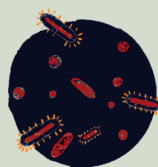
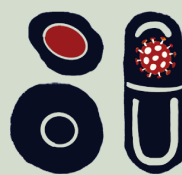


ANNUAL REPORT 2019-2020



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.



ट्रांसलेशनल स्वास्थ्य विज्ञान
एवं प्रौद्योगिकी संस्थान

TRANSLATIONAL HEALTH SCIENCE
AND TECHNOLOGY INSTITUTE

THSTI ANNUAL REPORT 2019-2020

The cover page of this Annual Report is largely inspired from the linocut graphics and effects. Linocut is a relief printmaking process that is like woodcutting. In this variation of woodcut, the designer draws an image on the surface of the wood block, and the negative space in a design is the cut way, leaving a raised image that can be coated with ink and stamped on paper and other surfaces. Linocut derived its name from the surface it used- linoleum! Because they are hand-cut and inked, linocuts often have a soft and vintage look.

In the current issue of THSTI's Annual Report, visual science communicator, Dr. Lipsa Pandahas tried to mimic the linocut through digital ways instead of the traditional ways and has introduced the concept of representation of 'subjects' with simple and geometric shapes. The blue and white prints in the thematic pages recreate the linocut style. She has also introduced the modern design concepts of the Japanese style by adding the elements such as dots, lines, and triangles. These components together tie the composition and reflect the essential elements of the thematic area of the research done by various groups at THSTI. Similarly, the cover page has assembled the components from each thematic area and represented in similar design style which captures both rustic and modern design mimicking the ideology of THSTI which embarks its position in translational research and yet ensuring traditional ethics and principle of research.

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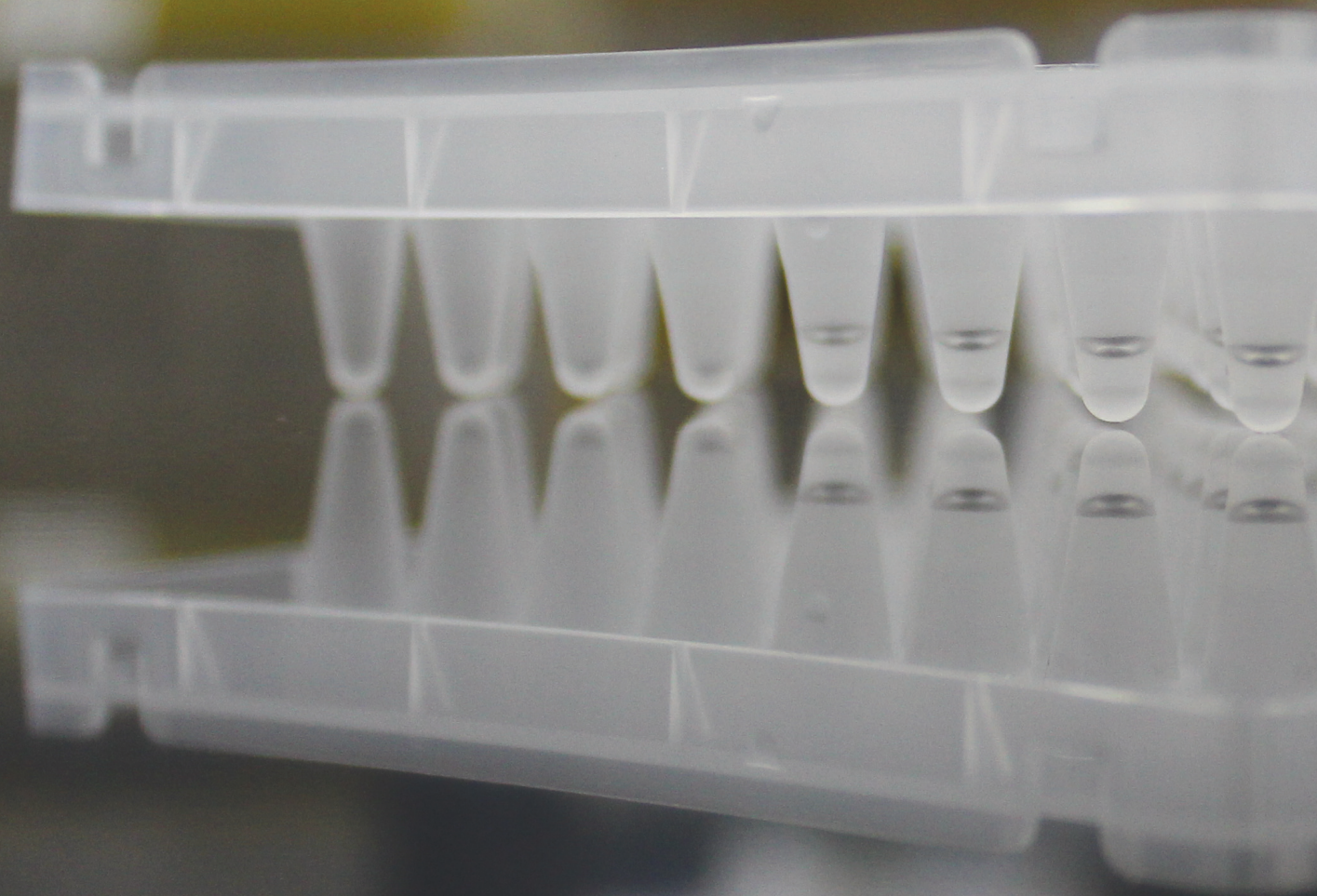
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THSTI SOCIETY

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Secretary, DBT



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DG, ICMR, New Delhi



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Additional Secretary and
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Prof. Gagandeep Kang
Executive Director,
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Dr. Amulya Panda
Director, NII,
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Dr. Jyoti Malik Logani
Scientist-E -DBT &
Nodal Officer, THSTI

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Managing Director,
ENDIYA Partners,
Hyderabad

FROM THE EXECUTIVE DIRECTOR

On January 26, 2020, Professor Maharaj Kishan Bhan, who conceived and developed the Translational Health Science and Technology Institute (THSTI) passed away. The community of THSTI lost its founder, mentor and its most enthusiastic champion. The interdisciplinarity that underpins how THSTI works to be a model translational health institute is his legacy. To honour his memory the institute launched an award to celebrate and reward interdisciplinary collaborations to pursue important goals in public health.



THSTI completed a decade as an Autonomous Institute of the Department of Biotechnology in 2019. The reorganization into a theme-based structure with four strategically prioritized thematic programmes complemented by four core facilities under the Translational Research Program (TRP) funded by DBT was implemented. We now hope for further expansion as the Governing Body has approved the proposal to begin Phase-3 construction work for the expansion of research and development activities.

Under the thematic programmes, we are delighted to welcome Dr. Anna George as Professor Eminence and a mentor for the large and diverse team in infection and immunology. With the funding support through DBT's IndCEPI mission, we are equipping ourselves to strengthen our epidemic preparedness and make India globally competitive when it comes to vaccine development. Our bioassay laboratory is growing as a platform for clinical development of vaccines and biologicals and building partnerships globally. Building the ferret facility for research on respiratory illnesses and BSL3 facility also gained support from the mission. The COVID-19 pandemic is an opportunity for THSTI to prove itself as a supporter of the diagnostics and vaccine ecosystem and we have begun efforts that will show our capabilities in the coming year.

While THSTI works through its faculty and collaborations in India, international benchmarking is one route to ensuring that our programmes are world-class and that we can contribute as widely as possible to public health research, to product development, relevant to the times. A case in point was an MoU with the Foundation for Innovative New Diagnostics (FIND), where we established a framework under which FIND and THSTI will jointly undertake projects in diagnostics. This agreement went on to be a very productive relationship when SARS-CoV2 arrived, because we were able to initiate a programme for validation of kits and assays for FIND, which supports WHO pre-qualification of these made-in-India products. We will describe next year the work done by THSTI researchers on COVID-19 assays, but at the end of the 2019-2020,

we are well on the way to significant collaborations with industry and international partners. In the areas where THSTI has unique capabilities, the development of aptamer-based assays to differentiate wet and dry snake bites is progressing well and we hope to see good results as the problem remains a critical one for India.

The GARBH-INI cohort that has been following pregnant women to develop early interventions for preterm birth since 2015, has reached its target of recruiting 8000 women and is now ready to focus on greater laboratory investigations that will hold the key to develop novel biomarkers and interventions to prevent children from being born too soon. Another important intervention that was taken up this year was immediate Kangaroo Mother Care (KMC). iKMC was evaluated in extremely low birth weight babies. A randomized controlled trial showed an improvement in survival and the plan is to communicate this and develop facilities for iKMC in prenatal care centres across the country. The maternal and child health program's drive to develop new, innovative tools resulted in the establishment of the Aryabhatta Data science and AI program (ADAPT). The program continues to exceed our expectations of a model translational programme in the able hands of Prof. Shinjini Bhatnagar.

In the area of non-communicable diseases, with Dr. Madhu Dikshit's focus on building strategic partnerships with industry, we joined hands with Jiva Ayurveda to utilize what AYUSH can teach us about non-alcoholic fatty liver disease (NAFLD) and use this knowledge to develop solutions for this disease that affects a sizable proportion of people in India. Our partnership with Dabur India Ltd. that we embarked on last year has started paying dividends as we are making progress towards having a therapeutic formulation for NAFLD.

In our effort to build a clinical research ecosystem in India, CDSA developed a clinical trial toolkit to help researchers who plan to conduct clinical trials in India. This toolkit was launched during the first DBT-BIRAC's Global Bio-India event in 2019. The Regulatory Science and Policy Unit also helped to set up the India Volunteer Infection Research Consortium with scientists, experts on ethics and others to formulate guidelines and a white paper for CHIM studies in India. The white paper was released at a symposium on the sidelines of the Ministry of Health and Family Welfare and WHO organised 3rd International Meeting on Access to Medical Products.

We have identified and are developing four support systems at THSTI as core facilities that serve not just the institution but also the NCR Biotech Science Cluster, academic institutions and industry. The Biorepository now houses over a million specimens and has infrastructure and processes that meet global standards. The Data Management core is supporting studies in maternal and child health and more. A recent study that extends THSTI's reach into the community is an interventional study for addressing vaccine knowledge and hesitancy among ASHA workers in Haryana, for which the data management staff went beyond the call of duty to support our partners. The Bioassay Laboratory is well on its way to accreditation which will be a landmark achievement for all the staff who have had a steep learning curve and invested a lot of effort, as well as for the institution. The Small Animal Facility is functional and increasingly used,

but we hope in the coming year to be able to upgrade the facility so that it can match world class facilities and work towards international accreditation. The preparedness of our core facilities is something that has paid off as THSTI became a key centre for clinical and translational research for DBT against COVID-19.

This past year we have had many important visitors. Dr. Trevor Mundel, President (Global Health), Gates Foundation and Prof. K. VijayRaghavan, Principal Scientific Advisor to the Government of India were among those who honoured us by visiting the campus and engaging with our scientists and students. We were fortunate to have Bharat Ratna Prof. C.N.R. Rao to deliver THSTI's Foundation Day address.

The administration and the research teams were faced with the enormous challenge of a reorganization last year and have new recruitments to conduct this year. By functioning as teams, we have been able to build core strengths in our faculty, complemented by Professors of Eminence who are looking after the institution and providing senior leadership. This has ensured that our young people grow and take up different areas of responsibility.

Our outreach programmes for schools and colleges by our teams' participation in science festivals and college visits continued in addition to research activities.

We have explored new ways of doing science, bringing in partners in India and across the world in the last ten years. To flourish in the next ten years, we have to take up new challenges, keep learning, create opportunities and build capacity to be able to continue to offer solutions for public health in India.

Gagandeep Kang

Executive Director

SUMMARY OF ANNUAL REPORT 2019-20

Research Highlights

Maternal and Child Health

- Observational studies to develop novel biomarkers and interventions to facilitate the translational pipeline conducted. GARBH-INI cohort enrolled 7878 participants into the cohort as on March 22, 2020. First trimester dating formula (called Garbhini-1) developed found to be more sensitive and accurate than global formulae.
- Analyses showed that maternal body mass index and biomass fuel use and/passive smoking has higher risk for preterm birth. GARBH-INI has been invited to join the Multi Omics of Maternal & Infants Global Consortium (MOMI) where multi omics signatures will be identified in over 1500 mother infant dyads pooled across five global cohorts.
- Around 2500 infants have been enrolled across seven hospitals in the ongoing RCT evaluating efficacy of oral zinc in young infant sepsis for reducing case fatality and subsequent 12-week mortality; 12-week overall risk of death is 8%.
- Immediate KMC was evaluated in very low birth weight new borns in an RCT for improving survival in the first month of life in 4000 babies enrolled across five countries.
- The Aryabhatta Data science and AI Program at THSTI (ADAPT) was established and follows the IIT Madras MoU on data analytics.

