Tandem, OMSSA, MassWiz, and MSGFplus (**Figure 4.19**). All the pipelines take a folder containing spectra files, FASTA database with search parameters and conduct the target-decoy searches and statistical validation through FDR in an automated manner. There are several similarities conceptually, but operationally there are some differences in the various tools. Broadly the steps are divided into:

- **FASTA database handling** – This requires, either an in-built or a manual step of creating a decoy database for statistical validation (required after search). The provided FASTA database is reversed to create another FASTA with all

the sequences from input (also called target database) in reverse order (called decoy database).

- **Search parameters** – These are the parameters that define how the algorithms will create theoretical protein digestion, peptide creation and then match the theoretical spectra of *in silico* generated peptides with the experimental data. The various parameters are instrument type and mass errors – both at peptide (MS1) and fragment level (MS2), enzyme used (like trypsin), number of allowed missed cleavages to account for incomplete digestion, fixed modification (induced at every occurrence of an amino acid during sample preparation step), variable modifications, FDR cutoff etc, among others. Some algorithms also require some parameters for spectra filtering and peak picking to remove bad spectra or pick significantly high-intensity peaks respectively.

- **Database searching** – The actual step wherein targets and decoy databases are searched (as two separate searches) to assign a peptide to each spectrum that satisfies all the given input parameters and instrument error. Some algorithms use indexing to speed up this step.

- **Post-processing and FDR** – In this step search output is filtered to pick the best matching peptide for each spectrum (called a peptide spectrum match or PSM), the target and decoy results are compared and processed to estimate the false discovery rate (FDR). The threshold is then applied at FDR 1% and all results that do not reach the predefined threshold are discarded or labelled as unidentified.

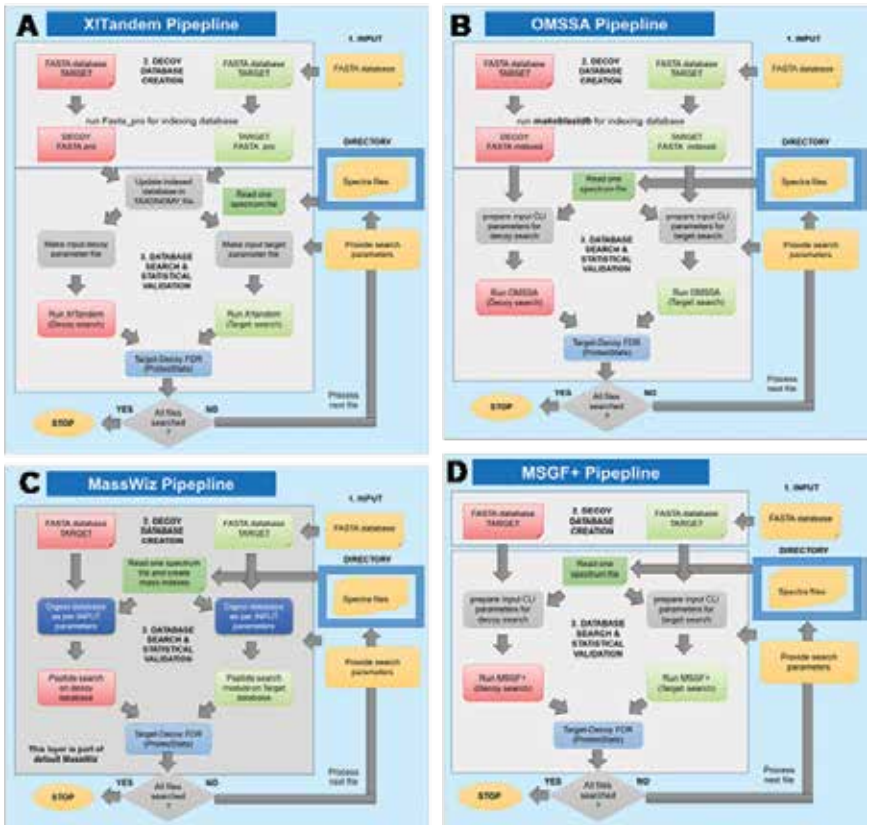


Figure 4.19(A-D): Overview of Pipelines created for the search algorithms (A) X!Tandem (B) OMSSA (C) MassWiz (D) MSGF+. The pipelines can be executed in automated manner after providing search parameters, FASTA database and a directory full of arbitrary number

These pipelines have been created to carry out large-scale database searches in an automated turn-key approach. These pipelines can take in MS data as input, and process these files through the tools once as target and other as decoy searches, then calculates false discovery rate (FDR) for statistical validation through ProteoStats library (Yadav et al., 2013). These have been checked through MIX3 dataset from 18mix and validated the code for the pipelines by comparing manual results with pipeline outputs. They also wrote the file format interoperability module to be able to use mgf, mzML file formats for uninterpreted MS/MS spectra, and mzID, pepXML and tandemXML file formats for search results to process results for FDR into CSV/TSV files.

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L-R: Pankaj Sharma, Arun Kumar, Dr. Dinesh Mahajan, Nidhi Sharma, Vikas Phagna, Gayatri, Ganesh Hegde

L-R: Front row – Suruchi Agarwal, Payal Gupta; Back Row – Dr. Nishant Kumar Soni, Dr. Amit Yadav

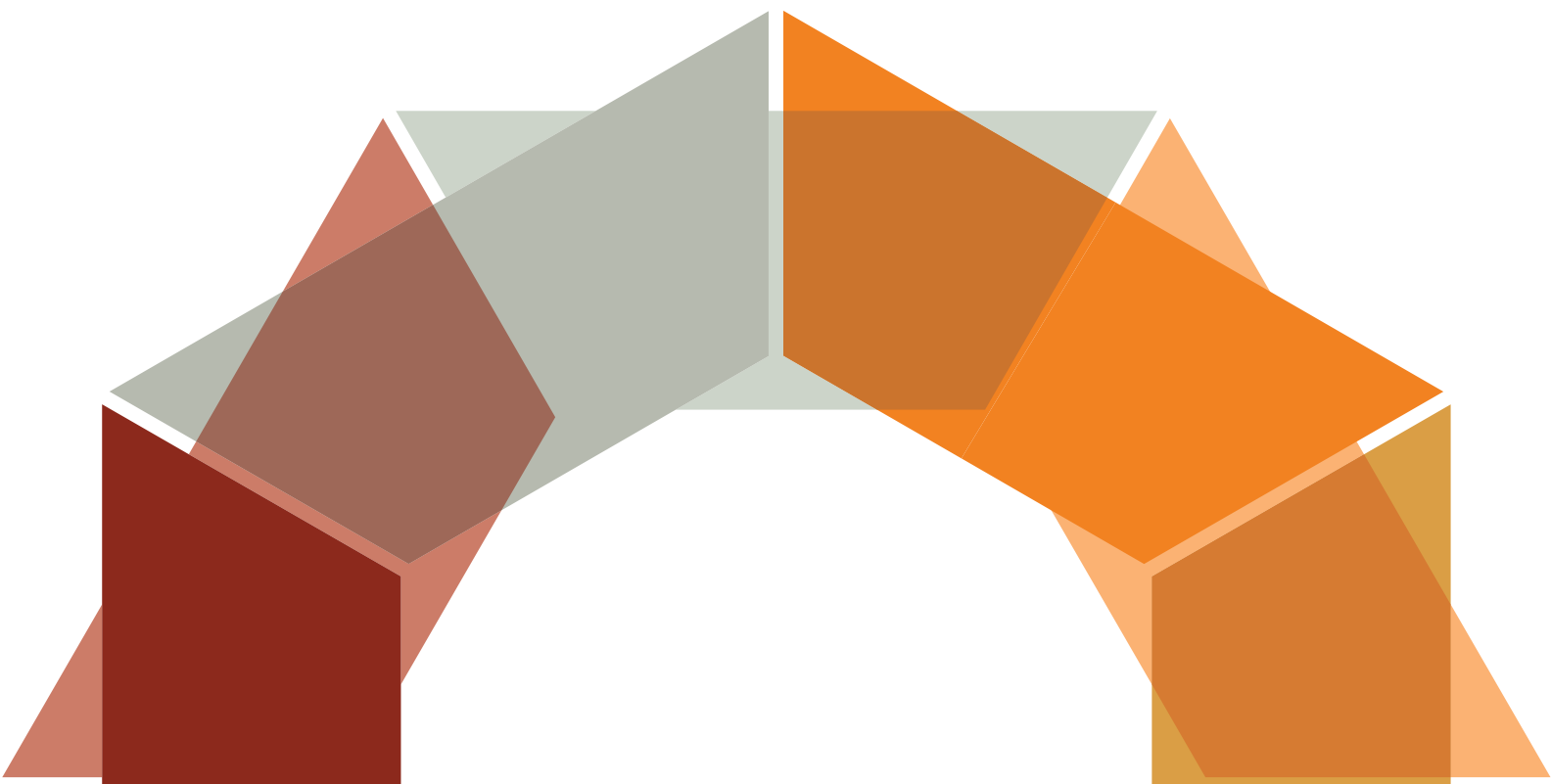
L-R: First row – Komal Sharma, Sahifa Siddiqui, Garima Arora, Dr. Sumana Biswas, Dr. Sonali Porey Karmakar, Second row – Dipanka Tanu Sarma, Dr. Samrat Chatterjee, Ankur Gupta, Krishan, Abhijit Paul, Shivam Kumar, Suvankar Haldar

L-R: First row - Hina Lateef Nizami, Dr. Sima Kumari, Deepika Kumari, Parul Kamboj, Dr. Sanjay Banerjee; Second Row – Ubaid Tariq, Roshan Kumar, Ayushi Purohit, Md. Jahangir Alam, Soumalya Sarkar, Last row – Aman Sharma, Bugga Paramesha

L-R: Neema Bisht, Dr. Yashwant Kumar, Sonu Kumar

L-R: Ankit Gupta, Dr. Sameena Khan, Sudha

TRANSLATIONAL RESEARCH PROGRAM (TRP)



BIOREPOSITORY

THSTI established a biorepository in 2015 under the DBT funded project, GARBH-Ini. Currently, it has ~700,000 biospecimens collected from the participants of the study cohort. These include maternal serum, blood, plasma, DNA, maternal blood in PAXgene tubes, saliva, urine, high vaginal swabs, cord blood, umbilical cord tissue, placental tissue punches, placental membranes, paternal saliva and neonatal heel prick venous blood as dried blood spots that are being collected across pregnancy, at delivery and post-delivery. All the biospecimens are barcoded with numeric codes and the participant IDs are de-identified by the use of Unique Identification numbers.

All the specimens that are reposited under the GARBH-Ini project are processed using a set of customized Standard Operating Protocols (SOPs) and validated lab processes. There are liquid cryo vessels with low-level alarms, dry shippers, 23 deep freezers that are housed in an area of 2766 sq. feet, all equipped with real-time remote temperature monitoring with electronic remote alerts and uninterrupted power supply. The deep freezers have been validated for their Installation Qualification (IQ), Operational Qualification (OQ) and Performance Qualification (PQ). All the equipment is accredited by the National Accreditation Board for Testing and Calibration Laboratories (NABL), a Constituent Board of Quality Council of India. A technical team maintains storage and retrieval of biospecimens according to protocols developed using international norms.

The procedure to access the biospecimens stored at the THSTI biorepository encompasses an application process that involves the submission of a biorepository access form and a two-page Letter of Interest to the Program Management Committee (PMC) of GARBH-Ini and Study Steering committee members for the approvals. The applications are reviewed by domain experts before final approvals are given. It is envisaged that this repository will serve as a platform for future studies on maternal and child health through a structured access policy.

For the next phase of the biorepository, the team plans to improvise on the sample management system to make it a web-based tool that could integrate seamlessly with the clinical data platform and the in-house lab management software that captures all the pre-processing critical variables.

Data Management Centre and Data Science Hub

Data Management Centre:

Purpose: The Data Management Centre (DMC) at THSTI provides state-of-the-art data management support to in-house intramural and extramural funded, clinical research projects.

The team: The core team comprises a data scientist, a programmer, a data manager, and two data entry operators.

The DMC has competence and experience to support studies including large cohorts and multicenter clinical trials, through the entire cycle of the study, beginning from data capture plan to consistent and time-bound delivery of accurate, analysis-ready data.

The DMC has proficiency and provides both paper-based and eCRF data capture support. The **Clinical Data Management Systems (CDMS)** developed in-house for both types of data capture are robust validated and ensure reliable data quality with an audit trail. The CDMS at THSTI is installed in a secure and validated IT environment and the DMC provides customized data management services on the platform, which include development of data management plan; case report form (CRF) designing; preparing study-related technical documents like annotated CRF, data validation plan; database development through the development of electronic data capture systems; developing standard operating procedures for quality adherence and customized CRF filling guidelines; Serious Adverse Events (SAE) data reconciliation; data import set-up for third party (central laboratory, images, etc.); customized reports; procedures for data sharing, database lock prior to analysis and data archiving.

The data management centre has developed an in-house **Laboratory Management System (LMS)** using barcodes. This helps track the journey of a biospecimen from collection till the time it is analyzed or stored in the biorepository.

Data security and storage: It is important that all data be held securely, be robustly backed up and its confidentiality safeguarded. Data integrity and confidentiality of data at the DMC are maintained within the CDMS and LMS by robust program security which gives restricted access to users, requires a login and password to access the program, monitors user activity, has a time-stamped audit trail system to track changes to data. Data generated or collected is de-identified using unique identification codes and then entered in the database. In addition to this, there is controlled access within the DMC and the data archival space.

All the data entered in the database is transferred to the server at THSTI. At least one mirror backup of the data is kept in the server at THSTI, protected by a password

and accessible to only authorized users. Additional processes for data backup like the use of external hard disks for time-bound backup every 15 days are in place. The external hard drive is kept at a location away from the DMC at THSTI.

Data access and ownership: The following principles apply: 1) ownership of data is decided by the PI and other stakeholders generating the data; 2) access of data to all stakeholders for the conduct of the project.

Currently, the DMC is managing the data of one large longitudinal cohort (GARBH-Ini) of 8000 pregnant women, one large multicenter academic clinical trial enrolling 4200 young infants with sepsis, several smaller studies within the GARBH-Ini cohort and one longitudinal cohort study on neonatal immune system development and maturation.

Data Stored: 2000 MegaBytes (2 TB); **Data Entry** is being done for seven ongoing studies (1000 x 15000 fields)

Bioassay Laboratory

A bioassay laboratory has been established for the clinical development of vaccines and biologicals. The bioassay laboratory is intended to meet the global standards in Good Clinical Laboratory Practice (GCLP) and will be applying for accreditation by National Accreditation Board for testing and calibration Laboratories (NABL) for vaccine development and testing.