Infection and Immunity

- With funding support from IndCEPI, THSTI will provide resources/reagents for vaccine

development, establish a ferret facility, the first one in the country and a CHIM study to advance novel vaccine candidates to Phase I and Phase IIa clinical trial in partnership with CMC, Vellore.

- Host-pathogen interactions in Dengue - RSV infection modulates zinc homeostasis by enhancing intracellular labile zinc pools, Development of AG 129 mouse model, established the Dengue structural E protein platform to support antibody-based therapeutic discovery and development.
- The TB repository created plasmid constructs for >150 targets along with many knockdown strains of proven efficacy. Researchers at NII, Delhi, NIPER, Mohali, CSIR-IMTECH, Chandigarh, and NCBS, Bengaluru, among others, have availed these.
- Screening for anti-mycobacterial agents revealed NU-6027 as a good candidate and may be optimized further for the development of anti-mycobacterial agents.

Multidisciplinary Clinical and Translational Research

- The team reported development of whole blood compatible UCNP-based LFA for the detection of HBsAg and anti-HCV antibodies. The UCNP-LFA is superior in terms of analytical sensitivity as well as clinical sensitivity compared to the commercial LFA for HBsAg.
- In-house anti-NS1 monoclonal antibodies used to generate dengue NS1 dipstick assay with an analytical sensitivity at least three times higher than the commercial POCTs for each DENV serotype.

- A panel of aptamers developed against crude venom of *Bungarus caeruleus* (Indian Krait). Both aptamers (K6 and K8) can detect as low as 2 ng venom in the serum background.

Non-Communicable Diseases

- Under a collaborative agreement with Jiva Ayurveda, THSTI will work to understand non-alcoholic fatty liver disease (NAFLD) in Indian patients. The intent is to create a scientific bridge and understanding between “AYUSH” and “Modern Science” for NAFLD by collecting

information on disease phenotype in Indian population across the geography.

- Standardization and validation of *in vitro* and animal models of (NAFLD), to identify therapeutic leads: THSTI's collaboration with Dabur India Ltd has embarked on developing a therapeutic formulation for NAFLD.
- Identification of stage-specific molecular signature for diagnosis/ prognosis of dyslipidemia, inflammation and fibrosis associated with NAFLD.

Bioassay Laboratory

Technical SOPs, quality management protocols in place by December 2019.

Generated an income close to INR 7,00,000.00 by providing assay services to renowned companies/institutes.

Only DBT AI to be designated COVID-19 testing laboratory in Haryana.

Small Animal Facility

THSTI initiated the process of establishing a ferret holding and experimentation facility in the to support research on respiratory viruses like influenza.

Infectious Disease Research Facility (IDRF) was made functional as a specialized animal biosafety level III containment facility for TB and HIV research

Biorepository

Recognized as one of the five “National Bio-Resource Centres for COVID-19” by Department of Biotechnology, Government of India.

As directed by NITI-Aayog and DBT, the team participated in developing policy guidelines for sharing biospecimen and data for research related to COVID-19.

Data Management Centre & Data Science Hub

Active participant in the Computer-Assisted Low-Cost UltraSound (CALOPUS) project with the University of Oxford. Big data management, cutting-edge analytical approaches using artificial intelligence and machine learning.

Experience in clinical data management placed the DMC in the best position to take up the challenge of setting up clinical studies in response to COVID-19 pandemic.

- Ninety-two publications (including eight book chapters) were reported during 2019-20. THSTI granted a patent in January 2020 for a method for preparing a chitosan polymer for drug delivery. Fifteen patents have been filed in 2019-20. Thirty-six projects were funded both by national and international funding agencies.
- Two online e-learning courses on Good Clinical Practice and Medical Devices, co-developed with CDSCO and CDSA launched and conducted. The Regulatory Science and Policy Unit (RSPU) established at CDSA has set up the India Volunteer Infection Research Consortium (IVIRC) which comprises of scientists, ethicists and representatives from WHO and CDSCO to formulate draft guidelines and a white paper for CHIM studies India.

CDSA

- Building the Clinical Research Ecosystem in India - A clinical trials toolkit to guide researchers who are interested in conducting clinical trials in India was launched in November 2019 during DBT-BIRAC's Global Bio-India event in 2019.
- CDSA supported the launch of ICMR TB vaccine trial for healthy household contacts of TB patients. This is the first-ever government led vaccine trial after the BCG vaccine trial that was undertaken decades ago. After a detailed landscape analysis of the available vaccine candidates, two potential vaccines were shortlisted for an ICMR led Phase III trial of

12,000 healthy household contacts of sputum smear positive TB patients. Participants will be recruited from twelve sites in six states—Delhi, Karnataka, Maharashtra, Orissa, Tamil Nadu and Telangana.

Academics

Fifty-seven students are enrolled for THSTI's doctoral program as on March 2020. Ten new students joined in 2019-20 and nine were awarded their doctorate degrees. Fourteen postdoctoral fellows joined. Forty-three students from different

UG colleges were trained in the Short-Term Training Program.

Employee welfare

To recognize exceptional research work and contribution to the overall development of the institute, THSTI instituted awards by creating an endowment deposit supported through the extramural non-governmental funds including Dr. M.K. Bhan Group Award for the most impactful collaboration in memory of Dr. M. K. Bhan. These awards will be distributed every year during the Foundation Day celebrations.

S. No	Head	Op. Balance	Re-appropriation	Receipt	Expenditure	Balance
1	GIA Manpower	160.80	0.00	750.00	783.10	127.70
2	GIA General	-7.64	0.00	1950.00	1942.36	0.00
3	GIA Capital	-55.65	0.00	2750.00	2689.85	4.50
	Total	97.51	0.00	5450.00	5415.31	132.20

The Finance section has adopted various digital methods for payment disbursements/collections in order to avoid the cash transactions. During the FY 2019-20, the institute has generated internal revenue to the tune of Rs 183.86 lakhs and also received the non-governmental funds from abroad of Rs 1295.16 lakhs in addition to the Govt. of India funding, as shown above.

Important events

- Second edition of THSTI's Immunology course from 1st to 3rd April 2019
- THSTI's tenth foundation day Celebrations marked by Prof. CNR Rao's talk, Presidential Address by DBT Secretary and outreach event
- THSTI and AIIMS, Delhi co-organize One-Day symposium on NAFLD therapeutics and diagnostics
- Fifth collaborators meeting of the Rotavirus Vaccine Impact Assessment and Intussusception Surveillance Study at THSTI
- Third Annual Meeting of South-East Asia Regulatory Network (SEARN) on 24th and 25th April 2019
- Course on Vaccinology for Clinical and Public Health Practice
- Workshop on Innovative Solutions for Maternal and Child Health Using Medical Image Analysis and Artificial Intelligence on International Women's Day
- Dr. Trevor Mundel (President, Global Health, BMGF), Prof. K. VijayRaghavan (Principal Scientific Advisor to the Prime Minister) and the team from Coalition of Epidemic Preparedness and Innovations (CEPI) were among important visitors to THSTI during 2019-20.
- THSTI hosted two NCR-BSC cluster events.
- Nineteen lectures for students and faculty members by national and international speakers.
- Three Open Days were organised – on THSTI's Foundation Day, as a pre-event of the India International Science Festival 2019 and to celebrate National Science Day 2020. Teams attended India International Science Festival 2019, DBT-BIRAC's Global Bio-India event in 2019, and Haryana Gyanotsav 2019.



INFECTION AND IMMUNITY

INFECTION AND IMMUNITY

TUBERCULOSIS

About 25% of the world's population is infected with latent *Mycobacterium tuberculosis* (*M. tuberculosis*) bacilli. The bacilli can reactivate during an immunocompromised state and cause active TB. Besides, there has been a constant increase in the number of drug-resistant TB (DR-TB) cases over the past few years. Tuberculosis (TB) kills 2 million people globally every year. *M. tuberculosis* is a highly successful pathogen because of its ability to subvert host antimicrobial pathways and persist in host tissues. All of these have made TB and *M. tuberculosis* focus of study at THSTI.

Understanding TB pathogenesis and identification of novel anti-TB therapeutic targets



The molecular switches that slow down the metabolism of *M. tuberculosis*, causing it to enter into a dormant or latent state, are not known.

Dr. Nisheeth Agarwal's

team is working: (i) to identify molecular pathways that enable the bacteria to persist in host tissues and (ii) to perform phenotypic and target-based screening to identify novel scaffolds with anti-tubercular activity. The primary focus of his lab is to understand how the host responds to mycobacterial infection and identification and characterization of

M. tuberculosis genes. These genes regulate essential metabolic pathways and so can be explored as new druggable targets. Specific objectives are:

- comparative analysis of the effect of infection with virulent and avirulent mycobacteria on host phosphoproteome profile,
- characterizing the landscape of *M. tuberculosis* persisters, and
- understanding the mechanism of proteostasis by emphasizing the role of Clp proteases.

The team has also initiated a nationwide program to facilitate TB research in the country. The team provides TB researchers with defined CRISPRi-based knockdown plasmid constructs and mutant strains. The initiative is in collaboration with CSIR-IMTECH (Institute of Microbial Technology), Chandigarh, and the Department of Biotechnology (DBT) funds it.

1. **Effect of infection of virulent and avirulent mycobacteria on host phosphoproteome profile:** In the previous year it was reported that *M. tuberculosis* and BCG infections modulate phosphorylation profile of several proteins associated with actin metabolism, nitric oxide synthesis, dendritic cell maturation, protein degradation together with many signaling proteins involved in mTOR, GPCR, integrin, MAPK, AMPK signaling pathways. The RIG-I protein regulates

type 1 interferon signaling, and exhibits differential phosphorylation in response to *M. tuberculosis*, but not against BCG infection. Dr. Bill Bishai's group at Johns Hopkins University, USA, collaborated to understand the role of this phosphoprotein. The work showed that *M. tuberculosis* infection in primary human macrophages activates RIG-I. The team undertook several studies using RIG-I knockout macrophages. This revealed that *M. tuberculosis*-mediated activation of RIG-I promotes IFN- β , IL-1 α and IL-1 β levels, dampens autophagy, and facilitates intracellular *M. tuberculosis* survival.

2. **Understanding the landscape of *M. tuberculosis* persisters:** Previous work showed CRISPRi-mediated silencing of essential genes, *gyrA/B* leads to induction of phenotypic drug tolerance in *M. tuberculosis*. Expression of various SOS-response genes, including LexA-RecA regulons, were found upregulated in a microarray experiment performed with *gyrA/B*(-) knockdown strain. Moving forward, we next planned. They set up an *in vitro* model of persistence to understand the landscape of *M. tuberculosis* persisters at the protein level. A bi-phasic growth curve, typical of *M. tuberculosis* persisters, resulted in the presence of drugs such as INH and Rif. The next step is to obtain enough RNA and proteins from a small fraction of the drug-tolerant persister subpopulation for further analysis.
3. **Understanding the mechanism of proteostasis in *M. tuberculosis*:** Biogenesis, folding, trafficking as well as degradation of proteins within and outside the cell are inter-regulated, thus maintaining protein homeostasis. The overall rate of protein synthesis and degradation determines the shelf life of a protein in the cell. Dr. Agarwal's

team characterized the Clp proteolytic machinery known to be crucial for bacterial survival. This will help them understand protein homeostasis (or proteostasis) in *M. tuberculosis*. Early findings showed that proteins are degraded irrespective of their molecular masses or isoelectric points. Importantly, the degradation of putative Clp substrates was found to be sequence-nonspecific and rely on the conformation of target proteins. They further observed that a majority of ClpP1P2C1-regulated proteins contain disorder-promoting residues near the termini. One such protein was a small heat shock protein, Hsp20, which exhibits disorder C-ter end. By performing a series of biochemical and biophysical studies, they showed that the disordered C-ter end is critical for interaction and subsequent degradation of Hsp20 by Clp machinery.

4. **Building up a repository of CRISPRi mutant strains of mycobacteria:** So far, the repository created has plasmid constructs for >150 targets along with many knockdown strains of proven efficacy. Researchers at NII, Delhi, NIPER, Mohali, CSIR-IMTECH, Chandigarh, and NCBS, Bengaluru, among others, have availed these.