Infrastructure: A laboratory measuring around 2000 sq. ft. has been set up as per the guidelines of WHO and NABL. Eight separate rooms have been created to maintain unidirectional workflow and segregate the activities such as flow cytometry, serology, reagent preparation, nucleic acid isolation, template addition, PCR and post-PCR rooms and animal cell culture for virus cultivation. New equipment such as CO_2 incubator, biosafety cabinets, PCR hoods, flow cytometer has been procured and installed. All the equipment has been calibrated and validated as per NABL requirements.

Documentation: Draft copies of quality manual, quality system procedures, equipment operating procedures, standard operating procedures, technical operating procedures as per the requirement of ISO 17025:2017 have been prepared.

Manpower: A technical team of six members has been assigned to work in the bioassay lab and the team has been trained in ISO17025:2017 and other modules such as measurement of uncertainty, inter-laboratory comparison, and proficiency testing.

Assays: The scope of assays as part of phase I activities (2018-20) is given in **Table 5.1**.

TABLE 5.1: SCOPE OF ACCREDITATION – THSTI BIOASSAY LABORATORY

Serial No.	Type of samples examined/ tested	Specific tests/ examination performed	Standard (method), Principle/Methodology or technique used	Range of testing/ Limit of detection
1	Human serum	Dengue NS1, Dengue IgG and IgM ELISA	Panbio® – Abbott	IgM > 11 and IgG > 22
2	Human serum	Dengue plaque reduction neutralization test	Guideline for plaque reduction neutralization testing of human antibodies to dengue virus (WHO/IVB/07.07)	Inhibition ≥ 50% in viral titers
3	Human whole blood/serum	Dengue viremia	The method developed in-house as per peer-reviewed publications	10^2 to 10^8 copies
4	Human whole blood/serum	Dengue serotyping	The method developed in-house as per peer-reviewed publications	Qualitative

Small Animal Facility

The Small Animal Facility (SAF) at THSTI is responsible for breeding and maintenance of quality laboratory animals and thus catering to the animal-related research requirements of THSTI and Regional Centre for Biotechnology (RCB). SAF provides veterinary care and offers the necessary technical support and training on animal care, handling and experimental techniques to both new and experienced animal researchers.

The SAF at THSTI was established in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forest and Climate Change, Government of India and registered with CPCSEA vide registration number 1685/GO/ReBi/S/2013/CPCSEA for "Research for Education & Breeding in-house use of small animals i.e. Guinea Pigs, Mice, Rat & Rabbit". All animal experiments are performed only after approval by the Institutional Animal Ethics Committee (IAEC).

The facility supports the research community by;

- Breeding and maintaining genetically-defined strains of mice and rats;
- Facilitating researchers in developing various experimental animal models. Currently, the SAF houses animals to support research in areas of i) auto-immune diseases, ii) infectious diseases, iii) cancer biology, iv) gut inflammation, v) cardiovascular and metabolic disorders, vi) muscle development and disorders, and vii) hemolytic diseases and
- Supporting specialized animal biosafety containment facility (ABSL-III) undertaking animal research for infectious diseases such as TB and HIV.

Since the facility started its operations in September 2016, it has come to house 1 rat stock and 26 mice strains inclusive of inbred, transgenic, knock-out, knock-in and immunodeficient strains. Last year nine mice strains including one immunodeficient strain were added to the breeding colony.

Standard animal quality control measures such as routine health, genetic and environmental monitoring are being followed to ascertain the quality of the animals housed in the facility. Expression of interests was called for during this period to have on-board a consultant to upgrade the existing facility to meet international standards for animal research and management. An appropriate pest control program has been implemented in the facility. The wash area was equipped with a bedding disposal station to safeguard and protect personnel from animal allergens and contaminants while cleaning the used animal cages. As a part of the up-gradation process the facility was equipped with electrically operated heavy duty, large capacity hydro spray automated cage, rack and bottle washer.

The Infectious Disease Research Facility (IDRF), a specialized animal biosafety level III containment facility was made functional to aid research on infectious diseases such as TB and HIV. The mandatory training required for working in Animal Biological Safety Level-III (ABSL-III) facility was accomplished for the end-users and presently *in vitro* work is being carried out in the facility. The animal research work will begin in due course of time.

Future plans

THSTI is in process of implementing a plan of up-gradation of this existing facility to achieve the applicable national and international standards related to animal research and animal facility management. A reputed and experienced firm will be selected to provide consultation and assist in the up-gradation plan for implementing international standards required in animal research and animal facility management. THSTI aims to develop and establish SAF as a national resource that will act as a nodal centre for nation-wide collaborators who require laboratory space and technical expertise to work with small laboratory animals.

The CCTV surveillance monitoring, access control system and molecular-based diagnostic screening for pathogens will be implemented at the facility in due course. A separate area or room will be developed in the facility to carry out animal experiments involving infectious diseases that do not require ABSL-III and above containment level.

Currently, one of the floors is operationalized. Procurement of more strains/stocks of lab animals including immunocompromised strains is under process. Necessary support will be provided to house and expand the animal colonies. If required, additional floors will be made operational to accommodate future research needs.

CLINICAL DEVELOPMENT SERVICES AGENCY (CDSA)

CLINICAL DEVELOPMENT SERVICES AGENCY (CDSA)

Clinical Development Services Agency (CDSA) is an extramural unit of THSTI with a mandate to support and nurture clinical product development and clinical research capacity in India. It is the only public agency created by the Government of India to facilitate the development of affordable healthcare products for diseases of public health importance. CDSA is uniquely positioned to address the needs of the Indian clinical research ecosystem in order to support end-to-end research and development for public good.

The main objectives of CDSA are:

1. To support and undertake conduct of cost-effective services for clinical research and clinical product development:

- Clinical operations for phase I-IV, project management, data management and biostatistics, quality assurance and monitoring, pharmacovigilance, site, and clinical research preparedness activities

2. To undertake human resource capacity building for clinical development and other related activities with an aim to strengthen health systems and clinical research in India
3. To coordinate a network of centres of excellence in India to conduct clinical trials
4. To develop regulatory science in India to provide tools and approaches to support researchers, regulators, health policymakers, and industry

CDSA Organogram and Structure

CDSA provides support and services for the various activities through its core team of 12. In addition, project staff is recruited based on the project requirements. The operational oversight of CDSA is provided by THSTI faculty (Dean-Clinical Research and an Assistant Professor) who report to the Executive Director of THSTI. Since November 2017, CDSA is also working closely with Medical Research Council Clinical Trials Unit (MRC CTU) at University College London (UCL), UK, which is at the forefront of resolving internationally important questions and delivering a swifter and more effective translation of scientific research into patient benefits. A Senior Professor from MRC CTU is committed to helping and mentoring CDSA as the Strategy Lead.

During the past year, CDSA has successfully created a robust institutional platform and a governance structure, developed a performance-based contract career path to attract talented professionals, recruited high-quality professionals, developed an ecosystem for training and capacity building in clinical research and provided support services to several ongoing projects.

The successful delivery of a program or clinical study is a multidisciplinary team effort. The various departments or disciplines within CDSA (listed and described

next), each with their distinct role and competence, work in an integrated manner for the successful delivery of a high-quality program.

Clinical Portfolio Management:

The Department of Clinical Portfolio Management (CPM) has leveraged strong capabilities centred in clinical study development and delivery. The CPM department manages projects, supports investigators in preparing sites for the conduct of research and independently monitors clinical data for quality. It provides advisory support to researchers/ academicians/ small and medium enterprises (SMEs) for effective implementation of research projects and undertakes data management. This includes contributing to grants as a co-applicant, reviewing the project proposal with special emphasis on operational aspects, project planning, tracking of milestones, risk identification and efficient implementation.

The CPM department with support from the regulatory science and training departments has successfully provided study support to more than 15 clinical studies across a variety of disease areas. Although the focus and expertise are on large multicenter late phase clinical trials, the team has provided support to all kinds of studies: clinical trials including regulatory trials, longitudinal cohort studies, and surveys. CDSA was the national monitoring agency in India's first Comprehensive National Nutrition Survey (CNNS), supported by UNICEF and MoHFW. The survey was conducted in 28 states across India. CDSA was responsible for quality monitoring in field and laboratory.

In the last one year, the CPM department has expanded its portfolio to include trial design/ conduct and led initiatives to improve the clinical research environment, in particular for clinical trials. It has initiated a risk-based methodology for monitoring. This has made quality monitoring more efficient and effective.

The specific studies/ trials and the role of CDSA in each are detailed in **Table 6.1**.

TABLE 6.1: Summary of specialized clinical study support services provided by CDSA

Serial No	Project Title (Funding Agency)	Principal Investigator / Institute	Role of CDSA	Contribution
1.	Inter-Institutional Program for Maternal, Neonatal and Infant Sciences: A translational approach to studying preterm birth (DBT)	Prof Shinjini Bhatnagar, THSTI; other collaborating institutes: RCB, NIBMG, AIIMS, SJH, MAMC, CDSA, General Hospital Gurgaon	• Study start-up support • Quality Management • Clinical and laboratory monitoring	• GCP-compliant study documents, ICD, CRF, SOPs and data collection tools. • Site set-up as per project requirements • GCP and GLP training of project team.
2.	Post marketing study to assess the safety and tolerability and immunogenicity bOPV in healthy Indian infants (DBT)	Mr. SK Tyagi, BIBCOL	• Coordinating PI • Regulatory advisory • Project management • Clinical operations • Data management • Medical writing • Quality monitoring • Biostatistics support	• Regulatory compliance approvals (approval for protocol, amendments, study progress, safety reporting) • GCP-compliant study documents, ICD, CRF, SOPs and data collection tools.

Serial No	Project Title (Funding Agency)	Principal Investigator / Institute	Role of CDSA	Contribution
3.	Efficacy and Safety of an Innovative and Affordable Goat Lung Surfactant for the treatment of RDS in preterm neonates (Wellcome Trust)	Dr. Ramesh Agrawal, AIIMS, Delhi	• Co-applicant/ Co-PI • Regulatory advisory • Project management • Medical writing • Medical monitoring • Clinical operations • Site Management • Data management	• Successful grant receipt for the project • Regulatory compliance approvals (approval for protocol, amendments, study progress, safety reporting) • GCP and CDSCO compliant study documents, ICD, CRF, SOPs and data collection tools. • Site set-up as per project requirements • GCP trained project team
4.	Zinc as an adjunct for the treatment of clinical severe infection in infants younger than 2 months (Research Council of Norway through GLOBVAC and CISMAC)	Dr. Nitya Wadhwa and Prof. Shinjini Bhatnagar, THSTI	• Study start-up support • Quality Management • Clinical monitoring	• GCP compliant study documents, ICD, CRF, SOPs and data collection tools • Study execution as per project requirements • GCP trained project team
5.	Immediate Skin-to-Skin Contact (Immediate Kangaroo Mother Care) Study (WHO/ BMGF)	Dr. H. Chellani, Safdarjung Hospital, Delhi	• Co-applicant/ Co-PI • Study start-up support • Internal Quality Management	• Successfully supported TOT and anthropometric standardization workshop for all participating countries • GCP compliant study documents • Study execution as per project/ WHO requirements • GCP trained project team
6.	Investigation of Rheumatic Atrial Fibrillation Using Vit K Antagonists, Rivaroxaban or Aspirin (PHRI)	Dr. G. Karthikeyan, AIIMS, Delhi	• Study start-up support • Project management • Clinical data monitoring • IP management • Safety reporting reconciliation	• GCP-compliant study documents, ICD, CRF, SOPs and data collection tools.
7.	Accelerating the application of stem cell technology in human disease – ADBS Study (DBT)	Dr. Sanjeev Jain, NIMHANS, Bengaluru	• Quality Management	GCP compliant study documents ICD, CRF, SOPs and data collection tools
8.	Iron Supplement in Infant Phase 2 Clinical Trial (NIH)	Dr. Sanjiv Amin, Rochester University	• Co-applicant/ Co-PI • Clinical operations • Medical monitoring	• HMSC Approval and CDSCO submission • Site set-up as per protocol requirements

Serial No	Project Title (Funding Agency)	Principal Investigator / Institute	Role of CDSA	Contribution
9.	An open-label, non-randomized, two-stage, dose-finding study of Verapamil [IR] tablet formulation in adult tuberculosis patients in Continuation phase of anti-tuberculosis treatment (DBT)	Dr. Padmapriyadarsini, NIRT, Chennai (02 sites across India)	• Medical monitoring and writing support • Quality Management	• Regulatory compliance and approvals • GCP and CDSCO compliant study documents, ICD, CRF, SOPs and data collection tools. • Site set-up as per project requirements
10.	Evaluation of the Efficacy and Safety of a Combination regimen of Bedaquiline, Delamanid, Linezolid and Clofazimine in Adults with Pre-extensive (Pre-XDR) and Extensively Drug-resistant Pulmonary Tuberculosis (XDR-TB): Prospective Cohort Study" (USAID)	Dr. Padmapriyadarsini, NIRT, Chennai (05 sites across India)	• Clinical data and Medical monitoring • Quality Management	• Regulatory compliance and approvals • GCP and CDSCO compliant study documents, ICD, CRF, SOPs and data collection tools. • Site set-up and study execution as per project requirements • Data and Medical Monitoring
11.	Comprehensive National Nutrition Survey (CNNS)	UNICEF/ Population Council	Concurrent monitoring of the clinical laboratory component	Study successfully completed and report submitted to UNICEF and MOH
12.	A Phase III, Randomized, Double-blind, three arm Placebo controlled Trial to Evaluate the Efficacy and Safety of two vaccines VPM1002 and Immuvac (Mw) in Preventing Tuberculosis (TB) in Healthy Household Contacts of Newly Diagnosed Sputum Positive Pulmonary TB Patients (ITRC-ICMR)	Dr. Manjula Singh, India TB Research Consortium ICMR (14 sites across India)	• Study start-up support • GCP training workshop at ICMR-HQ • Designed and Finalized feasibility questionnaire/ site preparedness checklist • Conduct of Site preparedness visits (one visit per site) • Review and finalization of appropriate study-specific logs, forms and SOPs • Pre-initiation site visit to ensure site preparedness (one visit per site) • Assist the sites in IEC dossier preparation and submission • Submit site preparedness report to ITRC on monthly basis	• Regulatory compliance approvals • GCP and CDSCO compliant study documents, ICD, CRF, SOPs and data collection tools. • Site set-up as per project requirements • GCP and GCLP trained project team
13.	Burden of multidrug-resistant neonatal sepsis in district hospital settings in India	Dr. Ramesh Agrawal, AIIMS, New Delhi (05 sites across India)	• Project Management • Data Management • Data and Process Monitoring	• Study in startup Phase • Regulatory compliance and approvals

Serial No	Project Title (Funding Agency)	Principal Investigator / Institute	Role of CDSA	Contribution
14.	Translation Research Consortium for establishing platform technologies to support prophylactic and therapeutic strategies for Dengue Discovery to Proof-of-Concept (TRC)	Dr. Gagandeep Kang, THSTI, Dr. Anmol Chandele, ICGEB, Dr. Nimesh Gupta, NII, Dr. Manidipa Banerjee, IIT, Delhi Dr. Nitya Wadhwa, CDSA, Dr. Rakesh Lodha, AIIMS, Delhi, Dr. Asha Abraham, CMC, Dr. Arvindkumar Govindakarnava, MAHE	• Program management • Quality Management	Initiation of discussions and GLA signed from all collaborators

Training

The training department undertakes regular short courses and programs related to clinical research, ethics, and regulatory processes. It has developed and conducted courses on Good Clinical Practice (GCP), Good Clinical Laboratory Practice (GCLP), Good Laboratory Practice (GLP), Laboratory Quality Management System (LQMS), ethics in biomedical research, clinical research methodology, regulations: new drugs, medical devices, IVDs, vaccines, phytopharmaceuticals, biopharma, stem cells and blood products. The department works closely with the national regulatory body of the country, Central Drugs Standard Control Organization (CDSCO) for the programs on regulatory requirements and processes. The team developed a program, where they had an interactive platform for the SMEs, industry, and researchers to interact with the regulators. These programs have been done in collaboration with the Department of Biotechnology (DBT), Biotechnology Industry Research Assistance Council (BIRAC) and more recently as a NITI Aayog initiative.

The department worked with the Indian Council of Medical Research (ICMR) and DBT, developed and successfully conducted a program for the national dissemination of new ethical guidelines and stem cell guidelines. As part of our Clinical Investigators Development Program, CDSA conducted NIH Courses on Principles and Practice in Clinical Research in April 2018. The program was unique as faculty from both National Institutes of Health and India were in attendance.

In 2018-19, CDSA conducted 19 programs across 14 different cities with various important stakeholders like CDSCO, DBT, BIRAC, ICMR, National GLP Compliance Monitoring Authority (NGCMA), Department of Science and Technology (DST), Quality Council of India (QCI) imparting training to various biomedical researchers, clinicians, scientists and ethics committee members **(Table 6.2)**.

In the last one year, CDSA initiated a comprehensive GCP training program to be conducted bi-annually at the THSTI campus. The first program this year was attended by 56 participants from across India.

TABLE 6.2: Year-wise summary of training programs conducted by CDSA across INDIA

	Year								Total
	2009-12	2012-13	2013-14	2014-15	2015-16	2016-17	2017-18	2018-19	2009-19
No. of training events	3	10	14	17	21	29	17	19	**130**
Cities	2	5	10	10	9	15	12	14	**77**
Faculty	11	112	146	175	233	236	120	149	**1182**
Participants	41	436	894	1241	1906	1510	4476	1344	**11848**
Institutions	10	117	222	428	536	391	409	418	2531

Figure 6.2: A snapshot of the website of the online course on Regulatory requirements for medical devices and IVDs in India

CDSA launched India's first online course on drug regulations

CDSA recently launched India's first online courses on drug regulations. The two courses (names mentioned below) were designed and developed by CDSA, reviewed and recorded by CDSCO. These courses were launched with technical support from the National Programme on Technology Enhanced Learning (NPTEL).

Online course 1: Current regulatory requirements for conducting a trial in India

Online course 2: Regulatory requirements for medical devices and IVDs in India

The courses were launched in February 2019 and were hugely successful. 1049 participants enrolled for course 1 while course 2 had 658 participants. A proctored online examination was conducted at the end of four-weeks by IIT Madras at 140 cities across the nation. Many aspirants took the certification exam.

Program with NIH (USA) on 'Principles and Practice of Clinical Research'

In support of DBT and ICMR's mission to train the next generation of clinician-scientists and health professionals involved in clinical research, a one-week course on the "*Principles and Practice of Clinical Research*" was organized under the aegis of the Indo-US Vaccine Action Programme and National Biopharma Mission of DBT-BIRAC in April 2018. The program was unique as this extensive program was steered by faculty both from NIH and India. A total of 100 participants representing 89 institutions across India were selected on merit basis. This program was attended by DCG(I), CDSCO and US Consulate General at Hyderabad.

Delivering training at Special Areas (North East, Himalayan, LWE, island territories and other less served areas:

CDSA, since its inception, has been consistently working in special areas like the north-east to extend the body of knowledge beyond the boundaries of metro cities and bigger towns. CDSA makes special efforts in reaching out to more distant regions and underrepresented areas like the north-east region and smaller tier 2 or 3 cities. This year CDSA conducted a training program in Regional Institute of Medical Sciences (RIMS), Imphal, Manipur training 55 participants, mainly ethics committee members of RIMS, faculty, investigators, clinicians among others.

National Workshop Series on Good Laboratory Practice (GLP) sensitization

CDSA contributed as an important stakeholder and knowledge sharer with National GLP Compliance Monitoring Authority (NGCMA), DST and QCI to run a series of six workshops across the nation.

Beyond borders

With funding from the Centre for Intervention Science in Maternal and Child Health (CISMAC), a research consortium anchored at the Centre for International Health,

University of Bergen, Norway, CDSA conducted two training programs on GCP and GCLP at Institute of Medicine (IOM), Kathmandu, Nepal.

CDSA trained a team of more than 50 senior regulators of NMRA (**National Medicines Regulatory Authority**), Sri Lanka on Laboratory Quality Management System and international laboratory accreditations in February 2019.

Invited by DBT to steer program-NITI Aayog recommendations

The *NITI Aayog had* recommended DBT and the Indian drug regulators, CDSCO to work together to facilitate innovation by working with the start-ups/incubators. To take this forward, a six cities' national workshop on regulatory compliances for accelerating innovations was planned. CDSA played a lead role in the successful conduct of the program across India. The series was funded by DBT through BIRAC-NBM.

Clinical research methodology course for biomedical researchers

Every year, CDSA conducts a course that meets the requirements of biomedical researchers. This year it was conducted for all Wellcome DBT fellows. The program was attended by 14 participants.

Other Support Services

Quality assurance:

- CDSA is supporting the THSTI Bioassay laboratory for the development of a laboratory quality management system to meet the international laboratory accreditation standards, IS/ISO 17025: 2017
- Dr. Sucheta Kurundkar from CDSA was invited by the National Medicines Regulatory Authority, Sri Lanka to support them to seek laboratory accreditation for drug testing laboratory

Collegium of Centers of Excellence:

To expand our reach for improving the clinical research ecosystem in the country, CDSA established a network of six centers of clinical research excellence called the Collegium of Centers of Excellence (CoE). Each centre has its unique expertise in research. The network is engaged in clinical research activities of globally acceptable standards and jointly works towards the common goal of developing clinical research capacity in India in the area of public health.

Co-branded training courses:

- A course on 'Public Health Nutrition Research Methods' co-branded by Centre for Chronic Disease Control, New Delhi and CDSA was conducted in 2018
- 'Basics of Research Methodology and Statistical Analysis using SPSS' co-branded by JSS, Mysuru, and CDSA was conducted in 2018
- Another program on 'Critical thinking and GCP' co-branded by JSS, Mysuru, and CDSA was conducted in 2018

New initiatives to enhance the clinical research ecosystem in India

In the past year, CDSA has been involved in a number of initiatives aimed at improving the clinical research environment, in particular, clinical trials**.**

Enhancing the Clinical Trials Unit/ Clinical Research Unit capability within CDSA

In this effort to enhance its CTU capability, CDSA is collaborating with MRC CTU. This will empower CDSA with the competence to collaborate with researchers and SMEs/ industry to deliver a program of high-quality and more regulatory compliant multicenter clinical trials/ studies. To further this, Dr. Nitya Wadhwa, faculty in-charge CDSA visited the CTU in March 2019 to understand better the structure and processes that underlie the functioning of the unit.

Academic-Clinical Research Unit (A-CRU) Network

CDSA is leading an effort to create a network of clinical trials/ research units to deliver programs of high-quality research. The first brainstorming meeting was held on July 2018 where at least nine institutions expressed interest in being a part of this network. The network of Academic Clinical Research Units (A-CRUs) will enhance the ability to deliver practice-changing trials and help build an Indian evidence base of patient safety and efficacy of new treatment strategies and novel Indian healthcare products. MRC CTU will support the network through knowledge-exchange and mentoring and help build multidisciplinary teams, adopt common standards, processes and quality management systems.

Clinical Trials Toolkit

This is a collaborative effort by CDSA, THSTI and MRC CTU. It is based on the National Institute for Health Research (NIHR) clinical trials toolkit UK and is a practical guide for researchers in India in designing and conducting publicly-funded clinical trials/ research. An interactive roadmap will guide them through the various steps required for conducting clinical trials in India. It incorporates the best practices in trials in India and globally, and outlines the current legal and practical requirements for conducting clinical trials in India. This will be launched in 2019.

Ethics application and review process

CDSA worked closely with ICMR Bioethics Unit in their efforts to develop a standard ethics application form for India. The form is available on the ICMR website for ethics committees to adopt.

Integrated electronic research application platform

CDSA is working with Forum for Ethics Review Committees in India (FERCI) and PATH to develop an integrated electronic research application platform for clinical research approvals. The first module developed is an online workflow management software for the efficient functioning of ethics committees which helps them track submissions, generate queries, assign reviewers and ensure security of study documents. As the next step, an electronic application form will be created using the common form developed in collaboration with ICMR, which connects to the online document management system software for ethics committees. This will help applicants to submit their applications to the ethics committees online. A one-stop online platform for submission for all approvals – DCGI, HMSC, CTRI, IEC is the goal.

Regulatory Science and Policy Unit

Over the last year, the Regulatory Science and Policy Unit (RSPU) has developed into its new strengthened avatar. Some of the activities where the RSPU has contributed are:

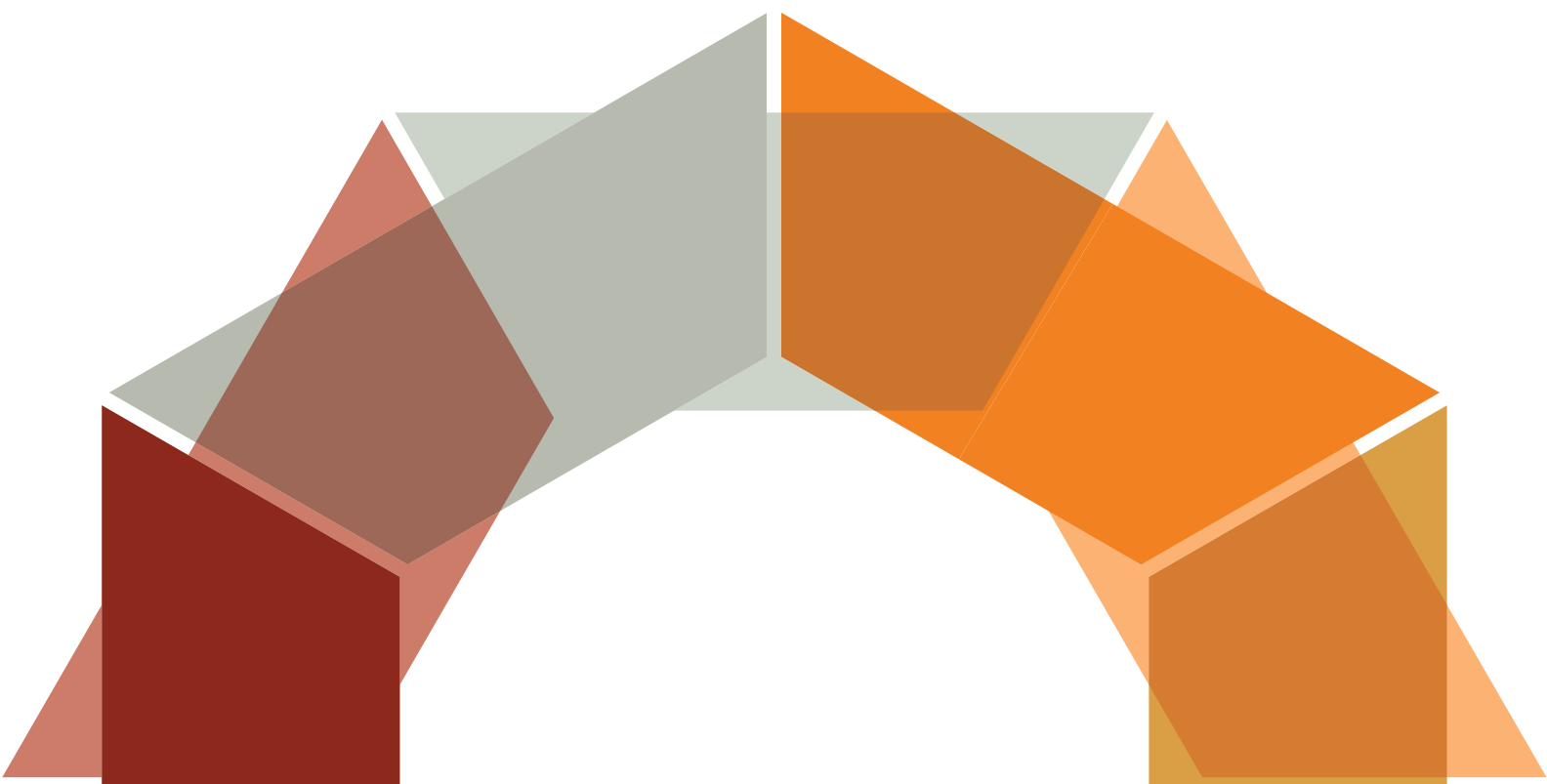
- Development of guidelines on nanopharmaceuticals. This was released by CDSCO
- Preparation of vaccine manufacturing landscapes of India for DBT

- Organising consultations to understand the barriers and enablers for experimental research in healthy human volunteers

Consultancy Services

Project Title (Funding Agency)	Principal Investigator / Institute	Role of CDSA
Clinical Trial Regulatory Advisory and Data Safety Consultancy Services	Dr. Nitya Wadhwa, CDSA	• Resourcing and planning • Training • Scientific Review of Trial Related Documents • Quality Management Assurance • Evaluation of the trial sites • Co-monitoring of trials • Safety data monitoring • Regulatory Advice • Review of validation data of immunogenicity assays

ACHIEVEMENTS



PEER-REVIEWED PUBLICATIONS

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2. Bhatia, D., Sinha, A., Hari, P., Sopory, S., Saini, S., Puraswani, M., Saini, H., Mitra, D.K., Bagga, A. (2018). Rituximab modulates T- and B-lymphocyte subsets and urinary CD80 excretion in patients with steroid-dependent nephrotic syndrome. Pediatr Res, 84(4), 520-526.

3. Chawla, A.S., Kanodia, P., Mukherjee, A., Jain, V., Kaur, G., Coshic, P., Chatterjee, K., Wadhwa, N., Natchu, U.C.M., Sopory, S., Bhatnagar, S., Majumder, P.P., George, A., Bal, V., Rath, S., Prabhu, S.B. (2018). Cell-intrinsic regulation of peripheral memory-phenotype T cell frequencies. PLoS One, 13(12), e0200227.