Way ahead: The lab is working to characterize novel host pathways that are differentially regulated upon infection and control mycobacterial growth in the host cells. They are also identifying potential target genes in *M. tuberculosis* itself to understand the cause(s) of the emergence of drug-resistance in bacteria. They intend to come up with potential drug targets and new scaffolds for future evaluation against drug-resistant as well as susceptible populations. The progress in recognizing a handful number of target genes in

M. tuberculosis with promise for screening small molecule inhibitors has been significant. Efforts are ongoing to identify and explore putative causal genes and the underlying mechanisms that are critical for the formation of persister-like cells in *M. tuberculosis*. Next, they plan to characterize the role of some of these genes in *M. tuberculosis* pathogenesis using the mouse model of infection. This is to identify the molecular determinants of *M. tuberculosis* virulence. Besides, they are also putting efforts towards building up a repository of CRISPRi plasmids and knockdown mutants that TB researchers outside THSTI can freely avail.



Enzymes regulating Polyphosphate homeostasis

Stringent response pathways involving inorganic polyphosphate (PolyP) are critical for

bacterial stress adaptation and virulence. The intracellular levels of polyphosphate (PolyP) are modulated by the activities of polyphosphate kinase-1 (PPK1), polyphosphate kinase-2 (PPK2), and exopolyphosphatases (PPXs). The genome of *Mycobacterium tuberculosis* encodes two functional PPXs that share an identity of 23% and 27% with *E. coli* homolog, respectively. **Dr. Ramandeep Singh's** team showed that both PPX enzymes displayed exopolyphosphatase, GTPase, and ATPase activity. To understand the role of exopolyphosphatase in *M. tuberculosis* physiology, drug tolerance, and virulence, they generated single *Dppx1*, *Dppx2*, and double mutant strains (*dkppx*) of *M. tuberculosis*. They were generated using temperature-sensitive mycobacteriophages. The mid-log and late-log phase cultures of *dkppx* strain had higher

intracellular PolyP levels in comparison to the parental strain. The double mutant strain displayed a defect in biofilm formation, survival in nutritional stress, low oxygen conditions, and also in macrophages. Furthermore, in concordance with previously published findings by the team, PolyP accumulation in *dkppx* strain resulted in increased tolerance to isoniazid, a cell wall inhibitor. To further unravel the collective biological function of exopolyphosphatases, *ppx1*, and *ppx2* in *M. tuberculosis* persistence, they performed *in vivo* infection of parental and *dkppx* mutant strain of *M. tuberculosis* in guinea pigs. Relative to the parental strain, the lung bacillary loads were reduced by 300.0-fold in guinea pigs infected with *dkppx* mutant strain at 8 weeks post-infection. The *dkppx* mutant strain was cleared more rapidly from spleens of infected guinea pigs at 8 weeks post-infection. This attenuated phenotype associated with the *dkppx* strain was also evident in the histological analysis of the lung sections. Transcriptional profiling on RNA isolated from mid-log phase cultures of WT and *dkppx* mutant strains of *M. tuberculosis* helped to understand the physiological role of intracellular PolyP. The detailed analysis of the RNA-seq data revealed that DosR regulated dormancy genes known to be upregulated in *M. tuberculosis* exposed to hypoxia were significantly downregulated in *dkppx* mutant strain. These observations suggested that PolyP accumulation might result in inhibition of DosS or DosT-mediated regulation of dormancy associated genes in mid-log phase cultures. To test this hypothesis, they studied the effect of PolyP on autophosphorylation of DosT and DosS *in vitro*. In concordance, with RNA-seq data, they found that PolyP inhibited DosT and DosS autophosphorylation activity in a dose-dependent manner. Using Microscale thermophoresis

(MST) assays, in the presence of an increasing concentration of PolyP₁₇, K_D of 1.52 ± 0.13 mM and 1.64 ± 0.11 mM were determined for DosT and DosS binding, respectively. Taken together, they demonstrate that PolyP accumulation in *M. tuberculosis* results in suppression of the expression of dormancy-associated genes, which might be responsible for the observed attenuation of the *dkppx* strain in guinea pigs.

Phenotypic screening to identify small molecule inhibitors against *M. tuberculosis*

Previously, Dr. Singh's team had screened a library of pharmacologically active compounds (Lopac-1280) to identify anti-mycobacterial compounds. The phenotypic screen identified a few novel small molecules, including NU-6027, a known CDK-2 inhibitor. The team demonstrates that NU-6027 inhibits *Mycobacterium bovis* BCG growth *in vitro* and also displayed cross-reactivity with *M. tuberculosis* protein kinase D and protein kinase G. Comparative structural and sequence analysis along with docking simulation suggest that the unique binding site stereochemistry of PknG and PknD is likely to accommodate NU-6027 more favorably in comparison to other *M. tuberculosis* Ser/Thr protein kinases. They go on to show that NU-6027 treatment induces the expression of pro-apoptotic genes in macrophages. Finally, they demonstrated that NU-6027 inhibits *M. tuberculosis* growth in both macrophage and mice tissues. These results implicate that NU-6027 may be optimized further for the development of anti-mycobacterial agents.

In addition to the screening of Sigma Lopac 1280 library, they also performed phenotypic screening of small molecule libraries from NIH against *M. bovis* BCG. They identified 24 scaffolds that exhibited MIC₉₉ values of at least 2.5 mM. The most potent

small molecule identified in their study was a nitroso containing pyrazole derivative, NSC 18725. Dr. Singh's team observed a significant reduction in viable bacilli load of starved *M. tuberculosis* upon exposure to NSC 18725. The action of NSC 18725 was "synergistic" with isoniazid (INH) and "additive" with other drugs in checkerboard assays. Structure-activity relationship (SAR) studies of the parent compound revealed that pyrazole derivatives without a functional group at the fourth position lacked anti-mycobacterial activity *in vitro*. The derivative with *para*-chlorophenyl substitution at the first position of the pyrazole ring was the most active scaffold. The team also demonstrated that NSC 18725 could induce autophagy in differentiated THP-1 macrophages. NSC 18725 also inhibited the survival of both fast and slow-growing mycobacteria inside human macrophages. These observations support the identification of NSC 18725 as the lead molecule against *M. tuberculosis*, which synergizes with INH, is active against non-replicative mycobacteria and induces autophagy in macrophages.

Targeting the L-arginine biosynthesis pathway as a target for the discovery of potent anti-tubercular agents

Microbial amino acid biosynthetic pathways are validated, but underexploited pathways for the discovery of anti-bacterial agents. N-acetyl glutamate synthase (ArgA) catalyzes an important step in an essential metabolic pathway and is an attractive target for the discovery of anti-tuberculars. The identified hit molecule was found to have low micromolar activity. Efforts are on to identify computationally active molecules from virtual compound libraries, follow the possible mechanism of inhibition, and to validate the computationally found active compounds in *in-vitro*

assays. Furthermore, the most likely binding site of identified compounds and possible mechanism of inhibition is explored through extensive molecular dynamics simulation studies.

Understanding the function and regulation of the complex network of TA systems from *M. tuberculosis*

TA systems are bicistronic genetic elements with tightly coupled translation and transcription. They consist of a stable toxin that interferes with essential cellular functions and a labile antitoxin that inhibits toxin activity. The toxins are invariably translated into a protein, whereas antitoxin can either be protein or RNA. Various bioinformatics and phylogenetic analyses have revealed that *M. tuberculosis* genome encodes for a large repertoire of TA systems. The conservation of this extensive repertoire of TA systems in species belonging to the *M. tuberculosis* complex suggests that TA systems regulate metabolic pathways that enable *M. tuberculosis* to adapt to the host. These mostly belong to Type II families such as VapBC, MazEF, ParDE, RelBE, and HigBA.

Using an inducible vector system, Dr. Singh's team has extensively characterized VapBC TA systems from *M. tuberculosis*. Based on growth patterns obtained, they classified these VapC toxins as strongly active, moderately active, or inactive toxins. Using a guinea pig model of infection, they showed that VapBC3, VapBC4, and VapBC11 are essential for *M. tuberculosis* to establish infection in guinea pigs. Now, they have also characterized the VapBC22 TA system from *M. tuberculosis*. Using *in vitro* assays, they show that these VapC22 encodes for a ribonuclease that cleaves MS2 RNA in an Mg^{2+} and Mn^{2+} dependent manner. Analytical ultracentrifugation studies revealed that both

these toxins and antitoxins are dimeric in solution. Transcriptome analysis revealed an alteration in levels of ~447 transcripts in the presence of free VapC22. Functional annotation analysis revealed that the majority of the differentially expressed genes belonged to either intermediary metabolism and respiration or conserved hypothetical protein. The global transcriptional response upon ectopic overexpression of VapC22 overlaps with the mycobacterial response to multiple stresses. These pleiotropic changes might be attributed to either (i) ribonuclease activity associated with VapC22 and other induced non-cognate toxins or (ii) increased transcript levels of various regulatory proteins in the overexpression strain.

Further, examining the *DvapC22* mutant strain showed that in comparison to the parental strain, the mutant strain was more susceptible to oxidative stress. Still, other stress conditions remained unchanged. The mutant strain was attenuated for growth at both 4- and 8- weeks post-infection in the guinea pig and mice model of infection. Histopathology analysis revealed chronic inflammation in the lungs of parental strain infected guinea pigs at both time points. In contrast, lung sections of *DvapC22* infected animals showed fewer granulomas with little evidence of inflammation and larger alveolar space.

In agreement, the mutant strain was also attenuated for growth in mice, and this growth phenotype was partially restored in the complemented strain. Global proteomic profiling of the *DvapC22* strain and comparison with the parental strain of *M. tuberculosis* gave mechanistic insights into the physiological role of VapC22. Fifty-eight proteins showed differential expression between the mutant and parental strains. Among the proteins with reduced expression, the majority

of the proteins are involved in cell wall processes or lipid metabolisms such as ESX-I or ESX-V secretion system. Also, the expression of cognate antitoxin was overexpressed in the mutant strain. The team hypothesized that the increased expression of VapB22 in the mutant strain might be associated with the observed *in vitro* and *in vivo*. In agreement, they also observed that overexpression of VapB22 (i) reduced the expression of various virulence factors such as EsxA and EsxB, (ii) enhances the susceptibility of *M. tuberculosis* upon exposure to oxidative stress conditions and (iii) reduces the virulence of *M. tuberculosis* in mice and guinea pigs. To further delineate the mechanisms associated with the attenuated phenotype of the mutant strain, total RNA was isolated from lung tissues of uninfected, wild type infected and *DvapC22* infected mice and subjected to transcriptome analysis. They observed that approximately 145 genes had differential expression in lung tissues of mice infected with parental and *DvapC22* strain at 4 weeks post-infection. The host-transcriptomic analysis revealed that in comparison to the parental strain, infection with *DvapC22* mutant strain resulted in an enhanced innate immune response. Higher infiltration of neutrophils, eosinophils, dendritic cells, and suppressed Th₁ response in lung tissues implicated the elevated immune response. These observations suggest that innate immune mechanisms can clear *DvapC22* *in vivo*. In contrast, wild type *M. tuberculosis* resists innate immune response and proliferates inside the host tissues.

Mechanistic understanding of persistence in mycobacteria

Successful TB treatment is challenged by slow-growing, non-replicating, metabolically inactive “persister” population of bacilli inside host cells that requires an extremely long treatment regimen. *M. tuberculosis* enters a dormant state leading to latent infection. This ensures its survival inside the host, successfully delaying the efficacy of currently available therapies. Dr. Amit Pandey and his team believe that altering the metabolic state of dormant *M. tuberculosis* could increase the effectiveness of antibiotics and shorten treatment duration. They hypothesize 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. Genetic and high-dimensional informatics approaches in their work have aided the identification of nutrient-specific pathways critical for the generation of persisters in mycobacteria. These efforts could lead to 1) a better understanding of host-pathogen symbiosis and 2) designing of novel intervention strategies targeting persisters (**Figure 1.1**).