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EXTRAMURAL GRANTS

Dr. Amit Awasthi

Project: Understanding the transcriptional landscape of IL-10-producing anti-inflammatory T cells in IBD
Funding agency: DST-SERB

Dr. Bhabatosh Das

Project: Effect of probiotics on whole gut microbiota and clinical course in patients with critical care illnesses - A pilot study

Funding Agency: ICMR, Govt. of India

Duration of sanction: 2019-2022

Amount: INR 46.0 Lakhs

Collaborating institute(s)/Individual(s): AIIMS, New Delhi

Dr. Dinesh Mahajan

Project: Application of CO2 technology for process development

Funding Agency: Private Company (Penam Laboratories)

Duration of Sanction: Nov-2018 to July-2019

Amount: INR 76.11 lakhs

Collaborating institute(s)/Individual(s): Penam Laboratories Pvt Ltd

Project: Chemical investigation and therapeutic evaluation for linking marker compound(s) with anti-diabetic potential of young shoots of *Wendlandia glabrata* D.C. and fruits of *Phoebe cooperiana*, used by indigenous ST people of Arunachal Pradesh

Funding Agency: DBT

Duration of Sanction: 2018-2021

Amount: INR 81 Lakhs

Collaborating institute(s)/Individual(s): IASST Guwahati

Dr. Jayanta Bhattacharya (Drs. Huma Qureshi and Gagandeep Kang are other investigators)

Project: Developing HIV broadly neutralizing antibodies as a prevention product for global access through antibody half-life extension engineering.

Funding Agency: GLOBVAC grant: ***The Research Council of Norway. (Global Health & Vaccination Research)***

Collaborating institute(s)/Individual(s): University of Oslo, Neutralizing Antibody Center, International AIDS Vaccine Initiative at The Scripps Research Institute, La Jolla, California, USA

Project: Translational Research Consortium for Establishing Platform Technologies to Support Prophylactic and Therapeutic Strategies for Dengue Discovery to Proof-of-Concept

Funding Agency: BIRAC

Duration of Sanction: 2019-2023

Collaborating institute(s)/Individual(s): Drs. Sweety Samal, Tripti Srivastava, Huma Qureshi, Rajesh Kumar, Shubbir Ahmed and Supratik Das

Dr. Milan Surjit

Project: Investigation of the mechanisms controlling synthesis and function of the ORF4 protein of genotype-1 Hepatitis E virus

Funding Agency:

Duration of Sanction: 2018-2021

Amount: INR 22.14 Lakhs

Drs. Niraj Kumar & Susmita Chaudhuri

Project: Development and evaluation of a rapid phenotypic antimicrobial susceptibility test for clinical use

Funding agency: BIRAC

Duration of sanction: 2019-2020

Amount: INR 44.96 Lakhs

Collaborating institute(s)/Individual(s): Central Salt and Marine Chemical Research Institute, Bhavnagar; Christian Medical College, Vellore

Dr. Nisheeth Agarwal

Project: Understanding the roles of ClpX and ClpC1 components of Clp proteolytic machinery in *Mycobacterium tuberculosis*

Funding Agency: Department of Biotechnology, India.

Duration of Sanction: 2018-2021
Amount: INR 72.35 Lakh

Dr. Nisheeth Agarwal and Dr. Ramandeep Singh

Project: Structural and Functional Biology of Membrane Proteins from *Mycobacterium tuberculosis*: Implications in the design of new anti-TB agents

Funding Agency: DBT

Duration of Sanction: 2019-2022

Amount: 2.03 Crores INR

Collaborating institute(s)/Individual(s): Dr. Bichitra Kumar Biswal, National Institute of Immunology

Dr. Pallavi Kshetrapal

Project: Role of Hydrogen Sulphide as a pharmacological modulator in adverse pregnancy outcomes like preeclampsia.

Funding Agency: Department of Science and Technology

Duration of Sanction: 2018-2021

Amount: INR 50 Lakhs

Collaborating institute(s)/Individual(s): All India Institute of Medical Sciences, Delhi

Dr. Samrat Chatterjee

Project: Effect of time lag and stochastic perturbation on the calcium oscillation in cardiomyocyte: Study based on Mathematical model

Funding Agency: SERB (MATRICS)

Duration of Sanction: 2019-2022

Amount: INR 6.6 Lakhs

Dr. Sanjay Banerjee

Project: Role of platelet activation in the development of systemic inflammations in the patients with type 2 diabetes.

Funding Agency: DBT

Duration of Sanction: 2019-2022

Amount: INR 76.11 lakhs

Collaborating institute(s)/Individual(s): RCB, Faridabad; AIIMS, New Delhi

Dr. Sankar Bhattacharyya

Project: Study effect of Dengue virus infection on in vitro Megakaryopoiesis

Funding Agency: Science and Engineering Research Board (SERB), Department of Science and Technology, Govt. of India

Duration of Sanction: 2018-2021

Amount: 30 Lakhs INR

Project: Mechanism of rapid propagation of dengue virus during infection

Funding Agency: Department of Biotechnology, Govt. of India

Duration of Sanction: 2018-2021

Amount: INR 100.1 lakhs

Dr. Shailaja Sopory

Project: Identifying innate immunological signatures in a Zinc supplementation trial in infants less than 2 months of age with severe infection

Funding Agency: SERB

Duration of Sanction: 2018-2021

Amount: INR 53 Lakhs

Collaborating institute(s)/Individual(s): Safdarjung Hospital, Maulana Azad Medical College and Kasturba Hospital

Prof. Shinjini Bhatnagar

Project: Non/minimally invasive methods for postnatal gestational age estimation using dating ultrasound as a gold standard under the grant A "bench to bedside" model for clinical and translational science between academic research institutes and hospitals focused on fetal growth restriction and preterm birth.

Funding Agency: Department of Biotechnology (DBT), under the Glue Grant

Duration of Sanction: 2018-2023

Amount: INR 6.8 Crores

Collaborating institute(s)/Individual(s): Gurugram Civil Hospital, Maulana Azad Medical College, Regional Centre for Biotechnology

Dr. Suchitra Gopinath

Project: Molecular mechanisms mediated by vitamin D signaling on skeletal muscle function.

Funding Agency: DBT

Duration of Sanction: 2019-2022

Amount: 62.06 lakhs

Collaborating institute(s)/Individual(s): National Institute of Immunology

Dr. Tarun Sharma

Project: Aptamer-based Tuberculosis Diagnostics Toolbox - as PI (Industry-academia collaboration)

Funding Agency: DBT under call for TB diagnostics

Duration of Sanction: 2018-2020

Amount: INR 35 lakhs

Collaborating institute(s)/Individual(s): AIIMS, Delhi and PGI, Chandigarh

Project Aptamer-based rapid diagnosis of snake bite: an unmet Indian need

Funding Agency: DBT under Innovative Young Biotechnologist Award

Duration of Sanction: 2018-2020

Amount: INR 66 Lakhs

Project: Aptamer based rapid test to differentiate venomous snake bites from non-venomous and dry snake bites

Funding agency: BIRAC

Duration of sanction: 2018-2020

Collaborating institute(s)/Individual(s): Tezpur University, Assam

PATENTS

Serial No.	Title	Application No.	Filing date	Inventors
1	A cyclic peptide and pharmaceutical composition comprising the same for inhibiting proliferation of HEV.	PCT/IN2018/050475	25/07/2018	M. Surjit, S. Anang
2	Aptamer against *M.tb* MPT51 and uses thereof.	PCT/IN2018/050581	07/09/2018	T.K. Sharma, J.S. Tyagi, A. Dhiman, R Das
3	A herbal composition from *Premna herbacea*, useful for prevention of obesity and type 2 diabetes and a method for its extraction.	201831046382	07/12/2018	N.C. Talukdar, S.K. Banerjee, Ajay Kumar et al.
4	A pharmaceutical composition for the treatment of Th17 cells mediated autoimmune inflammatory diseases and uses thereof.	201811032275	29/08/2018	A. Awasthi, Z. A. Rizvi, A. Pandey, S. Asthana, C. Suri, K. Srikanth.
5	A pharmaceutical composition for the treatment of IL9 mediated autoimmune inflammatory diseases and uses thereof	PCT/IN2018/050575	16/09/2018	A. Awasthi, S. Roy
6	A pharmaceutical composition for the treatment of IL9 mediated autoimmune inflammatory diseases and uses thereof	201811022084	13/06/2018	A. Awasthi, S. Roy
7	Method of Converting Carbon Dioxide into Carbonyl Compounds	PCT/IN2018/050648	11/10/2018	D. Mahajan, V. Kumar, A. Rana
8	Aptamer-based electrochemical biosensor for the rapid diagnosis of Tuberculosis Meningitis	201811042593	13/11/2018	T.K. Sharma, J.S. Tyagi, A. Dhiman, R Das

HONORS AND AWARDS

Awards

- Dr. Susmita Chaudhuri bagged the TiE – BIRAC WInER Award for Women in Entrepreneurial Research, from Tie-BIRAC, 2018.
- Drs. Jayanta Bhattacharya, Huma Qureshi, Rajesh Kumar and Suprit Deshpande received the MVP 2018 Award from IAVI, USA for "excellent and outstanding leadership efforts towards successfully establishing the B cell sorting and bnAb isolation platform" at the HVTR lab in THSTI by closely working with NAC.
- Dr Jayanta Bhattacharya was recognized as one of the IAVI Key Influencers by the CEO, IAVI, Dr Mark Feinberg.
- Dr. Tarun Sharma was adjudged winner of technology showcasing competition at IKMC-2018 organized by IKP and DUPONT (2018).

Membership

- Dr. Amit Awasthi was elected Secretary of the India Immunology Society after the elections for the Society Office Bearers on 11th October 2018.
- Dr. Pallavi Kshetrapal was elected to the Scientific Review Committee (SRC) for Initiative for Research and Innovation in Science (IRIS) Program jointly initiated by Intel Technology India Private Ltd (Intel) with the, Department of Science & Technology (DST) Government of India, and the Indo-US Science & Technology Forum (IUSSTF).
- Dr. Sanjay Banerjee was elected the Council Member, International Academy of Cardiovascular Sciences (IACS-Indian Section), 2019.
- Dr. Rajesh Kumar was elected fellow of Antiviral Research Society (AVRS) for the year 2018, based on the outstanding research contribution in the field of viral vaccine.

Honors

- Dr. Tarun Sharma was nominated as a Future Science Leader by Secretary, DBT and STS-Japan (2018).

Travel Grants

Following scientists were awarded travel grants by various agencies during the session 2018-19; the visits paid and awarding agencies are listed here.

- Dr. Samrat Chatterjee went for a short visit of one month to Mathematics Department, University of Warsaw, Poland.
- Drs. Shubbir Ahmed, Sweety Samal, Rajesh Kumar and Dr. Tripti Srivastava attended HIV Research for Prevention 2018 (HIVR4P 2018), held on 21 - 25 October, 2018 in Madrid, Spain.
- Dr. Rajesh Kumar attended the Research-for-Cure Academy which will be held at the Wits Rural Facility, Bushbuck Ridge, South Africa from 31 October to 2 November 2018.

SEMINARS AND CONFERENCES

- Aggarwal, S., Kumar, A., Jamwal, S., Midha, M., Hamza, B., Yadav, A.K. Studying the temporal dynamics of host immune response to different strains of mycobacteria for exploring host-directed therapeutic strategies. Immunocon 2018, THSTI, Faridabad, India. 01-03 November 2018
- Aggarwal, S., Banerjee, S.K., Talukdar, N.C., Yadav, A.K. The Sirtuin interactors and the role of PTMs in cardiovascular diseases. ICPCBMM - PSI Conference 2018, NCCS, Pune, India. Dec 14-18, 2018.
- Singh, K., Gupta, I., Khan, S. Understanding the cellular and regulatory role of human protein degradation machinery key players. 10th Young Investigator Meeting, Kerala, 2018.
- Srivastava, M., Suri, C., Asthana, S. What Modulates the Usp7 Function, A Dynamic Pocket or Inter-Regulatory Domains? Annual meeting of Biophysical Society, USA, 2019.
- Kumari, A., Pathak, D.P., Asthana, S. Structural insights into identification of small molecules that can modulates the lipid homeostatsis, Science Day symposium, NII, 2019.
- Suri, C., Srivastava, M., Prajapat, S., Sadhu, S., Chugh, S., Kalia, M., Awasthi, A., Singh, R, Chand, A., Jamwal, S., Asthana, S. Rational design of novel protein-protein interaction inhibitors to induce autophagy- A therapeutic approach to stimulate innate immunity, International Immunocon Conference, 2018.
- Mittal, L., Awasthi, A., Asthana, S. Identification of check-point inhibitors through peptidomimetics by targeting the PD-1/PD-L1 interface, International Immunocon Conference.
- Mittal, L., Venkatanarayana M, Kaur, T., Krishnaprasad G, Bhattacharya, S., Gundala, R, Asthana, S. Inhibition of dengue virus RNA-dependent RNA polymerase by binding of a novel inhibitor at the dsRNA exit site. RNA viruses: Immunology, pathogenesis and translational opportunities, EMBO symposium 28 – 30 March 2018.
- Kumar, N., Chaudhuri, S., Bhatnagar, S., Kang, G. Rapid Pathogen Identification and Phenotypic Antimicrobial Susceptibility Testing. 4th WHO Global Forum on Medical devices. Vishakhapatnam, India 2018
- Dr. Tarun Sharma attended the International Knowledge Millennium Conference (IKMC)-2018, Organized by IKP-Hyderabad.
- Dr. Sankar Bhattacharyya attended the 87th conference of the Society of Biological Chemists (India) at School of Life Sciences, MAHE, Manipal from November 25-27th, 2018.
- The 6th Molecular Virology Meeting at IIT Kharagpur from 28th February to 2nd March 2019 was attended by Drs. Jayanta Bhattacharya, Sankar Bhattacharyya and Supratik Das.