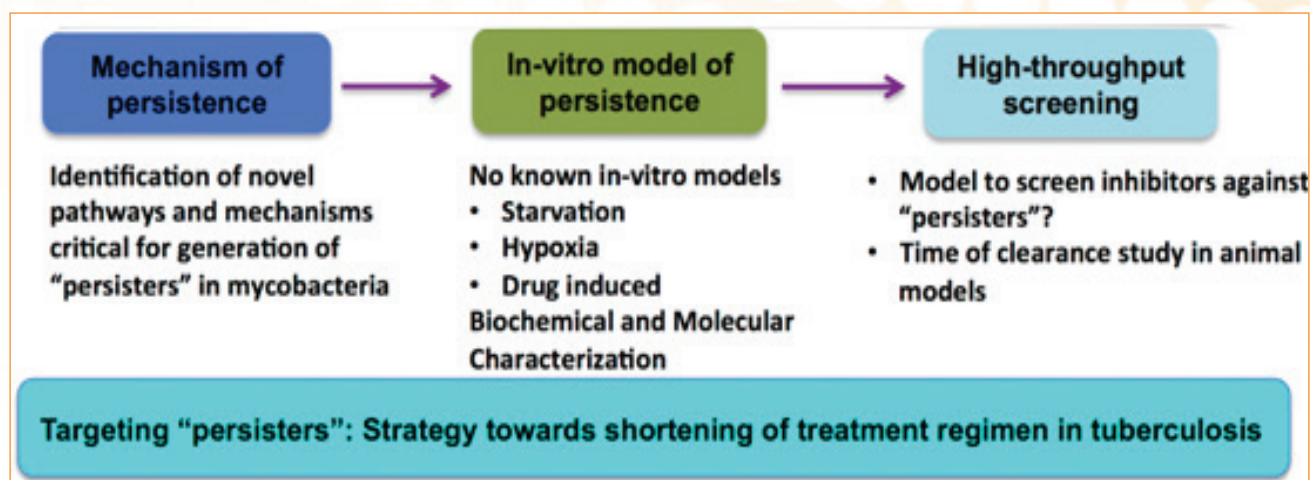


Figure 1.1: Schematic representation of the strategy employed towards shortening of treatment regimen in tuberculosis.

Dr. Pandey's lab successfully 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 as a potential target against persisters.

- a. Cholesterol utilization pathway: *M. tuberculosis* is an intracellular pathogen and entirely dependent on the host for its nutritional requirements. 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 successfully identified one of the *M. tuberculosis* pathways critical for the generation and enrichment of persisters during mycobacterial infections. 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 a new target against "persisters."
- b. Pathways regulating iron (Fe) homeostasis: Although iron is essential for most of the

bacteria, excess of intracellular free iron is toxic. Failure to regulate iron levels might lead to death either due to iron deficiency or toxicity. Since iron deprivation is also one of the antimicrobial strategies that the host adopts, both pathogen and the host compete for the limited iron during infection. Dr. Pandey, for the first time, has demonstrated that the *M. tuberculosis* transcription repressor protein SufR_{TB} regulates the ISC operon. The repressor protein has a role in controlling the intracellular iron homeostasis in *M. tuberculosis*. Disruption of the iron homeostasis in ΔsufR_{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 the growth of *M. tuberculosis* under oxidative and nitrosative stress conditions. The enhanced biofilm production phenotype observed in ΔsufR_{TB} is intriguing. It 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.

I. 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. These strains are potential *in vitro* models 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.

They are using both target and phenotype-based screening approaches to identify potential hits. Their goal is to validate these molecules in both *in vitro* and *in vivo* model of tuberculosis infection. X-ray crystallography and homologous modeling, has successfully solved the crystal structure of the vapBC12 ribonuclease complex (**Figure 1.2**). Their long-term goal is to identify inhibitors against the vapC12 toxin by performing *in silico* docking experiments.

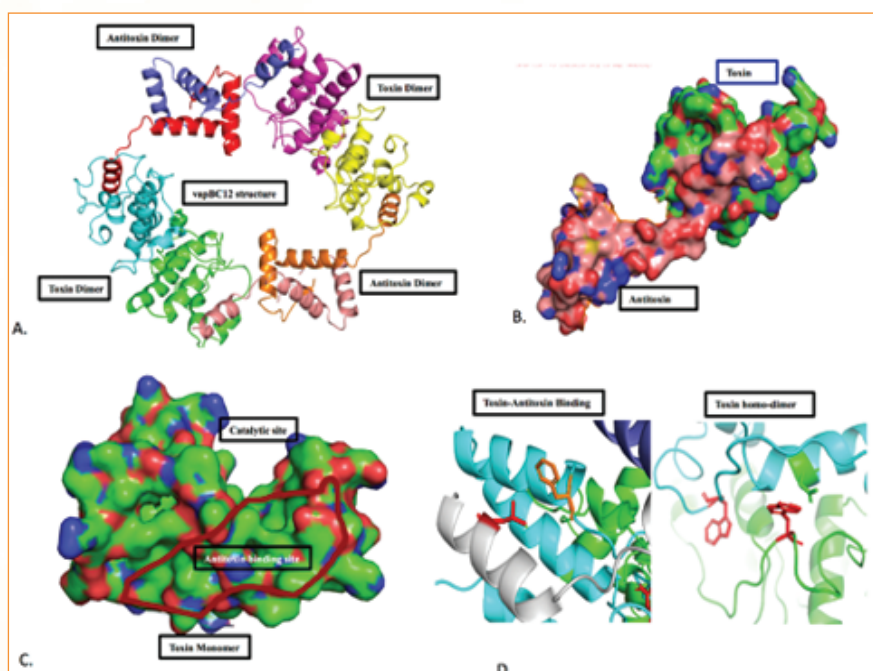


Figure 1.2: A. Crystal structure of vapBC12 complex. B. Toxin-Antitoxin heterodimer. C. Toxin monomer depicting the catalytic and antitoxin binding sites. D. Critical residues identified at the Toxin dimer and Toxin-antitoxin interface.

The strategy of including inhibitor of persisters as an adjunct could help increase the efficacy of the existing treatment regimen, thereby shortening the treatment duration significantly. **Dr. Shailendra Asthana's** team is screening small molecule inhibitors against vapC12 toxin using the small molecule inhibitor library. The *in silico* screening of novel scaffold targeting vapC12 protein seems critical for the generation of persisters.

II. 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, eradication of tuberculosis has been challenging. Since *M. tuberculosis* is an obligate intracellular pathogen, a timely activation of the cell-mediated immune response by the host is significant for its prevention. The challenge is to design vaccine strains that are

a) safe, b) immunogenic, c) long-lasting, and d) 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. His lab has generated recombinant *M. bovis* BCG strains that potentially demonstrate enhanced antigenicity that could eventually translate into better and longer protection against tuberculosis.

Vaccines' development for tuberculosis

Dr. Krishnamohan Atmakuri's lab is working towards the goal of designing superior TB vaccines and exploring virulent proteins as novel therapeutic targets for reducing TB burden. Similar to several Gram-negative bacteria, mycobacteria, too, generate and release extracellular membrane vesicles. Such vesicles are potential candidate vaccines in other diseases. Dr. Atmakuri's group evaluates if mycobacterial-derived vesicles can act as subunit vaccine candidates in enhancing BCG's protective ability.



Comparative proteomic analyses of extracellular vesicles enriched from virulent, attenuated, and avirulent mycobacteria:

Previously, this group and others had discovered that similar to Gram-negative bacteria, mycobacteria too release extracellular vesicles (EVs) from their outer surfaces. They had, hitherto, characterized and compared the EVs proteome from virulent *Mycobacterium tuberculosis* (*M.*

tuberculosis, strain: H37Rv) and avirulent *M. smegmatis* (Msm; strain: mc²155) mycobacteria. Their earlier analyses had also indicated that 50% of Rv EVs protein orthologs are also present in Msm EVs. Additionally, they had also reported that EVs of both Rv and Msm comparably trigger Th-1 pathway-specific pro-inflammatory cytokine responses *in vitro* in macrophages cell line THP-1.

A host must establish a long-term immunological memory of pathogen proteins before pathogen attack to protect itself from any infection. Since EVs of Rv could be a good source of the virulent protein cocktail, the group enriched the same. Detailed proteome analyses by mass spectrometry showed that several candidates from its established virulent protein families were missing as EVs content despite them expressing in the cells. These included (i) ESAT6; (ii) PE/PPE/PGRS; (iii) Mammalian cell entry; (iv) siderophores; and (v) surface lipoproteins families. Further, Rv being a BSL3 pathogen, enriching EVs from it in large quantities is highly impractical. Hence the group evaluated if EVs of a close relative of Rv that is well attenuated and BSL2 compatible would be a good source. A competing group from the US had shown earlier that EVs derived from vaccine strain BCG may not be well suited as a subunit vaccine candidate since they promoted pathogen growth in TB mice model. Hence, this group had previously evaluated if avirulent Msm could serve as an alternate EVs source.

Detailed comparison of Rv and Msm-derived EVs proteome indicated that several proteins common to Rv and Msm EVs belong to the core house-keeping family of proteins. Some orthologs to Rv EVs virulent proteins that exist in Msm EVs have significantly altered structural confirmations. Thus, they may not serve good as representative

immunogens of pathogenic-virulent proteins. To bypass these constraints, Dr. Atmakuri's group enriched EVs from a close, but an attenuated relative of Rv referred commonly to as Ra. Upon detailed comparative proteome analyses between

Ra, Rv, and Msm EVs, they found the following:

- Similar to Rv and Msm EVs proteome content, Ra EVs proteins too majorly fall into several functional categories (**Figure 1.3**)

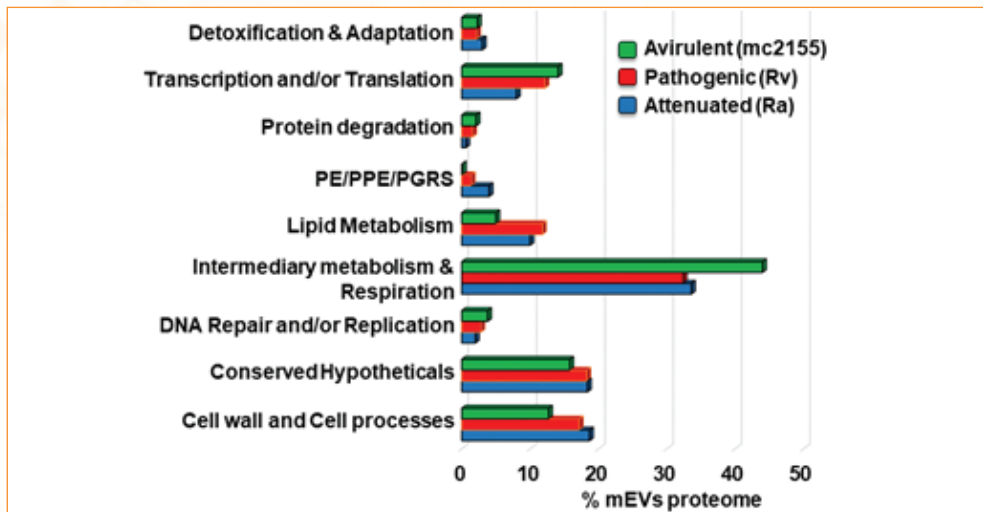


Figure 1.3: Functional categories (Y-axis) into which Ra, Rv and Msm EVs proteome fall. Green, Msm; Red, Rv; Blue, Ra. % mEVs proteome: X-axis

- Around 50% of Ra EVs proteome contains 91% of the Rv EVs proteome (**Figure 1.4**)

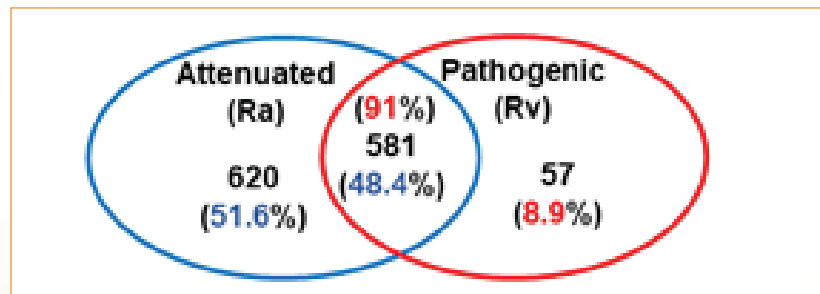


Figure 1.4: Total number of distinct and common proteins in EVs of Ra and Rv. (Red, Rv; Blue, Ra)

- Most of the virulent proteins missing in Rv EVs are present in the other 50% of Ra EVs proteome (**Figure 1.5**)

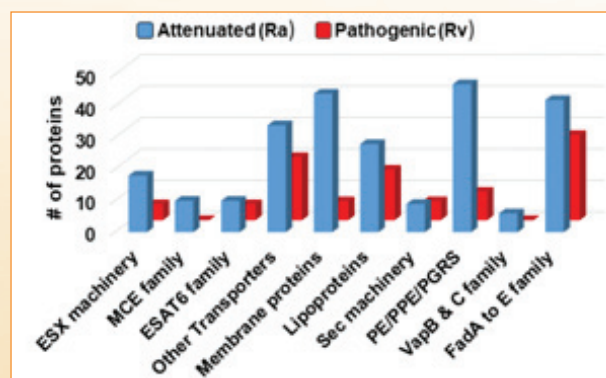


Figure 1.5: Comparative analysis of virulence protein (numbers: Y-axis) families (X-axis) present in Ra (blue) and Rv (Red) EVs