INVITED TALKS AND PANELS

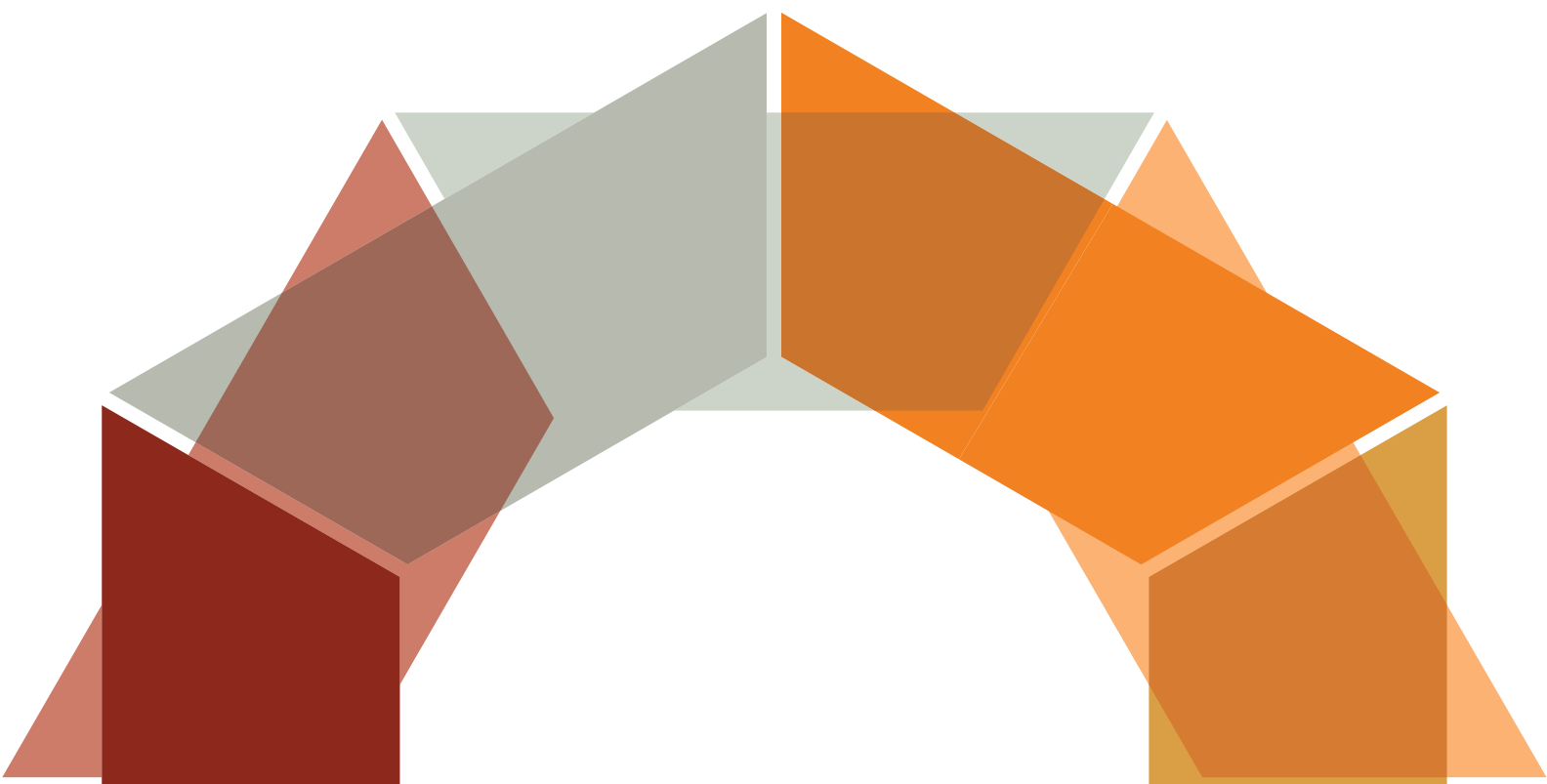
Individual	Title of the Talk	Hosting Institute
Dr. Prabhanshu Tripathi	Importance of Gut microbiota and probiotics in immune response	Ram Lal Anand College, University of Delhi
Dr. Amit Yadav	Field guide to analyze proteomics data - identification & quantitation; The RCB-Mass Spectrometry and Proteomics Workshop	RCB, Faridabad
	Characterizing the cogs in the machinery of life	The Institute of Advanced Study in Science & Technology, Guwahati, Assam
Dr. Amit Pandey	Host cholesterol utilization induces mycobacterial peristence during infection	"The EMBO workshop-Bacterial Persistence and Antimicrobial Therapy", Ascona, Switzerland
	Targeting "Persisters": A new paradigm for tuberculosis drug development	International Conference on Molecular Basis of Disease and Therapeutics (ICMBDT-2019); Central University of Rajasthan, Ajmer
	Evolution and transmission of drug-resistance tuberculosis in Agartala, Kohima and Imphal population	ICGEB, Delhi
	"Drug development in tuberculosis"; AICTE sponsored Quality Improvement Programme (QIP) for attending faculty, academician working in Pharmacy colleges throughout India.	Delhi Institute of Pharmaceutical Sciences & Research (DIPSAR), New Delhi
Dr. Guruprasad Medigeshi	Interactive session on grant-writing skills; skill shop on S&T communication	CSIR-Human Resource Development Centre
	Zinc homeostasis in RNA virus infections; 6th Molecular Virology Meeting	IIT- Kharagpur
	Host factors in dengue virus life-cycle; International Conference on Molecular Basis of Disease and Therapeutics	Central University of Rajasthan, Ajmer.
Dr. Jayanta Bhattacharya	Role of antibodies in prevention and treatment of HIV- 1; Prof. V. V. Modi Memorial lecture and One Day International symposium on Vaccine and Infectious Diseases	Department of Microbiology and Biotechnology Centre, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat

Individual	Title of the Talk	Hosting Institute
	Dissecting conformational and functional properties of HIV-1 trimeric env proteins obtained from elite neutralizers; International Conference on Biology and Therapeutics of HIV & Associated Infections	University of Hyderabad
	Role of antibodies in prevention and treatment of HIV; 6th Molecular Virology Meeting	IIT Kharagpur
	Engineered mAb-mimetic capable of neutralizing broad spectrum of HIV-1" jointly presented with Dr Ashish Ganguly, IMTECH, Chandigarh; CSIR led Brainstorming Meeting on New Millennium Indian Technology Leadership Initiative (NMITLI) scheme to develop project on "Drugs and Diagnostics for HIV"	CSIR Center, New Delhi
	Panel member; One-day symposium on the 'the role of innovative technologies in the vaccine development - Session 1: Application of Technology to Overcome Barriers of Vaccine Development'	MSD-Wellcome Hilleman Labs, at India Habitat, New Delhi.
	Genetic, Antigenic and Immunogenic Properties of an HIV-1 Envelope Protein obtained from an Elite Neutralizer; International symposium of infectious diseases	Regional Center of Biotechnology and Jamia Hamdard University
Dr. Krishnamohan Atmakuri	TB vaccines discovery and challenges, where do we stand globally; World TB Day Symposium, AIIMS, India 2018	AIIMS, New Delhi, INDIA
	Novel approaches to insights on neonatal sepsis; PRESIDE – annual meeting 2018	St Johns' Hospital, Bengaluru, INDIA
	TB vaccines: Deficiencies, challenges, discovery and alternates; Vision 2025: Mission TB free India 2019	Jodhpur, INDIA
Dr. Milan Surjit	Identification and characterization of antiviral activity of zinc against the Hepatitis E virus	BARC, Mumbai
	In the pursuit of a therapy against the hepatitis e virus (hev): identification of the anti-hev properties of a cyclic peptide and zinc	IIT, Guwahati

Individual	Title of the Talk	Hosting Institute
	Exploiting the host machinery for survival: lessons learnt from the hepatitis E virus; Molecular virology meeting-2019	IIT, Kharagpur
	In the pursuit of a therapy against the hepatitis e virus (hev): identification of the anti-hev properties of a cyclic peptide and zinc; International symposium on infectious diseases	RCB, Faridabad
	Studying the effect of CRISPRi-mediated DNA gyrase depletion in *Mycobacterium tuberculosis;* 10th DAE-BRNS Life Science Symposium -2019 (LSS-2019)	BARC, Mumbai
Dr. Nisheeth Agarwal	Gene silencing by CRISPR interference in *Mycobacteria;* Seminar series on "CRISPR/Cas9 in Genome Editing"	Nirma University, Ahmedabad
	Our first-hand experience with CRISPR interference approach for gene silencing in mycobacteria; Distinguished Seminar Series organized by Faculty of Life Sciences and Biotechnology,	South Asian University, Delhi
	Characterizing the essential genes of unknown functions in *Mycobacterium tuberculosis*: impact of CRISPRi approach; Koshika-Zoological Society	Department of Zoology, Daulat Ram College, Delhi University, Delhi
	Nuts & Bolts of CRISPR Interference: Optimization & Implications in Mycobacteria; BIOMANIA-2018	Biochemistry Department at Daulat Ram College, Delhi University, Delhi
	Designing, optimization and implementation of CRISPRi approach for silencing the expression of genes in *Mycobacterium tuberculosis;* CRISPR/ Cas9 genome editing workshop	Shiv Nadar University, Greater Noida
	Implementation of CRISPRi approach for silencing the expression of genes in *Mycobacterium tuberculosis;* CRISPR/ Cas9 technology for targeted genome editing	Institute of Microbial Technology, Chandigarh
Dr. Pallavi Kshetrapal	Placenta: The tree of life; Science Day	K.L. Mehta Dayanand College for Women, Faridabad
	The unexpected joys of travelling the tricky terrains in translational research; 33rd "Foundation day function" of the Department of Biotechnology, Ministry of Science & Technology, Government of India	National Institute of Immunology, New Delhi

Individual	Title of the Talk	Hosting Institute
	Introducing Garbh-INi– interdisciplinary Group for Advanced Research on BirtH outcomes – DBT INdia Initiative; The Preterm birth international collaborative Australasia branch (PREBIC-AA)	Seoul, Korea
Dr. Susmita Chaudhuri	Microcon 2018, 42nd Annual Conference of Indian Association of Medical Microbiologists	NIMHANS, Bangalore.
Dr. Samrat Chatterjee	Studying host-pathogen interaction under the influence of *Mycobacterium tuberculosis* through mathematical models; 106 Indian Science Congress (ISC)	Lovely Professional University, Jalandhar, Punjab
	Revealing the significance of calcium dynamics in normal cardiac functioning using mathematical models; Faculty presentation in IMSc	IMSc, Chennai
	Revealing the significance of calcium dynamics in normal cardiac functioning using mathematical models; India biodiversity meet-2019 (IBM 2019)	Indian Statistical Institute, Kolkata
Dr. Sanjay Banerjee	Serum protein signature of CAD in T2DM; 16TH Annual conference of International Society of Heart Research (ISHR) Indian section.	RUHS College of Medical Sciences
	TLR4 activation promotes cardiac fibrosis in rats through p53-caspase pathway; IACS-INDIA 2019 International Conference on Translational Research in Cardiovascular Sciences.	Sri Jayadeva Institute of Cardiovascular Sciences and Research.

ACADEMIA



ACADEMIA

Doctoral Program

The Ph.D. program in biomedical and clinical research offered by THSTI is recognized by Jawaharlal Nehru University, Delhi. The institute is affiliated with the Regional Centre for Biotechnology (RCB), Faridabad for the THSTI – RCB Ph.D. program. THSTI is also recognized by the Jadavpur University (JU), Kolkata for the Ph.D. program in Mathematical Biology and Systems Biology. Students selected for the doctoral program with JU are required to undergo Ph.D. course work in Mathematics.

The broad domains of research Ph.D. students work on are:

- Vaccines and infectious diseases
- Translational research in maternal and child health
- Drug discovery and mathematical modeling to understand disease biology
- Human microbial ecology
- Diagnostics, devices and biodesign

Courses offered through THSTI – JNU PhD program:

- Laboratory Research Methods
- Quantitative Methods in Research
- Practical Training in Research Methods
- Viral Illnesses of Human Importance
- Bacterial Illnesses of Human Importance
- Advanced Epidemiology
- Immunology & Immunotechnology
- Essentials of Clinical Trials
- Introduction to Drug Discovery
- Bio-Mathematical and Computational Tools
- Introduction to Biodesign

Currently, 73 students are enrolled in the THSTI doctoral program. A list of students who joined in 2018-19, their supervisors and funding agency that supports them are mentioned next.

List of PhD students who joined in 2018-19

Serial No.	Name of the student	Current Supervisor	Funding agency
1	Ms. N. Sugandha	Dr. Amit Awasthi	DBT
2	Ms. Upasna Madan	Dr. Amit Awasthi	UGC
3	Mr. Vaibhav Kumar Nain	Dr. Nisheeth Agarwal	ICMR
4	Ms. Manisha Singh	Dr. Ramandeep Singh	CSIR
5	Ms. Garhima Arora	Dr. Samrat Chatterjee	THSTI
6	Ms. Shabnam Ansari	Dr. Milan Surjit	CSIR
7	Mr. Rahul Singh Mewada	Dr. Amit Kumar Pandey	DBT
8	Ms. Taruna Sharma	Dr. Amit Kumar Pandey	CSIR

Postdoctoral program

The various postdoctoral programs active at THSTI has attracted bright young researchers from across India. Table below lists the ones who joined us this year. Here's a list of postdoctoral programs:

- Innovation Award scheme in Biodesign
- Translational Research Award in Infectious Diseases (TRAIN)
- Early Career Medical Research Award (ECMRA)
- SERB – National Postdoctoral Fellowship
- DBT – Research Associateship

List of postdoctoral fellows who joined THSTI in 2018-19

Serial No.	Name of the fellow	Funding Agency
1	Dr. Jyoti Gupta	National Postdoctoral Fellowship (N-PDF) - SERB
2	Dr. Neeraj Kumar Chauhan	
3	Dr. Sima Kumari	
4	Dr. Zaigham Abbas Rizvi	
5	Dr. Rishabh Sharma	
6	Dr. Harleen Khurana	DBT Research Associateship – I
7	Dr. Jaya Gandhi	DBT Research Associateship – I

Short-term Training Programme (STTP)

THSTI hosts and trains graduate and undergraduate students from institutes across the country for their dissertations. Thirteen students were trained during the year 2018-19 including two trainees who were supported by the DST Knowledge Augmentation through Research in Young Aspirants (DST KARYA) program.

Academic achievements and other highlights


Dr. Sakshi Malik


Dr. Garima Arora


Dr. Rinki Kumar


Dr. Bhavya Khullar


Dr. Saumya Anang


Dr. S. Chandru

- Five candidates were conferred the doctoral degrees during this session – Dr. Garima Arora, Dr. Bhavya Khullar, Dr. Sakshi Malik, Dr. Saumya Anang, Dr. Chandru and Dr. Rinki Kumar.

- Mr. Abhijit Paul's poster was adjudged the best in the mathematical section of the 106th Indian Science congress held from 7th to 9th January 2019.

- Ms. Suyasha Roy was selected for the Newton Bhabha PhD Placement Programme 2017-18 at University of Oxford, United Kingdom; received Travel Grant Award from International Union of Immunological Society (IUIS) for an oral presentation on her PhD work at 5th European Congress of Immunology (ECI) held from 2nd-5th September, 2018 at Amsterdam, The Netherlands. She also received an International Travel Award from the Australasian Society of Immunology (ASI) to present her PhD work at the 7th Federation of Immunological Societies of Asia-Oceania (FIMSA) Congress held from 10th-13th November, 2018.

- Mr. Bugga Paramesha bagged the best poster presentation award in the workshop and symposium on "Translational Research in Biomedical and Agricultural Sciences", jointly organized by Indian Society of Translational Research (ISTR) and Department of Biochemistry, AIIMS, New Delhi and was held on 6th -7th July 2018.