- Around 45-50% of proteins from Ra and Rv EVs have orthologs in Msm EVs (**Figure 1.6**)

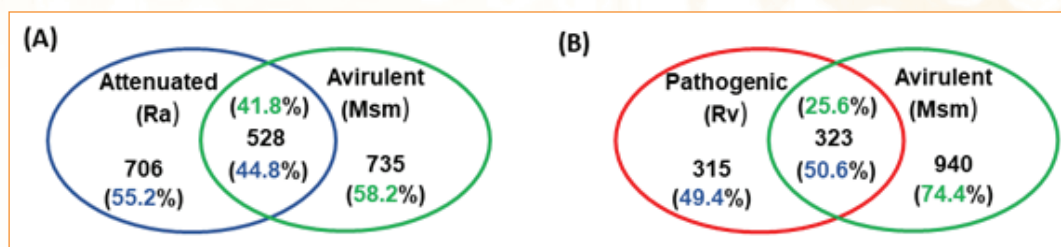


Figure 1.6: Total number of distinct and common proteins in (A): EVs of Ra and Msm; Blue, Ra; Green, Msm; and (B) EVs of Rv and Msm; Red, Rv; Green, Msm

- Ra EVs too can trigger comparable levels of Th1-specific pro-inflammatory cytokines *in vitro* in THP-1 macrophages (**Figure 1.7**)

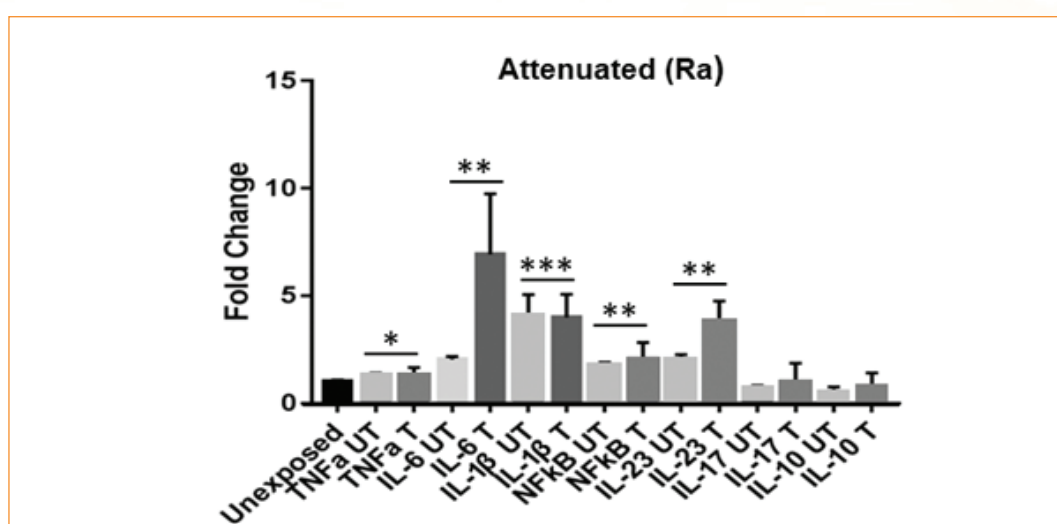


Figure 1.7: Ra mEVs trigger proinflammatory cytokines. Treated (T; intact EVs with DNase I; RNase A and Proteinase K) and untreated (UT) mEVs of Ra were added to differentiated macrophages (THP1) at 100:1 MOI. Five days later, cells were lysed, total RNA extracted and cytokine transcripts quantified 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. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.005$ (Paired T-test).

With these suitable features of Ra EVs, Dr. Atmakuri's group speculates that perhaps Ra EVs may be a suitable subunit vaccine candidate. Currently, comparative testing of Ra, Rv, and Msm-derived EVs as subunit booster to BCG-vaccinated Guinea pigs is ongoing.

Way ahead: Currently, the Ra, Rv, and Msm-derived EVs are being evaluated for their potency as subunit boosters to BCG-vaccinated Guinea pigs to evaluate if they can significantly boost BCG's protective response upon challenge by

Rv. The candidate(s) that can significantly boost BCG's protective response will be then evaluated for dosage and mode of delivery (of boosting). Then, they will be tested for efficacy in rabbits and monkeys before translation into humans. As described earlier, the best candidate will be first repeat tested. Then, different dosages and routes of delivery of boosting will be evaluated.

(B) **Mycobacterial EVs-specific aptamers to delineate mEVs functions:** Typically, to delineate functions of any protein, one of

the tools generated is an antibody specific to it. Given that (i) EVs contain several hundred proteins; (ii) typical yields (protein equivalent) of mEVs are low (per L of *in vitro* grown culture); (iii) generating EVs-specific antibody may take several months, and a vast quantity of EVs; and (iv) screening several thousands of potential antibodies may be very challenging, Dr. Atmakuri's group explored the identification of EVs-specific, small single-stranded DNA aptamers. In the recent past, aptamers have emerged as antibody rivals. Aptamers can be generated *in vitro*, do not require any animals, and unlike antibody, a little amount of target protein(s) is/are required to generate them. Since aptamers are synthetic molecules, they can be synthesized similar to primers at a vast scale with absolutely no batch-

to-batch variations. In collaboration with Dr. Tarun Sharma's group, that has expertise in screening for aptamers, using ~150 µg protein equivalent of Msm EVs as a target, employing Aptamer Linked Immobilized Sorbent Assay (ALISA), using BSA and skim milk as non-specific blocking reagents, they screened a massive SELEX library of aptamers to shortlist six potential binders (**Figure 1.8A**). Subsequent shortlisting led them to identify two specific aptamers that are highly specific to mycobacterial EVs (**Figure 1.8B**) with very weak to no binding (non-specific) to macrophages. The group is exploring the two shortlisted aptamers to understand the dynamics of EVs circulation in THP-1 macrophages and innate release of EVs by mycobacteria upon infection into alveolar macrophages.

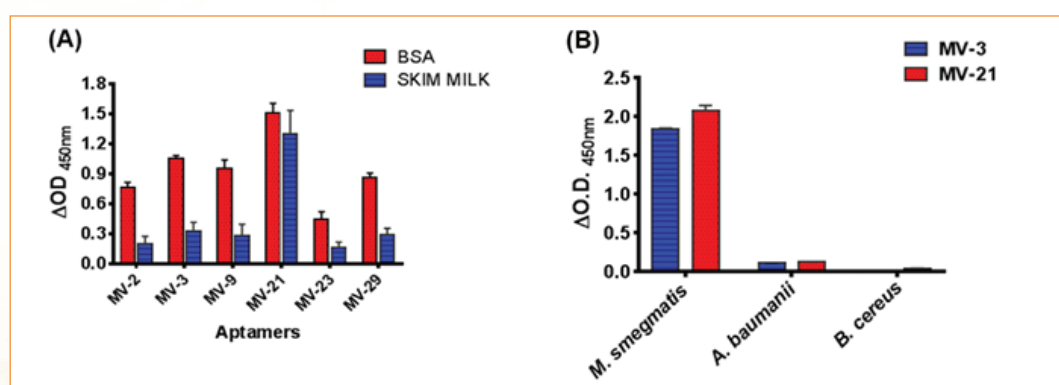


Figure 1.8: Comparative analysis of mycobacterial EVs-specific aptamers and their specificity of binding. Y-axis: quantitation with absorbance of binding at 450 nm. (A) X-axis: Shortlisted primers screened with either BSA (red) or skim milk (blue) as blocking agents. (B) Specificity screening of two (MV-3 (blue) and MV-21 (red)) high binders. X-axis: EVs of Msm, *Acinetobacter baumannii* (a G-ve pathogen) and *Bacillus cereus* (G+ve bacterium); present in Ra (blue) and Rv (red) EVs. The data are from three independent screens with different preparations of Msm EVs.

(C) **Role of HupB, a nucleoid-associating protein, and its evaluation as a therapeutic target:** Most therapeutic drugs employed to kill pathogens target essential components and their functions. Irrational use of drugs invariably leads to the emergence of resistant pathogens. Dr. Atmakuri's group hypothesizes

that targeting *in vivo* essential proteins, including virulent or secreted, dramatically reduces the emergence/selection of resistant pathogens. While the pathogen may not die, the target molecule effectively attenuates it, thus aiding in its quick elimination by the host immune system. Thus, in combination

with existing prophylaxis, these novel target molecules may also help reduce the total duration of treatment, especially for MDR and XDR-TB.

Previously, Dr. Atmakuri's group had reported the discovery of two small peptides that efficiently bind ESAT6. Currently, their evaluation of inhibiting ESAT6 *in vitro* in alveolar macrophages infected with pathogenic *M. tuberculosis* is ongoing. Here, they discuss the potency of another virulent, *in vivo* essential, nucleoid-associating protein of *M. tuberculosis*, HupB, as a therapeutic target. It is well established that in the presence of first-line drugs, *M. tuberculosis* increases its HupB levels 3-6 folds. They speculated that during treatment, HupB levels of the pathogen in TB patients increases, bind *M. tuberculosis* genome, and alters gene expression.

While it is complicated to evaluate directly in TB patients, Dr. Atmakuri's group first conducted *in vitro* testing of the dynamics of HupB protein levels under different conditions of stress that the host imparts during survival in host alveolar macrophages. In regular rich media used routinely for *M.*

tuberculosis growth across labs, HupB protein levels significantly increased as the bacterial growth gradually progressed to the late logarithmic phase. Interestingly, in minimal nutrient media, under oxidative stress and in the presence of the isoniazid (first-line drug), they found HupB protein levels accumulating significantly more than in rich media. However, HupB protein levels significantly dropped as Rv cells got exposed to low pH (5.5 and below), nitrosative stress, and Pyrazinamide (first-line drug) (Figure 1.9). These observations led them to generate a hupB-specific knock then out (KO; $\Delta hupB$). Contrary to Rv (wildtype, WT), the KO grew very slow with very smooth and sticky colony morphology (Figure 1.10). Given the altered morphology, they also tested KO's sensitivity to SDS, a detergent to which Rv is known to be fairly impermeable at a given concentration and found it to be highly sensitive (Figure 1.11). Based on the above observations, currently, the team is assessing the (i) the KO membrane composition; (ii) its sensitivity to antibiotics; (iii) its survival capability in TB mice model and (iv) whether targeting it with a known inhibitor alters the WT to exhibit KO phenotype. In the immediate

future, they will test if the KO fails to survive in mice.

Way ahead: As described earlier, the % survival of KO will be evaluated in mice in comparison to WT (Rv) and KO complemented with HupB. Assessment of the possible reasons for KO's slower growth is in progress. Assessment of (i) the KO's membrane composition, (ii) its sensitivity to antibiotics, (iii) its

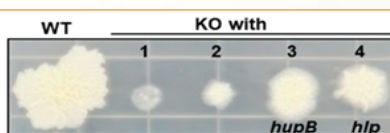
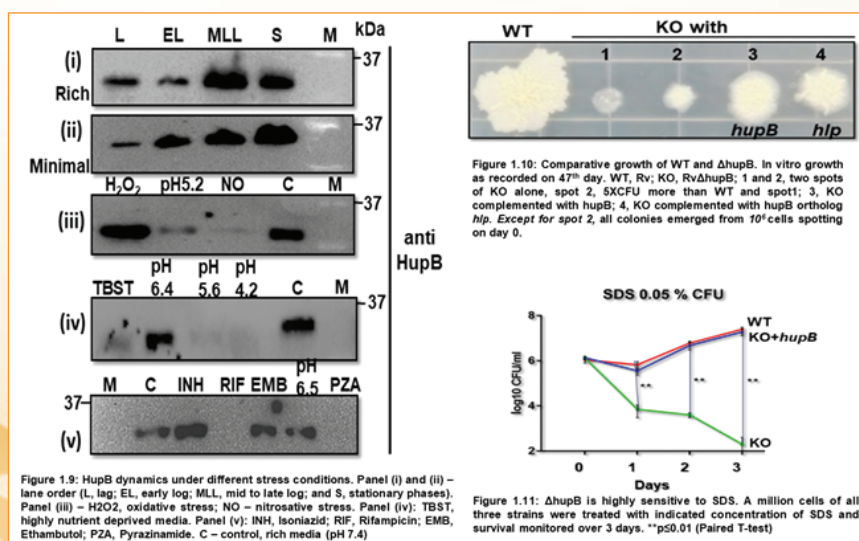


Figure 1.10: Comparative growth of WT and $\Delta hupB$. In vitro growth as recorded on 47th day. WT, Rv; KO, $\Delta hupB$; 1 and 2, two spots of KO alone, spot 2, 5XCFU more than WT and spot 1; 3, KO complemented with hupB; 4, KO complemented with hupB ortholog hlp. Except for spot 2, all colonies emerged from 10⁶ cells spotting on day 0.