- Ms. Hina Lateef Nizami was awarded the first prize in oral presentation at the Cardiovascular Research Convergence 2018 (CRC 2018) jointly organised by AIIMS, New Delhi, and ICGEB, New Delhi on 13th and 14th October 2018.

- Ms. Archana Pant won the first prize for a poster presentation at BioZest 2018 organized at the South Asian University, New Delhi on 6th December 2018.

- Ms. Hina Lateef Nizami and Mr. Parmeshwar Katare were awarded for poster and oral presentation (respectively) at the International Academy of Cardiovascular Sciences (IACS) India section 2019; International Conference on Translational Research in Cardiovascular Sciences. Ms. Hina received the N.S. Dhalla Best Poster award, while Mr. Parmeshwar bagged the award for being one of the finalists for the D.K. Agrawal Young Investigator award session.

- Dr. Satyabrata Bag won the Best Poster Award at the International Symposium on Infectious Diseases held during 12-14th November 2018. His winning poster was titled '*Insights into the Resistance Traits of Multidrug-Resistant Enteric Pathogens Isolated from India*'. The Symposium was jointly organized by the Regional Centre for Biotechnology and Jamia Hamdard University.

- Dr. Md. Jahangir Alam's poster was awarded at the 16th Annual Conference of International Society for Heart Research

EXTERNAL RELATIONS AND INSTITUTIONAL DEVELOPMENT OFFICE

The External Relations and Institutional Development (ERID) office supports researchers at THSTI in grants management, regulatory compliance for ethics committees, communications, and science outreach. While Ms. Vidhya Krishnamoorthy is in charge of the grants support and ethics secretariat functions, Dr. Siuli Mitra manages science communications and coordinates outreach programs.

ADMINISTRATION

The administrative, financial and human resource management, among other several functionalities, are provided for by the THSTI Administration and here's listing an account of activities accomplished in the session 2018-19.

THSTI Governance

THSTI conducted one Society, two Finance Committee and two Governing Body meetings. Recommendations made by individual committees were documented, circulated among concerned individuals/departments and eventually implemented.

THSTI Internal Committees

Various internal committees constituted to advise and support the Executive Director in decision making. All these committees completed their tenure on 31st March this year. The composition of the committees that were functional in the session 2018-19 are listed later in this report.

Right to Information:

In 2018-19, THSTI received 59 applications under the RTI Act, 2005.

Human Resource Management:

THSTI posted 39 recruitment notifications for filling 134 positions. The rolling advertisements continued to be advertised.

Finance and Accounts:

The department attends to the day-to-day financial matters, payments to contractors/suppliers, salary payments among other functions. The annual statement of accounts prepared by the department can be found later in this report.

Stores and Purchase:

The department is responsible for the purchase of scientific equipment, chemicals, reagents and other

consumables from local and overseas markets. THSTI has invested INR 14.27 CR on consumables and INR 8.03 CR on equipment and furniture in the financial year 2018-19.

Information and Technology (IT):

This year the IT department initiated the planning and implementation of ERP solution for THSTI that is inclusive of process automation of Academics, Accounts, HR, General Administration, and Purchase. THSTI became an EduRoam (Education Roaming) enabled institution, hosted in-house Payment Gateway application, DNS server, and ERP server. The IT section is responsible for smooth functioning of IT-based infrastructure in the institute that includes data center, campus-wide leased line network, auditorium, seminar room, lab-based workstations, laptops, printing services among others.

Engineering and Estate Management:

The engineering department develops and maintains the physical infrastructure of the institute and is primarily responsible for setting up research labs/offices and ensure continued functioning of equipment and infrastructure. 2018-19 saw a substantial transformation of infrastructure with new labs and office spaces coming up and the department was instrumental in completion of infrastructural updating for Bioassay laboratory, Biorepository, Aryabhata Data science and AI Program center, Infectious Disease Research Facility (IDRF). Within the NCR BSC campus they carried out implementation of the ecological work plan, fencing of 85 acres of cluster land, and functionalization of faculty housing among other assignments. The department successfully executed energy audit and took action to save electricity worth INR 20 lakh per annum. Currently underway are vertical extension of hostel building, construction of BSL-3 and Office of Connectivity buildings in the cluster, construction of stone boundary for 85 acres of the cluster land and preparation of a detailed project report for the development of this land. Installation of a solar power system of 500 KW capacity and a 66KV electric grid are being planned to reduce power consumption in the cluster and improve power supply respectively.

Intellectual Property Protection:

THSTI filed eight patent applications in 2018-19.

FINANCE AND ACCOUNT STATEMENTS

TRANSLATIONAL HEALTH SCIENCE AND TECHNOLOGY INSTITUTE,FARIDABAD

BALANCE SHEET AS AT 31ST MARCH, 2019

Amount (In Rs.)

LIABILITIES	Schedule	31.03.2019	31.03.2018
Corpus / Capital Fund	1	1,70,58,71,477	1,58,48,12,511
Reserves and Surplus	2	9,98,36,749	11,40,76,493
Earmarked/Endowment Funds	3	-	-
Secured Loans and Borrowings	4	-	-
Unsecured Loans and Borrowings	5	-	-
Deferred Credit Liabilities	6	-	-
Current Liabilities and Provisions	7	44,34,26,823	44,82,98,120
TOTAL		**2,24,91,35,049**	**2,14,71,87,124**
ASSETS			
Fixed Assets	8	1,76,43,18,802	1,60,37,86,179
Investment From Earmarked/Endowment Funds	9	-	-
Investment-Others	10	2,700	2,700
Current Assets, Loans, Advances etc.	11	48,48,13,547	54,33,98,245
Miscellaneous Expenditure (to the extent not written off or adjusted)		-	-
TOTAL		**2,24,91,35,049**	**2,14,71,87,124**
SIGNIFICANT ACCOUNTING POLICIES AND NOTES ON ACCOUNTS	24		
CONTINGENT LIABILITIES	-		

Schedules 1 to 24 form an integral parts of Accounts

As per our separate Report of even date attached
For Mehra & Sistani
Chartered Accountants

(BHAWANI SINGH)
CONSULTANT (F & A)

(M.V.SANTO)
HEAD ADMINISTRATION

(Dr.GAGANDEEP KANG)
EXECUTIVE DIRECTOR

(SANJIV RAI MEHRA)
PARTNER
M. No. 80402

TRANSLATIONAL HEALTH SCIENCE AND TECHNOLOGY INSTITUTE FARIDABAD

MEHRA & SISTANI * Chartered Accountants *

Place: Faridabad
Date: 30/07/2019

TRANSLATIONAL HEALTH SCIENCE AND TECHNOLOGY INSTITUTE

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31ST MARCH, 2019

Amount (in Rs.)

INCOME	Schedule	31.03.2019	31.03.2018
Income from Sales/ Services	12	1,15,71,668	1,25,08,532
Grants/Subsides	13	20,69,80,000	21,50,00,000
Fees/Subscriptions	14	1,40,000	68,000
Income from Investments	15	-	-
Income from Royalty, Publication etc.	16	-	-
Interest Earned	17	2,76,31,588	80,51,229
Other Income	18	30,95,842	25,61,477
Increase/(Decrease) in stock of Finished goods and works in progress	19	-	-
Deferred Income-Fixed Assets		9,41,24,776	9,16,34,124
TOTAL (A)		**34,35,43,874**	**32,98,23,362**
EXPENDITURE			
Establishment Expenses	20	8,12,60,554	8,40,09,924
Other Administrative Expenses etc.	21	16,26,90,407	14,52,50,684
Expenditure on Grants , Subsidies etc.	22	-	-
Interest	23	77,59,879	-
Depreciation (Net Total at the year-end-corresponding to Schedule 8)		9,41,24,776	9,16,34,124
Prior period Adjustment A/c (ANN-A)		-	-
TOTAL(B)		**34,58,35,617**	**32,08,94,732**
Balance being excess of Expenditure Over Income (A-B)		**(22,91,743)**	**89,28,630**
Transfer to special Reserve(Specify each)		-	-
Transfer to /from General Reserve		(22,91,743)	89,28,630
BALANCE BEING SURPLUS /DEFICIT CARRIED TO CORPUS/CAPITAL FUND		-	-
SIGNIFICANT ACCOUNTING POLICIES AND NOTES ON ACCOUNTS	24		
CONTINGENT LIABILITIES	-		

Schedules 1 to 24 form an integral parts of Accounts

As per our separate Report of even date attached
For Mehra & Sistani
Chartered Accountants

(BHAWANI SINGH)
CONSULTANT (F & A)

(M.V.SANTO)
HEAD ADMINISTRATION

(Dr.GAGANDEEP KANG)
EXECUTIVE DIRECTOR

(SANJIV RAI MEHRA)
PARTNER

Place: Faridabad
Date: 30/07/2019

TRANSLATIONAL HEALTH SCIENCE & TECHNOLOGY INSTITUTE (THSTI)
Faridabad

CONSOLIDATED RECEIPTS AND PAYMENTS ACCOUNT FOR THSTI,PROJECTS & FELLOWSHIP FOR THE YEAR ENDED 31ST MARCH, 2019

AMOUNT-IN-RUPEES

RECEIPTS	31.03.2019		31.03.2018	
OPENING BALANCE:-				
Fellowship	(78,62,510)		(1,34,98,813)	
Projects	40,76,25,305		29,16,07,010	
THSTI	12,07,51,771		11,81,78,864	
Grant-in Aid Received:-				
Fellowship	2,75,54,336		2,59,94,119	
Projects	41,63,45,445		58,12,99,022	
THSTI	41,94,32,000		29,48,31,000	
Other Receipts -THSTI				
Application Fees	25,750		68,000	
Admission Fee	31,500		-	
Earnest Money Deposit	47,11,938		67,17,779	
Guest House Receipt	2,54,708		2,32,852	
Income from Sales and Services	1,15,54,168		1,24,46,132	
Income Tax Refund Received	-		17,56,920	
Interest Received from Banks	1,98,71,709		78,95,639	
Interest Received from Income Tax	-		1,55,590	
Miscellaneous Receipts	13,673		83,500	
Other Receipts	-		35,772	
Penalty Receipt	1,71,675		1,62,599	
Recruitment Fee	1,95,700		4,300	
Recruitment Fee	1,40,000		-	
Donation	-		2,700	
HRA Recovery	20,99,836		18,43,404	
Vendor Registration Fee	1,08,000		67,000	
RTI Receipt	2,000		122	
Sales of Scrap	17,500		62,400	
Security / Hostel Deposit Received	12,88,235		33,54,093	
Tender Fee	1,93,000		1,65,000	
Accrued Interest Received	5,89,620		7,43,954	
Decrease in advances	78,03,843		1,08,85,103	
Govt. Dues Payable	9,39,737		18,78,974	
Other Liabilities/Payable	2,28,69,438		1,12,78,635	
TOTAL		1,45,67,28,377		1,35,84,51,670

AMOUNT-IN-RUPEES

PAYMENTS Particulars	31.03.2019		31.03.2018	
Fellowship	**2,67,61,340**		**2,03,57,816**	
Projects	**42,61,04,264**		**46,54,80,725**	
THSTI				
Work -in- Process- Building	17,44,75,059		3,00,00,000	
Fixed Assets	6,37,83,962		8,30,63,424	
Administrative Expenses	11,25,09,578		10,10,78,759	
Manpower	7,08,90,662		7,52,50,121	
Consumables	4,88,47,763		4,66,28,655	
Advances , Receivables & Liabilities	7,98,88,848		1,60,77,602	
Closing Cash & Bank Balance				
Fellowship	**(70,69,514)**		**(78,62,510)**	
Projects	**39,78,66,486**		**40,76,25,305**	
THSTI	**6,26,69,930**		**12,07,51,771**	
TOTAL		1,45,67,28,377		1,35,84,51,670

As per our separate Report
of even date attached
For Mehra & Sistani
Chartered Accountants

(BHAWANI SINGH)
CONSULTANT (F & A)

(M.V SANTO)
HEAD ADMINISTRATION

(DR. GAGANDEEP KANG)
EXECUTIVE DIRECTOR

(SANJIV RAI MEHRA)
PARTNER

PLACE: Faridabad
DATE: 30/07/2019

Mehra & Sistani

Chartered Accountants

New Delhi

AUDITORS' REPORT

To
Executive Director
TRANSLATIONAL HEALTH SCIENCE AND TECHNOLOGY INSTITUTE
FARIDABAD

1. We report that we have audited the Balance sheet of **"TRANSLATIONAL HEALTH SCIENCE AND TECHNOLOGY INSTITUTE "**as at 31 March 2019 and the relative Income & Expenditure Account and Receipt & Payment Account for the period ended on that date, annexed there to. These financial statements are responsibilities of the society management. Our responsibility is to express an opinion on these financial statements based on our audit.

2. We conducted our audit in accordance with auditing standards generally accepted in India. These standards require that we plan and perform the audit to obtain reasonable assurance about whether the financial statements are free of material misstatement. An audit includes examining, on a test basis, evidence supporting the amounts and disclosures in the financial statements. An audit also includes assessing the accounting principles used and significant estimates made by management, as well as evaluating the overall financial statement presentation. We believe that our audit provides a reasonable basis for our opinion.

3. Further to our comments as follows, we report that:

   a) We have obtained all the information and explanations, which, to the best of our knowledge and belief, were necessary for the purpose of our audit.

   b) In our opinion, proper books of account have been kept as required by law so far, as appears from our examination of those books.

   c) The Balance Sheet and Income & Expenditure Account and Receipt & Payment Account dealt with by this report are in agreement with the books of accounts.

   d) In our opinion, the balance sheet and income & expenditure account and Receipt & Payment Account deal with by this report comply with the Accounting Standards issued by the Institute of Chartered Accountant of India, to the extent applicable.

   e) In our opinion and best to the information and according to the explanation given to us, they said account a true and fair view in conformity with the accounting principles generally accepted in India

.....Contd/2

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I. In the case of the Balance sheet, of the state of affairs of the Institute as at 31'march 2019;and

II. In the case of Receipt & Payment Account, of the receipt for the period ended on that date.

III. In the case of Income and Expenditure Account, excess of Expenditure over Income for the period ended on that date.

For Mehra & Sistani
Chartered Accountants
F.R.N. 000409N

(Sanjiv Rai Mehra)
Partner
Membership No.080402
Date : 30th July,2019
UDIN: 19080402AAAACG9620

SCIENTIFIC EVENTS AND OUTREACH

Outreach programs for schools and colleges

The session of 2018-19 was exciting with new programs in outreach and various events were organized which saw enthusiastic participation from colleges. Here are a few highlights:

- A total of seven college visits were conducted during which faculty members and scientists from THSTI delivered lectures at these colleges.

List of college visits, names of the colleges, and the faculty members and scientists who visited the colleges.

At Acharya Narendra Dev College, University of Delhi

Serial No.	Date of visit	Name of college	Name of faculty members/ scientists who visited
1	4th May 2018	Ram Lal Anand College, University of Delhi, Delhi	Drs. T. Ramamurthy, Amit Awasthi, Bhabatosh Das, and Prahanshu Tripathi
2	7th June 2018	Department of Biotechnology, Manav Rachna International Institute of Research and Studies, Faridabad	Drs. Gaurav Batra, Niraj Kumar, and Tarun Sharma
3	31st August 2018	Acharya Narendra Dev College, University of Delhi, Delhi	Drs. Nitya Wadhwa, Pallavi Kshetrapal, Shailaja Sopory, Suchitra Gopinath, and T. Ramachandran
4	14th September 2018	Shaheed Rajguru College of Applied Sciences for Women (SRCASW), University of Delhi, Delhi	Drs. Sanjay Banerjee, Samrat Chatterjee, Dinesh Mahajan, Shilpa Jamwal, and Sameena Khan
5	29th September 2018	Kirorimal College, University of Delhi, Delhi	Drs. Shailendra Asthana, Samrat Chatterjee, Renu Goel, and Ajay Kumar
6	25th September 2018	Maitreyi College, University of Delhi, Delhi	Drs. Susmita Chaudhuri, Tarun Kumar Sharma, and Chandresh Sharma
7	5th February 2019	Shaheed Rajguru College of Applied Sciences for Women (SRCASW), University of Delhi, Delhi	Drs. Guruprasad Medigeshi, Nisheeth Agarwal, Milan Surjit, Amit Pandey, Krishnamohan Atmakuri and, Sankar Bhattacharyya.

In the picture - Three students from Shaheed Rajguru College of Applied Sciences for Women who shadowed Dr. Sankar Bhattacharyya for the Shadow A Scientist program at THSTI.

An all women team from THSTI visited K. L. Mehta Dayanand College for Women, Faridabad to mark Science Day 2019 and the International Day for Women.

- 22 students from six colleges across NCR participated in a very short-term training program, *Shadow A Scientist*, initiated for colleges. Ten scientists with different expertise (clinical scientist, molecular virologist, computational biologist, molecular biologist) working in maternal and child health, drug discovery for non-communicable diseases and infectious disease themes were part of the first leg of these attachments.
- Students in the First Year of BSc (Nutrition and Dietetics) of the Faculty of Applied Sciences, Manav Rachna International University visited THSTI on 25th July 2018. They received a brief introduction about the institute's goal and research programs by Dr. T. Ramamurthy, National Chair, THSTI. What followed was a short interactive session with other members of the Science Setu committee. The students got to interact with Ph.D. students and technical staff as they were taken around to visit laboratories and facilities in the institute.
- A team of five young women researchers along with Dr. Pallavi Kshetrapal visited the K.L. Mehta Dayanand College for Women at Faridabad to mark National Science Day 2019 and delivered lectures to a gathering of 300 college students. This was the first girls-teaching-girls program under the *Science Setu* program of THSTI.

THSTI – NIPGR Open Day to mark India International Science Festival (IISF) 2018

THSTI hosted the second Open Day with NCR BSC partner National Institute of Plant Genome Research at THSTI on 28th September 2018 as a pre-event of the 4th India International Science Festival. Executive

Students from Manav Rachna International School, Faridabad at the THSTI- NIPGR Open Day 2018

Director of THSTI, Prof. Gagandeep Kang declared the institute open in her address to more than 200 students and faculty from two schools and five colleges from across NCR in her welcome speech. She introduced the young audience to THSTI underlining that the institute's mandate is to not only carry out basic research to avert public health challenges but translate this knowledge to transform the diagnostic and therapeutic landscape in biomedical research. Noted columnist and author of *Jal, Thal, Mal,* Shri Sopan Joshi was the chief guest for the occasion and delivered the popular talk in Hindi to mark the Open Day. The day's highlight was a tour around laboratories of THSTI, Small Animal Facility and Biorepository, intermittent with demonstrations, interactive sessions with scientists and poster presentations. A contingent from THSTI also represented the institute at the final event in Lucknow 5th to 8th October 2018. The institute also sponsored the visit of a student enrolled in the Bachelor's program of Acharya Narendra Dev College along with the team from THSTI.

CDSA and THSTI organized a meeting to discuss the Common Review Process for multicentre studies in India

CDSA and THSTI organized this meeting in collaboration with the ICMR on 6th August 2018 at THSTI, Faridabad. It was attended by senior clinical researchers, trialists, members of ethics committees, independent ethics experts from different institutions in the country and a representative from CDSCO. A common review process for ethical approval of multicentre studies and a pilot undertaken using the process was presented. The discussions focused on how the proposed common review process would apply to multicentre clinical trials. There was much debate on the roles and responsibilities of the ethics committee chosen to undertake the common review (designated ethics committee) and of the ethics committees of the participating sites. The general consensus was in support of a common review process as it was felt that it would enhance the quality of the ethical review. The final recommendations will be added as an appendix to the recently released ICMR National Ethical Guidelines for Biomedical and Health research involving human participants released in 2017.

THSTI hosts two days' workshop on Basic Course in Medical Education

A 2-day workshop was organized by THSTI to impart a basic course in medical education for scientists from THSTI and RCB at THSTI on 6th and 7th August 2018. Faculty members from AIIMS visited the institute and were speakers and moderators for the workshop. Sessions conducted were on Group dynamics, Systems approach to educational process; Teaching-learning process and adult learning; Educational objectives – Taxonomy & Domains; Framing objectives, competences; Active learning - Principles; Small group teaching; Interactive large group teaching & lesson planning; Educational technology, e-learning and blended learning; Introduction to Microteaching; Skills teaching and simulation; Teaching and assessing communication skills; Quality improvement in medical education.

THSTI organized the NCR Cluster Seminar Series

The November's NCR Cluster Seminar Series was organized by THSTI at the NCR BSC Auditorium on November 19, 2018. A series of three lectures were organized on the theme Diagnostics: Recent Advances and Perspectives:

Lecture 1: Diagnostics for blood-borne infections and tropical febrile illnesses by Dr. Gaurav Batra

Lecture 2: Artificial Intelligence and Early Diagnostic marker for Alzheimer's disease by Prof. Pravat Mandal

Lecture 3: Plant diagnostics: Opportunities and Challenges ahead.

The lectures were followed by a Disease Detective game that saw participation by teams of Ph.D. students from NII, NIPGR, ICGEB, RCB, and THSTI.

THSTI Organizes Its First High Content Screening Workshop with Support from GE Healthcare

A two-day long workshop on high content screening (HCS) and image analysis were organized by Dr. Shilpa Jamwal and her team for HCS users and researchers with support from GE healthcare and tecan/bioscreen on 27th and 28th November 2018. Hands-on sessions were conducted for designing experiments and image analysis in HCS platforms. Researchers from four institutes participated. The participants

expressed their satisfaction with the workshop design and shared their interest in attending the upcoming workshops in the series.

THSTI had the privilege to organize the 45th Annual Meeting of the Indian Immunology Society at its premises in the NCR BSC, Faridabad from 1st-3rd November 2018. The theme of Immunocon 2018 was Immunotherapy and Advances in Immunology. The inaugural session saw luminaries from the field of Immunology declaring open the three-day event with their experiences of being a part of the society. Four keynote lectures, 11 plenary lectures, lecture sessions on T cell differentiation, clinical immunology, infection and immunity, immune-regulation, innate immunity, poster and discussion sessions were all organized over the span of three days. The institute hosted speakers and participants from national and international organizations. A good footfall with about 500 participants was seen.

Meeting to explore collaborative opportunities in Arbovirus research convened at THSTI

Scientists from the Dept of Virology, Armed Forces

THSTI hosts Immunocon 2018 – 45th Annual Meeting of the Indian Immunology Society

Research Institute of Medical Sciences (AFRIMS), Bangkok, visited THSTI on 8th February 2019 to explore collaborative opportunities to develop vaccines and diagnostic devices for arboviral infections. They were joined by experts on arbovirus epidemiology, diagnostics, vaccines and clinical research from premier Indian institutes such as National Institute of Virology, Indian Institute of Science, Christian Medical College, Bharati Vidyapeeth, International Centre for Genetic Engineering and Biotechnology and National Centre for Biological Sciences.

Other Events:

THSTI Celebrates Swachhata Pakhwada

THSTI is observing the Swachhata Pakhwada from 07th to 11th May 2018. The event began with a Swachhta pledge lead by the Head of Administration, Mr. M. V. Santo. This was followed by an extempore competition which saw participation across students, scientists and technical staff eloquently expressing their views on the significance and pertinence of cleanliness in general and at scientific organizations in particular.

NCR Biotech Science Cluster (BSC) observed the International Day of Yoga

THSTI and RCB observed the 4th International Yoga Day on June 21, 2018, at the NCR Biotech Science Cluster, Faridabad. The event received enthusiastic participation of people from the two institutes who practiced various *asanas* and meditation during the hour-long session.

THSTI celebrated the 9th Annual Foundation Day

As the institute completed its 9th year, the THSTI fraternity was joined by the Secretary, DBT, Dr. Renu Swaroop, Prof. Anil K. Gupta, Founder of Honey Bee Network and a renowned advocate of grassroots innovations along with dignitaries from DBT and other institutes to commemorate the Foundation Day. Prof. Gagandeep Kang, the current Executive Director ushered in the celebrations by briefing the audience about the institute's strengths, the proposed reorganization of THSTI into its original cabinet-approved structure, achievements in the past one year and where we see our self at different time points over the next 10 years while also emphasizing on nurturing partnerships within and beyond the cluster to accomplish our goals. The Foundation Day Address by Prof. Gupta educated the audience about 'a new grammar for translational research' to let context guide the content of our research, the significance of collaborations and involvement of end-users of research in its design. Secretary, DBT in her Presidential Address congratulated the institute on its efforts of knowledge generation through basic research and moving towards product development, high-quality students' output, remarkable national and international partnerships and emphasized the induction of entrepreneurial ventures and importance of collaborations to take the institute forward. She endorsed THSTI's efforts in putting in place a governance mechanism and modifying its policy to solidify and continue academia-industry collaborations.

Meeting to explore the role of Academia-led Clinical Trial Units (CTU) in India

CDSA and THSTI organized a meeting in partnership with the MRC Clinical Trials Unit of University College London on 23rd July 2018 at THSTI. In attendance were senior clinical researchers and trialists affiliated to 18 leading academic institutions in the country. It explored the need in India of Academic Clinical Research Units (A-CRUs) modeled on the MRC CTU at UCL that would collaborate with academic researchers and SMEs/industry to deliver a programmeof high-quality clinical research, particularly multicentre clinical trials/studies that specifically address the challenges of healthcare in India. The A-CRUs would support all aspects of design, conduct, close-out, analysis and publication of trials/studies.

NCR BSC organized a plantation drive

A plantation drive was inaugurated by Shri Amitabh Singh Dhillon (IPS), Commissioner of Police, Faridabad at the NCR - Biotech Science Cluster campus. The plantation drive was initiated to maintain an ecological balance in the campus that occupies a part of an area sprawling over 200 acres. Prof. Gagandeep Kang, Executive Director, THSTI and Shri Dhillon addressed the crowd emphasizing the significance of the plantation and encouraged plantation for a sustainable co-existence of the campus with the environment.

NCR Biotech Science Cluster observes Swachhta hi Sewa campaign

THSTI and RCB spearheaded the Swachhta hi Sewa campaign of NCR Biotech Science Cluster at Sainik Colony of Faridabad on 3rd October 2018. Students, scientists and other staff from both the institutes came together rallying in the locality and distributing pamphlets door-to-door to drive home the message - 'Sanitation is everyone's business.' Street play artistes from Delhi joined the group from NCR-BSC to perform a *Nukkad Natak* as part of the awareness campaign.

Training session on Fire Safety at NCR Biotech Cluster

A training session in fire safety was organized with evacuation mock drills, mock drills for fire-fighting along with a presentation of Fire Fighting systems by Fire Officer of Haryana Fire Services and some agencies. The session was attended by staff and students of THSTI.

THSTI IN MEDIA

IndCEPI and GARBH-Ini among 5 key missions announced by the Union Minister for Science & Technology on DBT's Foundation Day

The **Union Minister for Science & Technology** announced key missions at the foundation day ceremony of DBT including Atal JaiAnusandhan Biotech Mission - Undertaking Nationally Relevant Technology Innovation (**UNaTI**), which is expected to transform Health, Agriculture and Energy sectors during the next five years. This mission includes two of our collaborative ventures - GARBH-Ini and **IndCEPI**. While **GARBH-Ini** is a mission to promote maternal and child health and develop prediction tools for preterm birth, **IndCEPI** is aimed to develop affordable vaccines for endemic diseases. DBT celebrated its 33rd Foundation Day in New Delhi with "*Celebrating Biotechnology: Building Indian as an Innovation Nation*" as their theme.

The announcement of the Press Information Bureau can be found here:

http://pib.nic.in/newsite/PrintRelease.aspx?relid=188951

THSTI's zinc study covered by the Delhi edition of Indian Express

The Delhi edition of the popular daily The Indian Express covered THSTI's study that is evaluating zinc as an adjunct for the treatment of very severe infection in infants. The multi-center and multi-country study is being carried out in collaboration with the University of Bergen, Norway and Tribhuvan University, Nepal and is led by Prof. Shinjini Bhatnagar and Dr. Nitya Wadhwa who are the Principal Investigators of the project. "*Our previous study showed significant effects of zinc in reducing the risk of treatment failure… our current study in question is whether adjunct treatment with zinc will reduce the risk of death in infants with clinical severe infection*," the daily quoted Prof. Bhatnagar.