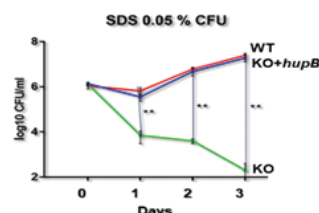


Figure 1.11: $\Delta hupB$ is highly sensitive to SDS. A million cells of all three strains were treated with indicated concentration of SDS and survival monitored over 3 days. **p<0.01 (Paired T-test)

survival capability in the TB mice model, and (I v) whether targeting it with a known inhibitor alters the WT to exhibit KO phenotype, are planned.

Dr. 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. They identified few computationally active peptides against ESAT6. They found that these peptides were among the possible host-interactors of ESAT6. A set of few peptides appeared as ESAT6 binders to modulate its activity. They confirmed it through Surface Plasmon Resonance (SPR) binding analysis.

FLAVIVIRUS INFECTIONS

Host-pathogen interactions in Dengue



Zinc homeostasis in RNA virus infection:

Zinc homeostasis is regulated by zinc influx and efflux transporters, which modulate cellular zinc levels in response

to various stimuli, such as inflammation, stress responses, and growth factors, and changes in intracellular labile zinc levels constitute an integral part of this modulation. Free zinc is proposed to act as a second messenger regulating signal transduction pathways, further underscoring the importance of zinc homeostasis in health and disease. Zinc supplementation in cell culture has been shown to inhibit various viruses, like herpes simplex virus, rotavirus, severe acute respiratory syndrome (SARS) coronavirus, rhinovirus, and respiratory syncytial virus (RSV). However, whether zinc plays a direct antiviral role in viral infections

and whether viruses have adopted strategies to modulate zinc homeostasis have not been investigated. **Dr. Guruprasad Medigeshi's** group focuses on the interaction of RNA viruses with intracellular zinc homeostasis and understand its role in virus life-cycle.

RSV infection modulates zinc homeostasis by enhancing intracellular labile zinc pools:

Free zinc acts as a second messenger regulating signal transduction pathways. This highlights the importance of zinc homeostasis in physiological functions. The group investigated the influence of RNA virus infections on zinc homeostasis. They demonstrate that RSV infection leads to increase in labile zinc levels in A549 cells, primary small airway epithelial cells and in epithelial cells isolated from nasopharyngeal aspirates of children with RSV infection. They utilized two different zinc-binding fluorophores fluozin-3 (FLZ-3) and zinpyr-1 (ZP-1) and measured labile zinc levels by flow cytometry in RSV-infected cells. They observed an increase in labile zinc levels in both FLZ-3 and ZP-1 staining in a time-dependent manner. They did not observe any change in labile zinc levels in cells infected with Dengue virus whereas RSV infection, in particular, led to increase in both cytosolic and vesicular labile zinc pools. To further verify whether this phenomenon is specific to A549 cells, they used primary small airway epithelial cells (SAEC) to further confirm these observations and observed a time-dependent increase in labile zinc levels in SAECs infected with RSV without any effect on cell viability (**Figure 1.12A and 1.12B**). Next, isolated nasal epithelial cells from nasopharyngeal washes collected from pediatric patients infected with RSV or healthy controls and observed a two-fold increase in ZP-1 signal in nasal epithelial cells isolated from RSV patients as compared to cells from healthy controls (**Figure 1.12C**) and as observed for A549 and SAECs, the viability of nasal epithelial cells, that were used for ZP-1 staining,

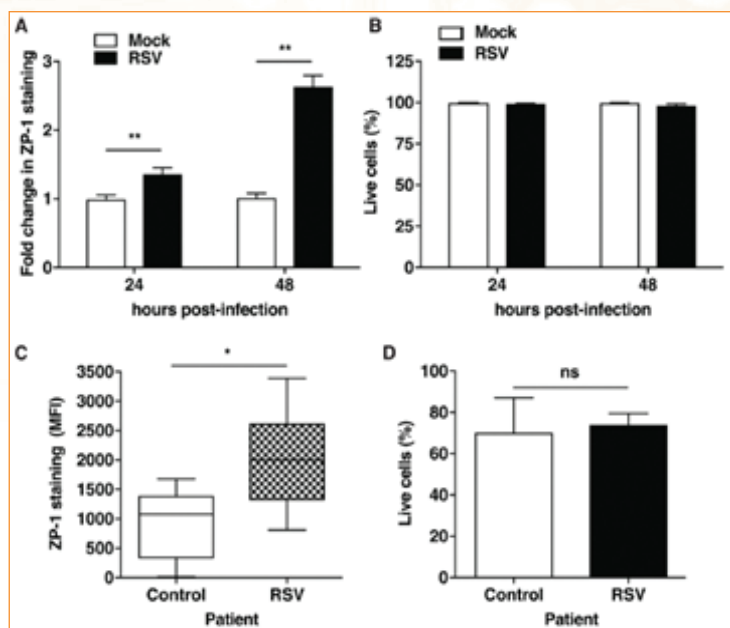


Figure 1.12: Labile zinc levels increase in primary epithelial cells. (A) SAEs were infected with RSV at 3 MOI and cells were stained with ZP-1 at 24 h pi and 48 h pi. (B) Cell viability was determined by staining with cells with Fixable viability dye eFluor780. (C) Nasal epithelial cells isolated from nasopharyngeal aspirates were stained with ZP-1. Labile zinc levels were presented as median fluorescence intensity. (D) Cell viability of NPA was determined by staining with cells with Fixable viability dye eFluor780. Control (n=10), case (n=7). Data from at least two independent experiments. * $p < 0.05$, ** $p < 0.01$.

was not compromised under the experimental conditions (Figure 1.12D). Overall, these data suggest that RSV infection leads to an increase in labile zinc levels in cell lines, primary cells and in nasal epithelial cells isolated from RSV patients.

Inductively coupled plasma mass spectrometry (ICP-MS) quantified total zinc content within the cell under RSV infection conditions. The team observed an increase in total zinc content in cells infected with RSV but no significant difference was observed in Mg or Mn content. Surprisingly, they also observed a significant increase in Cu content in RSV infection relative to uninfected cells (Figure 1.13A-D). This suggests that an increase in the total zinc content in RSV-infected cells is possibly due to zinc uptake.

Zinc homeostasis in cells is regulated by 14 influx (SLC39 or ZIP) and 10 efflux (SLC30 or ZNT) transporters and also by redistribution

between intracellular organelles. As ZIP family of transporters play key role in zinc uptake, the team next performed siRNA-mediated knockdown (KD) of ZIP1 which is a ubiquitously-expressed zinc uptake transporter localized to plasma membrane and ZIP8 whose expression is higher in lungs as compared to other tissues and is also mostly localized to plasma membrane. They show that downregulating ZIP1, which may impact zinc uptake from the extracellular medium, leads to better RSV infection suggesting that zinc may act as antiviral to perturb RSV infection.

The team continues to investigate whether PBMC subsets isolated from dengue patients show modulation of labile zinc pools. This would specifically link zinc homeostasis to innate and/or adaptive immune responses in dengue infection and further help us to focus on specific cellular functions regulated by zinc and how changes in labile zinc pools correlate with clinical outcomes of the disease. In addition, they are creating a repository of dengue isolates from India which will serve as a national resource.

Dr. Medigeshi's team had earlier identified three inhibitors for dengue virus (Salmeterol, Fluoxetine and N-Desmethylozapine). They are currently establishing AG129 mouse models to initiate animal work to generate proof-of-concept data for these antivirals. They will also characterize some of the dengue isolates from virus repository to study dengue pathogenesis and also to generate mouse-adapted virus strains for antiviral development.

Way ahead: Increase in labile zinc levels appears to be an antiviral response as zinc supplementation led to inhibition of RSV

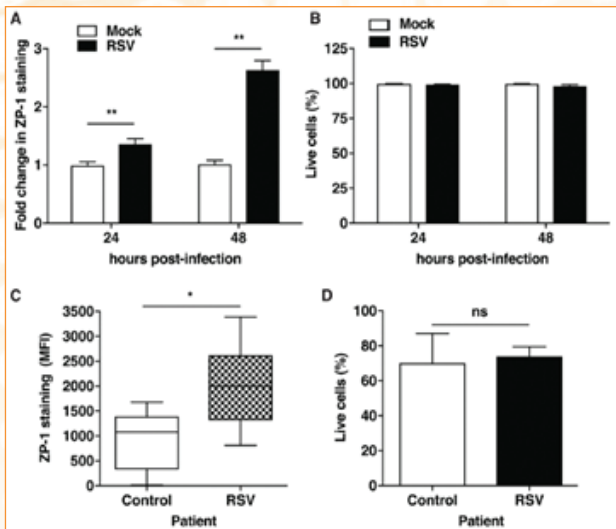


Figure 1.13: Labile zinc levels increase in primary epithelial cells. (A) SAEC's were infected with RSV at 3 MOI and cells were stained with ZP-1 at 24 h pi and 48 h pi. (B) Cell viability was determined by staining with cells with Fixable viability dye eFluor780. (C) Nasal epithelial cells isolated from nasopharyngeal aspirates were stained with ZP-1. Labile zinc levels were presented as median fluorescence intensity. (D) Cell viability of NPA was determined by staining with cells with Fixable viability dye eFluor780. Control (n=10), case (n=7). Data from at least two independent experiments. * $p < 0.05$, ** $p < 0.01$.

infection whereas mimicking zinc deficiency by a zinc chelator or ZIP1 knockdown proved to be beneficial for RSV infection. Increased oxidative stress is one of the clinical manifestations of zinc deficiency which could be corrected by zinc supplementation. Oxidative stress may influence viral infections in more than one way depending on the host pathways and compartments that the viruses utilize for genome replication, translation, assembly and egress. We are further investigating the link between oxidative stress response and RNA viruses so as to understand the fundamental aspects of host-pathogen interactions and to harness this information for developing therapeutics.

Effect of megakaryopoiesis on DENV replication

Thrombocytopenia associated with dengue virus infection is suggested to be the result of



multiple mechanisms that affect either the biogenesis or stability of platelets. Platelets are produced from megakaryocyte mother cells, infection of which has a suppressive effect on platelet biogenesis.

Dr. Sankar Bhattacharya's team is studying the effect of dengue replication on the progress of megakaryocyte differentiation and vice versa i.e. the effect of differentiation on viral replication. As a model system, they use cells of the human K562 cell line which differentiate into megakaryocyte-type cells when treated with phorbol esters. Quantifiable parameters of this differentiation include expression of platelets specific surface markers and endomitosis coupled with profound changes in pattern of gene expression. In the study, pharmacologically-induced differentiation of K562 into megakaryocytes promoted the replication of DENV in these cells. In contrast, DENV replication seems to inhibit the development of differentiation steps by specifically targeting certain crucial signaling axes, without showing any drastic effect on host cell survival. Analysis of the transcriptome in differentiating cells showed significant upregulation of inflammatory genes. Therefore, it was interesting to note that DENV replication was not only refractory to the expression of these genes but seems to benefit from the differentiation process. A comparison of the PMA-induced transcriptome changes between uninfected and virus-infected cells have been performed and is currently being analysed.

Way ahead: (i) Detailed analysis of comparative transcriptome data to score for reversal of gene expression associated with megakaryopoiesis, and (ii) Analysis of post-transcriptional gene

regulation changes that are imposed by DENV replication in differentiating cells.

Characterization of novel DENV RdRp inhibitor 'hit' compounds for pan-serotype activity

Dr. Bhattacharya's team has filed a provisional patent for two novel oxyindole compounds as 'hit' molecules active against DENV replication through inhibition of viral RNA-dependent RNA polymerase. The molecules have been shown to be active against DENV2 and currently efforts are ongoing to test their efficacy against the other three serotypes of DENV.

Way ahead: Using docking studies, the two molecules are predicted to interact with the RNA-template entrance site on the RdRp protein. They plan to perform site-directed mutagenesis studies to map the RdRp amino acids potentially interacting with the compounds. Comparison of the interaction between wild-type and mutant proteins with the compounds will be performed using bacterially over-expressed and purified protein, by Surface Plasmon Resonance (SPR).

Antigen and antibody design for viral pathogens



One of the key challenges in developing antibody-based therapeutics in Dengue virus (DENV) is the antibody-dependent enhancement (ADE). To circumvent ADE

and still develop potent therapeutic antibodies against DENV, **Dr. Shubbir Ahmad's** team went on to design and engineer single-chain variable fragment (ScFv) based broad multi-specific antibodies against DENV that can avoid the ADE

problem but also offer protection at the same time. One possibility in antibody-mediated therapeutic approach for DENV is to develop a multi-specific antibody to neutralize all the four dengue serotypes.

Strongly neutralizing and pan serotype-specific DENV monoclonal antibodies (mAbs) were selected to design and engineer ScFv pairs in various combinations to retain correct folding and functionality of each V_H - V_L pairs, such as tandem scFv format or in other possible permutation combination of the V_H - V_L fragments. The entire designed construct will also have to be optimized for suitable linker length to connect the subsequent V_H - V_L fragments. The objective is to maximize the correct pairing of V_H - V_L combination for all the four serotypes for optimal function and neutralization potential. Currently, designing of different multi-specific ScFv formats and optimizing their expression and purification are in the initial stage to obtain relatively pure designed constructs in both mammalian and bacterial expression systems that will need to be characterized further.

Way ahead: The designed scFv antibodies will be tested for their neutralization potential. Based on their neutralization breadth and potency, more design formats will be tested for optimal function. This approach will also be extended to other viral pathogens.

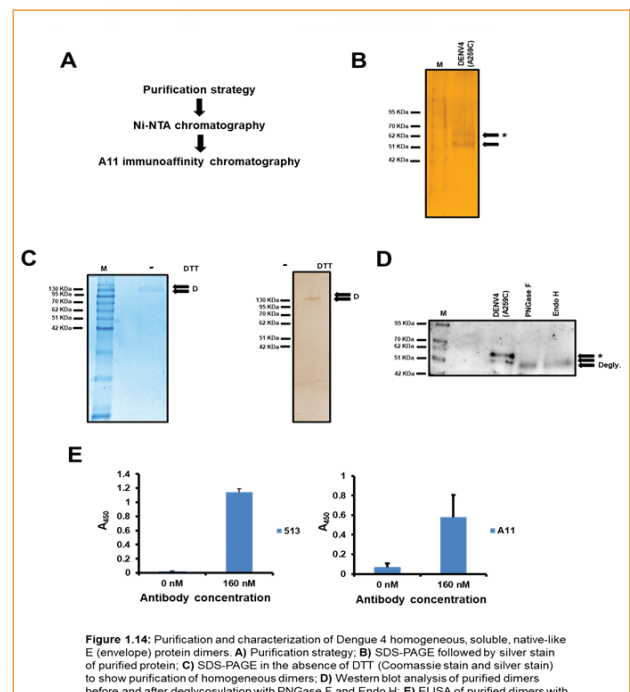
Prophylactic and Therapeutic Strategies for Dengue



Dr. Supratik Das's work involves the design, generation, production, and characterization of Dengue 3 and 4 soluble, dimeric, native-like Env

(envelope) antigens for isolation of mAbs from Dengue patient samples (**Figure 1.14**). Three strategies to generate soluble DENV3 and DENV4 proteins that are dimeric, native-like and display all the broadly neutralizing epitopes were pursued. These three strategies are 1) artificially cleaved DENV prME construct, 2) DENV E395 only and 3) naturally cleaved DENV prME construct. Of these strategies, strategy 3 was found to work best and was therefore pursued. The capsid (C), precursor membrane (prM) and envelope (E) proteins form the structural proteins of dengue virus. Furin-mediated prM cleavage is required for mature virus particle formation, rearrangement of E protein and viral infectivity. Due to inefficient processing of DENV prM by furin, DENV-infected cells produce high levels (30-40%) of prM-containing immature particles. In HIV-1, it has been observed, that efficient cleavage of the Env (envelope) gp160 precursor by furin into its constituent subunits gp120 and gp41 leads to preferential binding to broadly neutralizing antibodies. Furthermore, it has been reported that furin-mediated cleavage strongly influences the ability of soluble HIV-1 trimeric, Env immunogens to adopt native-like conformation. Recombinant protein expression entails overexpression of the protein which may further reduce cleavage efficiency. To circumvent this problem the R6 (RRRRRR) substitution was introduced to replace the furin cleavage site which leads to more significantly improved cleavage efficiency. Following Ni-NTA pull-down of the protein nearly 100% efficiency of cleavage was obtained. Using this strategy wild-type Dengue 3 and 4 E(envelope) proteins were successfully purified from mammalian expression system and found to be predominantly monomers. Cys-Cys

disulphide bonds in E protein were engineered to stabilize the E protein dimer. Using Ni-NTA chromatography followed by immunoaffinity chromatography Dengue 4 E protein dimer was successfully purified to homogeneity and currently Dengue 3 E protein dimer purification is in process. Currently, the antigenicity, stability and structural homogeneity of the proteins are being tested by using various biochemical, biophysical and structural experiments.



Way ahead: The purified and characterized Dengue virus 3 and 4 dimers are going to be further stabilized by structure guided Cys-Cys disulphide bonds and M protein to improve yield and thermal stability. Apart from monoclonal neutralizing antibody isolation, these dimers can be used for various studies like immunization in animal models, diagnostics and structure-function studies of virus-antibody co-evolution.

Development of AG 129 mouse model to facilitate Dengue research



Dr. Sweety Samal's group is working to establish AG129 mouse model that supports systemic DENV infection. This model will be used to test DENV vaccines, antivirals, and supportive therapies. They have received AG129 breeding pair from Marshall Bioresources and

have successfully been able to establish the mouse colony and expansion. They are now developing sub-lethal and lethal DENV-2, DENV-3 and DENV-4 models in AG129 mice.

AG 129 model for Dengue research: A Dengue animal model utilizing IFN α -KO AG129 mice is available to study virus pathogenesis. The team is optimizing the methodologies so that these models could be used in future for testing of vaccine, antiviral or potential monoclonal antibodies.



Dr. Tripti Srivastava's team established the Dengue structural E protein platform to support antibody-based therapeutic discovery and development. The

surface-exposed E protein on infectious mature dengue virus (DENV) plays a vital role in virus entry and is the primary target of neutralizing antibodies. Isolation of pathogen-specific antibodies using conventional approaches: Hybridoma, microneutralization, phage display are challenging due to lack of specificity, which makes the procedure extensively laborious, less efficient, and time-consuming. Dr. Srivastava's team followed a methodology employing antigen-

specific memory B cell isolation technology. It has the advantage of specificity, efficiency, and is a less laborious approach (**Figure 1.15**). Dengue envelop protein E, which is the primary target of antibodies on the surface of the virion is an ideal antigenic bait for the purpose. DENV E protein is present on the surface of virus in a dimeric conformation. Hence two WHO reference strains - DENV1 West Pac/74-Nauru 1974; DENV2 S-16803-Thailand 1974 respectively for Dengue serotypes 1 and 2 were targeted to express in various soluble form. They used PrM-E based expression cassette to express full length, and cysteine stabilized E protein dimers.

Dengue serotype 2, E protein, was successfully purified as a stabilized dimer through dimeric

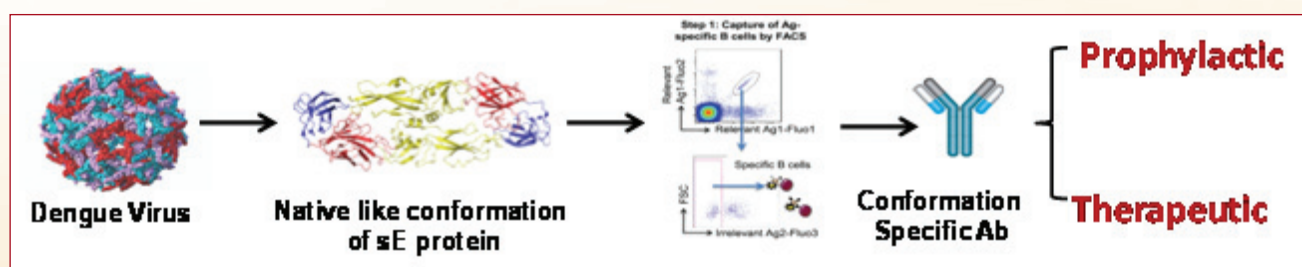


Figure 1.15: Recombinantly produced Dengue surface E protein dimers in conformation similar to as it is present on viral surface (dimers) will be an ideal bait for conformation specific antibody isolation

conformation-specific antibody affinity-based purification. However, a model structure-based prediction was done with energy minimized prefusion conformation of serotype 1 E protein to identify new combination of inter-subunit residues. If the residues could be mutated to cysteine, the E protein dimers may be stabilized (**Figure 1.16**). Different other soluble constructs of E protein from Dengue serotypes 1 and 2 been expressed and purified through a mammalian expression system. Domain III-based protein constructs expressing only domain III, which is known for serotype. Some pan neutralization activities also successfully expressed and purified for the characterization of binding specificity.

Way ahead: Protein expressed through mammalian expression system will target complex conformational epitopes directed pan serotype-specific antibodies with both therapeutic and prophylactic potential. Where a similar approach could be implemented for other infectious diseases, the present proposed concept in the long-term will establish a dengue reagent resource at THSTI. The resource will be an interactive, multidisciplinary platform to improve, standardize, and support platform for dengue research: vaccine evaluation, diagnostic, vaccine development as well as for antibody engineering and developability.



Figure 1.16: Structural superimposition of Dengue 2 and Dengue 1 surface Envelop protein showing difference in conformation orientation, inset showing energy minimized structure displaying identified residue which are at the closest approach once mutated to cysteine will form disulfide bond

VIRAL HEPATITIS



Dr. Milan Surjit's

laboratory investigates multiple aspects of hepatitis E virus (HEV) biology; (a) understanding the host-pathogen interactions involved

in HEV pathogenesis, (b) understanding the mechanism of viral translation, replication, and release, (c) identification of novel anti-HEV compounds which are potential therapeutic

drugs and (d) development of a recombinant vaccine against HEV. Recently, his laboratory has initiated studies to understand the mechanism of Severe acute respiratory syndrome coronavirus 2 (SARS CoV2) pathogenesis and evaluating the prophylactic potential of self-amplifying mRNA vaccine candidates against it. The ultimate goal of the laboratory is to generate sufficient knowledge/resources for an in-depth molecular understanding of the life-cycle of HEV and SARS-CoV2 and the development of efficient prophylactic and therapeutic products against these pathogens using the above knowledge/resources.

In the last year, his laboratory developed protocols to produce virus-like particles (VLPs) of HEV using *Pichia pastoris* and *E. coli* expression systems. His team is evaluating these VLPs for their potential as a candidate vaccine against HEV.

With the emergence of SARS-CoV2 as a global pandemic, his laboratory has initiated studies towards the development of a self-amplifying mRNA vaccine against the pathogen. Additionally, his group is exploring different aspects of viral replication to identify key processes, which may be targeted to develop specific antiviral therapeutics against SARS-CoV2.

Way ahead: In the coming year, his group aims at evaluating the protective efficacy of the vaccine candidates against HEV and SARS-CoV2 in suitable small animal models.

HIV VACCINE AND ANTIBODY TRANSLATIONAL RESEARCH

Dr. Jayanta Bhattacharya's lab primarily carries out basic, fundamental and translational research in (a) characterizing association between genetic, antigenic and neutralization properties of primary HIV-1 subtype C that is predominantly circulating



in India and (b) isolation and characterization of neutralizing antibodies elicited in natural HIV infection. The overall goal of the laboratory is to leverage their strengths in developing strategies to identify and optimize neutralizing monoclonal antibodies suitable for broadly dissecting the circulating HIV-1 clade C variants in India.

- a. **Geospatial diversity within HIV-1 subtype C gp120 gene and its association with predicted sensitivity to broadly neutralizing antibodies:** *HIV-1 subtype C, the globally predominant subtype, is responsible for the majority of HIV-1 infections in India, the third largest HIV infected population. His team examined the global diversity of the available HIV-1 subtype C gp120 sequences and assessed its likely impact on neutralization outcomes of global HIV-1 subtype C.* A total of 1814 full length HIV-1 C gp120 sequence from 37 countries were retrieved from Los Alamos National Laboratory HIV database (www.hiv.lanl.gov). Phylogenetically, sequences from Asian countries (including India) clustered together however differed significantly when compared with pan African subtype C sequences. Variable loop lengths within Indian and African clusters were distinct from each other, specifically V1V2 and V4 loops. Pairwise analyses at each of the 25 pNLG sites indicated distinct country specific profiles. Highly significant differences (Fisher's exact test, $p < 0.001^{***}$) were observed in prevalence of 4 pNLGS (N130, N295, N392 and N448) between South Africa and India. Compared to other bnAbs, the predicted sensitivities to VRC01, VRC03 and VRC13 were also found to be largely dissimilar between sequences from India and South Africa as well as to those reported in the CATNAP database. The differences in HIV-1 subtype C gp120 sequences indicate disparate and distinctly evolving clusters within subtype C with differential predicted responses to bnAbs. These data warrant expansion of existing representative HIV-1 gp120 sequences from India. There is also an urgent need to further

characterize the pre-existing bnAbs in relation to HIV-1 env diversity to enable design of optimal population-specific bnAb based intervention strategies.