Dr. Wadhwa went on to explain the methodology - "*The infant, once enrolled, is administered either zinc or placebo for 14 days with the standard treatment. The infant is followed up every 6 hours for signs of recovery or treatment failure. After discharge, the infant continues to be followed up for 12 weeks to measure effects of zinc on 12-week morbidity and mortality*".

https://indianexpress.com/article/lifestyle/health/new-study-hopes-to-understand-if-zinc-can-cut-infant-mortality-5496502/

Development of an Aptamer-based TB Meningitis Test by THSTI Faculty and an Entrepreneur Dr. Tarun Sharma

An aptamer-based diagnostic test for TB meningitis has been patented by AIIMS and THSTI and licensed to AptaBharat Innovation Pvt Limited, a THSTI start-up founded by Dr. Tarun Sharma. A diagnostic test for TB meningitis (the most severe form of TB) with nearly 100% sensitivity and about 91% specificity has been developed by a multi-institutional team led by Prof. Jaya Sivaswami Tyagi (Department of Biotechnology at AIIMS) and Dr. Tarun Kumar Sharma (THSTI) in collaboration with RML Hospital, IIT Indore and UTU, Uttarakhand. A rapid, point-of-care diagnostic test for TB meningitis that uses the DNA aptamer has already been adapted to a sensor format and is being evaluated in clinical samples. "*While antibodies have to be generated in animals and so will not be of uniform quality, aptamers can be produced in the lab*", the newspaper quoted Dr. Tarun on the advantage of using aptamers. https://www.thehindu.com/sci-tech/science/aiims-led-team-develops-highly-sensitive-portable-test-for-tb-meningitis/article24541516.ece

The Indian Express highlights THSTIs efforts towards HIV vaccine design

The HIV Vaccine Translational Research (HVTR) Laboratory at THSTI, in collaboration with the International AIDS Vaccine Initiative (IAVI), a global non-profit, is steering efforts to design a preventive

vaccine against HIV. "*Where prevention is concerned, this is one of the cleverest viruses we have ever seen. It can develop so rapidly that it can get past any immune defenses. It is a virus that attacks the immune system, so it needs to figure out most quickly how to protect itself from the immune system and it does that by rapidly mutating. This is why this research is so important*," said Prof Gagandeep Kang, Executive Director, THSTI, while speaking to the Indian Express. The laboratory aims to develop a vaccine that would elicit an immune response capable of neutralizing a broad spectrum of HIV immunogens. Dr. Jayanta Bhattacharya, Principal Investigator at the laboratory explained that there are individuals who make antibodies that can kill a wide array of viruses circulating globally. Work is underway to identify such individuals to develop the antibodies.

https://indianexpress.com/article/cities/delhi/at-a-lab-in-faridabad-efforts-to-develop-a-vaccine-for-hiv-5324951/lite/

Research on antibiotic resistance at THSTI receives wide media coverage

Antibiotic resistance is emerging as a major health challenge. Dr. Bhabatosh Das's research team has found that friendly gut bacteria are a reservoir of drug-resistant genes which when transferred to disease-causing bacteria may make the disease untreatable. "*It was surprising to see that friendly gut bacteria fostered resistance traits. This unexplored arm acts as a huge potential source of antibiotic resistance dissemination. We are currently working to resensitize these bacteria and make them antibiotic-sensitive*," said Dr. Das.

https://www.thehindu.com/sci-tech/health/a-nursery-of-drug-resistance/article24782373.ece

HupB-binding aptamer inhibits TB bacteria entry into host cells: A study by Dr. Tarun Sharma highlighted in The Hindu and Science Chronicle

In collaboration with researchers at AIIMS, Dr. Tarun Sharma's group found that aptamer-based inhibitors bind to HupB protein found on the bacterial cell surface, significantly inhibiting its entry into host cells. "*Compared with controls, the aptamer-treated bacteria showed reduced ability to enter the host cells. At 55%, the HupB-13T aptamer had a greater ability to inhibit TB bacteria entry than the HupB-4T (42%) aptamer. So, targeting the HupB protein using aptamer-based inhibitors can effectively block TB infection and will be effective in both drug-sensitive and drug-resistant TB patients*." the newspaper quoted Dr. Sharma.

https://journosdiary.com/2018/09/08/aptamer-inhibitor-tb-bacteria-into-cells/
https://www.thehindu.com/sci-tech/science/aptamer-inhibits-tb-bacteria-entry-into-cells/article24903152.ece

THSTI COMMITTEES

Serial No.	Committee	Members
1	Scientific Advisory Committee	Dr. Partha Majumder Dr. Raghavan Varadarajan Dr. Rakesh Gokhale Dr. Ashok Venkitaraman Prof. Judi Allen Dr. Sujata Srinivasan
2	THSTI Management Committee	Executive Director and Heads of all the centers **Chairperson - Executive Director**
3	Finance Committee	Financial Advisor, DBT Executive Director, THSTI Deputy Secretary (Finance), DBT Advisor/Scientist G, DBT, and Scientific Coordinator, THSTI Executive Director, RCB Dr. B. Ravindran, Emeritus Professor, Institute of Life Sciences Dean, THSTI Administrative Officer (Finance and Accounts), THSTI Head – Administration, THSTI
4	Maintenance Committee	Dr. Ramandeep Singh Dr. Bhabatosh Das Dr. Uma Chandra Mouli Natchu Dr. Niraj Kumar Dr. Shailendra Asthana Mr. G. R. Agarwal Mr. Vishal Gupta Mr. Narender Sharma Mr. C. B. Yadav **Chairperson – Dr. Ramandeep Singh/Dr. Bhabatosh Das**
5	Purchase Committee	Dr. Nisheeth Agarwal Dr. Sanjay Banerjee Dr. Amit Awasthi Dr. Gaurav Batra Dr. Shailaja Sopory Mr. Manoj Kumar Mr. C. B. Yadav **Chairperson – Dr. Nisheeth Agarwal/Dr. Sanjay Banerjee**
6	IT and Communications Committee	Dr. Guruprasad Medigeshi Mr. M. V. Santo Dr. Samrat Chatterjee Dr. Amit Yadav Mr. G. R. Agarwal Mr. Tushar Sharma **Chairperson – Dr. Guruprasad Medigeshi/ Mr. M. V. Santo**

Serial No.	Committee	Members
7	Institutional Ethics Committee – Human Research	Prof. Satinder Aneja Prof. Subir Kumar Maulik Dr. Ujjayini Ray Mr. Munawwar Naseem Ms. Jasmine Singh Ms. Vidhya Krishnamoorthy Mr. D. Raghunandan Dr. Ashutosh Tiwari Dr. Suvasini Sharma Dr. Sarmila Mazumder Dr. Tarun Batra Dr. Rajiv Janardhanan Dr. Sivaram Mylavarapu **Member Secretary – Ms. Vidhya Krishnamoorthy**
8	Institutional Ethics Committee – Animal Research	Dr. Sudhanshu Vrati Dr. Niraj Kumar Dr. Krishnamohan Atmakuri Dr. Amit Awasthi Dr. Amit Pandey Shri. M. T. Sambandam Mr. Ranvir Parashar Prof. Harbans Lal Dr. J. P. Mittal **Chairperson - Dr. Sudhanshu Vrati**
9	Institutional Committee for Stem Cell Research	Prof. Narinder K. Mehra Dr. Sujata Mohanty Dr. Ujjayini Ray Mr. Munawwar Naseem Dr. Prasenjit Guchhait Dr. Shailaja Sopory Prof. Nalin Mehta **Chairperson - Prof. Narinder K. Mehra**
10	Biosafety Committee	Dr. Nisheeth Agarwal Dr. Susmita Chaudhuri Dr. Shailaja Sopory Dr. Vinay Kumar Nandicoori Dr. Uma Chandra Mouli Natchu Dr. Anirban Basu **Chairperson – Dr. Nisheeth Agarwal**
11	Academic Committee	Dr. Guruprasad Medigeshi Dr. T. Ramamurthy Dr. Uma Chandra Mouli Natchu Dr. Manjula Kalia Dr. Amit Awasthi Dr. Samrat Chatterjee Mr. Joby Cyriac **Chairperson - Dr. Guruprasad Medigeshi**

Serial No.	Committee	Members
12	RTI Act Committee	Dr. Krishnamohan Atmakuri – Public Information Officer Prof. Shinjini Bhatnagar – Appellate Authority Mr. M. V. Santo **Executive Director – Public Authority**
13	Internal Complaints Committee	Prof. Shinjini Bhatnagar Dr. Nita Bhandari Dr. Manjula Kalia Dr. Monika Bahl Ms. Amandeep Kaur Ahuja (external member) Dr. Shobha Broor (external member) Mr. M. V. Santo **Chairperson - Prof. Shinjini Bhatnagar**
14	Student Welfare and Hostel Committee	Dr. Amit Pandey Dr. Nitya Wadhwa Dr. Sankar Bhattacharya Dr. Sucheta Kurundkar Mr. M. V. Santo Two student representatives **Chairperson – Dr. Amit Pandey/Dr. Nitya Wadhwa**
15	Tender Opening Committee	Mr. Satish Kumar Mr. Alok Kumar Gupta Mr. Abhishek Sharma
16	Building Committee	Dr. V. S. Chauhan Executive Director, THSTI Executive Director, RCB Director, NII Director, NIPGR Director, NBRC Dean, Clinical Research, THSTI Dr. Alka Sharma, Advisor, DBT Mr. Shrikumar Suryanarayan, Director-General, Association of Biotechnology Led Enterprises Dr. Partha Majumder, NIBMG **Chairman – Dr. V. S. Chauhan**
17	Grievance Redressal Committee	Dr. Chandrashekar Dr. Niraj Kumar Mr. M. V. Santo – Member and Nodal Officer – SC/ST **Chairperson - Dr. Chandrashekar**
18	Vigilance Officer	Dr. Guruprasad Medigeshi

CHAIR AND HONORARY FACULTY

Biotechnology Chair
Prof. John David Clemens, Professor,
Department of Epidemiology,
Founding Director, Centre for Global Infectious Diseases, UCLA School of Public Health, California

National Chair

Dr. T. Ramamurthy
THSTI, Faridabad

Dr. Madhu Dikshit
THSTI, Faridabad

Visiting Professor of Eminence

Prof. N. K. Ganguly
Former Director-General,
Indian Council of Medical Research, Delhi
Honorary International Visiting Faculty

Dr. Madhukar Pai
Associate Professor, McGill University, Canada
Associate Director, McGill International TB Centre, Canada

Prof. Salman Azhar
Associate Director of Research,
Geriatric Research Education and Clinical Centre (GRECC), USA

ADJUNCT FACULTY/ HONORARY VISITING PROFESSOR

Dr. Satyajit Rath
Agharkar Chair,
Agharkar Research Institute, Pune

Dr. Vineeta Bal
Visiting Faculty, Biology Division,
Indian Institute of Science Education and Research, Pune

Prof. Anil K. Tyagi
Vice-Chancellor, Guru Gobind Singh Indraprastha University, Dwarka

Dr. Navin Khanna
Group Leader, International Centre for Genetic Engineering and Biotechnology, Delhi

Dr. Nita Bhandari
Director, CHRD-Society for Applied Studies, Delhi

Dr. Amit Sharma
Group Leader,
International Centre for Genetic Engineering and Biotechnology, Delhi

Dr. Jaya Sivaswami Tyagi
Professor, Department of Biotechnology,
All India Institute of Medical Sciences, Delhi

Dr. Partha Majumder
Professor, National Institute of Biomedical Genomics, Kolkata

Dr. Ankur Mutreja
Senior University Lecturer, Department of Medicine, University of Cambridge, United Kingdom

Dr. Ranjith Kumar C.T.
Associate Professor, University School of Biotechnology, Guru Gobind Singh Indraprastha University, Delhi

Dr. Suchitra Devi Gopinath
Innovative Young Biotechnologist Award fellow, THSTI

Dr. Amit Singhal
Principal Investigator, Singapore Immunology Network, Singapore

Dr. Harshpal Singh Sachdev
Senior Consultant, Pediatrics and Clinical Epidemiology, Sitaram Bhartia Institute of Science and Research, Delhi

Dr. Usha Menon
Professor, Group Leader, Professor of Gynaecological Oncology, MRC Clinical Trials Unit, University College London, UK

Dr. Sagarika Halder
Assistant Professor, Department of Experimental Medicine and Biotechnology,
Postgraduate Institute of Medical Education and Research, Chandigarh

Dr. Anura Vishwanath Kurpad
Professor & Head of Physiology,
St. John's Medical College,
St. John's National Academy of Health Sciences, Bangalore

Dr. Jonathan D. Pillai
Jiva Sciences Pvt. Ltd.,
Center for Cellular and Molecular Platforms,
NCBS Campus, Bangalore

Dr. Ravindra Mohan Pandey
Professor and Head, Department of Biostatics,
AIIMS, New Delhi

Dr. UC Mouli Natchu
Associate Professor, Division of Infectious Disease,
St. John's Research Institute, Bangalore

Dr. Subhash Vinayak Kapre
Chief Executive Officer, Inventprise LLC, USA

Dr. Syed Khalid Ali
Associate Director, Clinical R&D, MSD-Wellcome Trust Hilleman Laboratories, New Delhi

Dr. Arup Banerjee
Associate Professor, Regional Centre for Biotechnology, NCR BSC, Faridabad

Dr. Manjula Kalia
Associate Professor, Regional Centre for Biotechnology, NCR BSC, Faridabad

SEMINARS AND MEETINGS

Date	Title	Speaker
06-04-2018	Vitamin D signaling in skeletal muscle mass maintenance	Dr. Suchitra Devi Gopinath
13-04-2018	Understanding foraging and feeding preferences in insects using *Drosophila melanogaster*	Dr. Pinky Kain Sharma, RCB
20-04-2018	Curtailing viral infections; T cell immunity or neutralizing antibodies?	Dr. Huma Qureshi, THSTI
23-04-2018	T and B cell signatures in preclinical islet transplant studies in non-human primate preclinical models	Dr. Amar Singh, Schulze Diabetes Institute, Department of Surgery, University of Minnesota
27-04-2018	Molecular insights into antimicrobial resistance of human gut microbiota	Dr. Bhabatosh Das, THSTI
11-05-2018	Proteostasis mechanism in neurodegenerative disorders	Dr. Tushar K. Maiti, RCB
25-05-2018	Time-resolved crystallography provides snapshots of the DNA synthesis reaction	Dr. Deepak T. Nair, RCB
22-06-2018	Deep interrogation in biology and disease: A Mass Cytometric approach	Dr. Amit Bhattacharya, Consultant Scientist, Premas Life Sciences Pvt. Ltd.
29-06-2018	Celebrating fifty years of science in India: Passion & Profession	Dr. Eswaran, RCB
06-07-2018	Rapid pathogen identification and antimicrobial susceptibility testing	Dr. Niraj Kumar, THSTI
30-07-2018	Bioentrepreneurship	Ms. Shreya Malik, Deputy Manager, BCIL and Ms. Aditi Kumar, BIG Programme Manager, Bio-Incubator, IIT Kanpur
03-08-2018	The role of unfolded protein response in flavivirus replication	Dr. Sankar Bhattacharyya, THSTI
30-08-2018	National Liver Disease Biobank services	NA
30-08-2018	How multiplex technologies promote biomarker discoveries?	Nilangi H Andurlekar, Product Manager for multiplexing and SMCxPro immunoassay system, Merck

Date	Title	Speaker
06-09-2018	Talks on Vaccine Research	NA
12-09-2018	Patenting Biotechnology Inventions - Hurdles and Solutions	Dr. Sharana Gouda, Asst. Controller of Patents & Designs, Head, Biotechnology Group, The Patent Office, Chennai,
26-10-2018	Metabolomics analysis for biomarker discovery	Dr. Yashwant Kumar, THSTI
11-03-2019	Planetary Health – linkages between global environmental change and human health	Prof Sir Andrew Paul Haines, Environmental Change and Public Health, London School of Hygiene and Tropical Medicine (LSHTM), Visiting Springer Nature Professor, Indian Academy of Sciences
13-03-2019	ClinEpiDB - A new platform for exploring clinical and epidemiological datasets	Dr. David S. Roos, E. Otis Kendall Professor of Biology, University of Pennsylvania School of Arts and Sciences

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