- b. **Structural features of a novel HIV-1 Indian clade C trimeric soluble Env SOSIP and the polyclonal neutralizing antibody responses developed in vaccinated rabbits.** Induction of protective immune response to genetically diverse HIV-1 remains a challenge. Dr. Bhattacharya's team examined the structural and antigenic properties of an HIV-1 clade C soluble envelope protein (Env; 1PGE-THIVC), prepared from primary sequence obtained from an Indian elite neutralizer, in solution and by polyclonal antibodies that it induced in rabbits. SAXS analysis of 1PGE-THIVC confirmed that molecules occupy a monodisperse globular profile in solution and shape restoration confirmed presence of 3-fold symmetry, albeit with some degrees of inherent molecular mobility which possibly regulate recognition by mAbs. EMPEM analysis was carried out with serum samples collected at weeks 6 and 12 following the first protein boost and at week 22 following the second protein boost. The common feature of all three serum samples was the presence of two non-neutralizing responses; gp41 base and an N611 glycan hole (resulting from inefficient posttranslational modification). The serum samples collected at weeks 12 and 22 demonstrated a C3/V5 directed antibody response, while the week 22 sample demonstrated a fourth antibody response that indicated "V1/V3" and "V2-like" antibody responses. However, these did not appear the same as that of canonical bnAb "V1/V2-apex" or "V3-glycan supersite." The potent autologous neutralization conferred

by vaccinated serum was mapped to C3/V4 region of viral Env, consistent with the generalized "C3/V5 epitope" response seen by EMPEM. They report three-fold symmetrical shape of a novel clade C SOSIP and identified subtle local differences that revealed new insights about its relative breathability in solution. The EM imaging revealed a diversity of epitopes targeted by polyclonal antibodies elicited by this novel SOSIP including ones that potentially neutralized sequence matched and unmatched autologous viruses.

- c. **Isolation & characterization of bnAbs.** In partnership with the IAVI Neutralizing Antibody Center (NAC), they isolated three mAbs by single B cell sorting from an African elite neutralizer with novel sequences that have demonstrated variable neutralization breadth and potency when tested against global HIV-1 Env-pseudotyped virus panel. They are currently assessing the extent that these novel bnAbs alone and in combination with other existing and novel bnAbs can demonstrate neutralization breadth against primary HIV-1 Indian clade C obtained from Indian patients. They have sorted and cloned a number of functional mAb clones from two Indian donors by antigen-specific B cell sorting, and they are currently being screened for their neutralization potential.

Variation in neutralization susceptibility of HIV-1 Indian Subtype C isolates to potent and broadly neutralizing monoclonal antibodies (bnAbs) targeting distinct epitopes.

Broadly neutralizing antibodies (bnAbs) have emerged as promising agents for HIV-1 prevention and treatment, as demonstrated by recent studies in non-human primate models and

humans. However, given substantial geographic variation of HIV-1 subtypes, it is important to understand the extent to which the lead bnAbs can neutralize the region-specific circulating HIV-1 subtypes/recombinants. Dr. Bhattacharya's team has initiated a study to investigate the extent of the susceptibility of a large number of pseudotyped viruses expressing HIV-1 clade C *envelopes* of Indian origin to a number of bnAbs having distinct epitope specificities. To date, they have examined 56 Env-pseudotyped viruses. At a concentration of 5 µg/ml, they identified several isolates (36/56, 64.28%) with resistance against one of these four bnAbs and some (17/56%) with resistance against two bnAbs. However, only four isolates were resistant to three of the four bnAbs. The degree of resistance of Env-pseudoviruses to CAP256.25 (14/25), PGT121 (15/56), and PGDM1400 (19/56), VRC01 (9/56) were found to vary. Detailed sequence analysis revealed that distinct known substitutions at key residues that are targets of these four bnAbs were associated with neutralization resistance. Non-susceptible Env-pseudotyped viruses with intact key contact sites for each of the bnAbs tested suggested novel yet unknown env sequence features associated with neutralization resistance. This study indicated that HIV-1 Indian subtype C strains sensitive to 1-2 bnAbs predominant among HIV infected population, however very few were resistant to all bnAbs. This warrants further assessment of bnAbs that are under clinical development, against a larger panel of subtype C strains currently circulating in different geographical regions and distinct populations in India. This will help understand whether these promising bnAbs will be useful individually and/or in combination against Indian subtype C.

They also examined *env* clones representing viral quasi-species isolated from contemporaneous cross-neutralizing plasma of two chronically infected Indian patients and having V1V2 specificity, for their degree of sensitivity to CAP256-VRC26.25 and PGDM1400 bnAbs, both of which targets the V1V2 apex region of viral envelope. The goal of this study was to examine the degree of heterogeneity of *envs* obtained from same individual at the same time point in their sensitivity to bnAbs with similar epitope specificity of HIV-1 subtype C contemporaneous *envelopes*. The plasma of both donors demonstrated cross-neutralizing activity and targeted the V1V2 region of viral envelope protein. While all 5 of the autologous *envs* obtained from donor 1 were found to be completely resistant to contemporaneous plasma antibodies, 4 *envs* obtained from donor 2 were found to be sensitive. All *envs* isolated from both the donors were resistant to sCD4 and 17b mAb, suggesting that they were expressed as compact near-native conformation. Natural escape variants from donor 1 were mostly found to be sensitive to PGDM1400 but resistant to CAP256-VRC26.25, while those isolated from donor 2 demonstrated a reciprocal phenotype. While the CAP256.VRC26.25 resistant *envs* were found to be associated with K169E substitution. All of them contain N160, the key epitope for PGDM1400 sensitivity, indicating that PGDM1400 resistance was associated with novel yet unknown env sequence features. The study highlights the development of neutralizing antibodies with different V1V2 specificities with unique escape pathways associated with neutralization heterogeneity of envelope proteins expressed by contemporaneous circulating HIV-1. The observation also underscores the need for dissecting the spectrum of neutralization properties of the clonal variation in better optimization of bnAbs for effective treatment.

Similarly, they examined an association between variation in *env* sequences isolated at two different time points from broadly cross-neutralizing plasma of a slow progressing Indian HIV+ individual (donor ID: G37112) and their sensitivity to bnAbs with distinct epitope specificities. Donor plasma samples were sourced from IAVI's Protocol G cohort in India. While *env* clones obtained at baseline were found to possess shorter V1 loop length with fewer N-linked glycans compared to those obtained from a second visit, longer V2 loop length with fewer N-glycans found in *envs* from baseline compared to those obtained in subsequent time point. N160, a key glycan in V2 apex targeted by both PGDM1400 and CAP256. VRC26 bnAbs were present in *envs* obtained at baseline but found absent in *envs* obtained in the second visit; however, no correlation on the degree of virus neutralization observed. Interestingly, *envs* with the same V1V2 loop lengths containing N332 glycan supersite in V3 differed in their sensitivity to PGT121, highlighting a new modality of HIV-1 resistance to PGT121. Moreover, variation in the signature motif (LDI/PDI) in the V2 reported facilitating gp120 attachment to $\alpha 4\beta 7$ integrin on CD4+T cells for productive infection. Finally, resistance to VRC01 of *envs* obtained from the second visit was found to be associated with the presence of N234/N276 glycans in the loop D and subtle insertion/deletion in the V5 loop. The study highlighted new insights into the distinct mechanism associated with diversities in the sensitivity of HIV-1 to bnAbs. This information will help understand the basis for immune evasion of circulating viruses in individuals who develop potent cross-neutralizing antibodies relevant for designing appropriate intervention strategies.

Way ahead: In continuation to ongoing efforts towards better characterizing the intra-subtype

diversity of the circulating HIV C in India towards developing effective a bnAb based intervention, the overall goal moving forward through inter-and intra-institutional collaborations are as follows:

- To examine the association between whole-genome diversity of HIV-1 subtype C with special reference to envelope (*env*) gene and degree of susceptibility to neutralizing antibodies in people living with HIV (PLHIV) in different risk groups, distinct geographical regions in India.
- To concurrently examine homeostatic and virus-specific immune signatures associated with virological suppression, reservoir dynamics that impact sensitivity to bnAbs.
- To optimize bnAb combinations and examine whether they can demonstrate enhanced neutralization breadth, potency of currently circulating clade C and recombinants in India.
- Discovery, optimization and development of bnAbs with improved breadth, potency and serum half-life

Development of a small molecule based new drug lead with novel pharmacology as a therapeutic option for HIV-1 infection with proof-of-concept studies in disease model.

Dr. Dinesh Mahajan and his group at THSTI are focused on medicinal chemistry and pharmacology development activities related to pre-clinical drug lead development for HIV-1 infection. 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 animal model of HIV

infection. Dr. Mahajan and his team identified and developed a small molecule based on new chemical entity (NCE) 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 was evaluated for various *in vitro* as well as *in vivo* pharmacological experiments (PK-ADME, off target studies, dose tolerance and toxico-kinetic evaluation) and currently being pursued for dose ranging animal efficacy studies in humanized mice infected with HIV-1. If successful in efficacy studies, 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: After successful proof-of-concept studies in animal model of HIV infection, his group would like to develop pharma collaboration for late stage pre-clinical and clinical development of this novel lead.

INFLUENZA

The goal of the Influenza program is to create a platform in India to accelerate influenza research in the country and develop preventive strategies to protect public health. The Influenza program includes multidisciplinary research across a large network of institutions, supporting the development of vaccine candidates by testing *in vitro*, pre-clinical studies in mice and ferrets and facilitating clinical trials and developing a roadmap for human challenge studies. The major thematic areas are *Virology, Vaccine development, Pre-clinical animal model*, and *Clinical virology*. Another aim of this program is to serve as a national resource

to the Influenza scientific community. Dr. Sweety Samal's team is building on resources/reagents to provide a repository for viral isolates, serum samples, antibodies, purified proteins, and reagents to facilitate development of universal Influenza vaccines for all influenza strains for both seasonal and pandemic Influenza strain. The long-term goals of the program are to establish a multidisciplinary collaborative team bringing in together the academic institutes, industry, start-ups, clinics to conduct translational research so as to accelerate development of new and improved control measures, serve as a resource for scientific and public health communities, and provide a research platform in the surge of epidemic or pandemic outbreak.

Development of Influenza A matrix 2 ectodomain soluble protein-based next-generation vaccine candidate using mammalian expression system as a proof-of-concept: Influenza A viruses remain a major public threat globally, causing severe respiratory tract infections. In addition, the co-circulation of influenza viruses among human and animal reservoirs and the antigenic variation of the viruses have caused major pandemic outbreaks in the past few decades, and seasonal outbreaks continue each year. For a country like India, where air pollution is another major threat to public health, it increases the risk and susceptibility to respiratory infections like influenza. Currently, licensed influenza vaccines are directed against the viral HA protein. The protein binds host receptors to mediate viral entry. However, the protection is strain-specific, and it needs annual evaluation and reformulation. Efforts are on to exploit the conserved epitopes in Influenza virus proteins in the development of a next-generation universal vaccine. Influenza Matrix 2 protein an integral structural protein on the surface of the viral

membrane, which serves as a protein-selective ion channel. The protein is homo-tetramer of 97 amino acid residues, out of which the Matrix 2 external domain or ectodomain (M2e) of 23 amino acid residues are highly conserved. Multiple copies of tandem repeats of the conserved region of M2e are linked together. They have been evaluated as a universal vaccine candidate. THSTI's team constructed a composite peptide, including either two or three or more tandem copies of M2e peptides fused with a six-histidine-tag sequence followed by a tetramerizing bio clamp C terminally. The proteins were expressed either in *E. coli* or 293T and 293Expi, purified and biochemically characterized. Six to eight weeks old BALB/c mice were immunized I/M with protein prime-boost regimens, and the humoral and cell-mediated immune response was evaluated. The hypothesis was that stabilizing and expressing M2e protein in a mammalian system might influence the antigenicity and immunogenicity of M2e with the added advantage to robustly produce a large scale of proteins with a native-like fold and hence can act as an efficient vaccine candidate. In an intramuscular protein priming and boosting regimen in mice, these proteins induced high titers of antibodies and elicited mixed Th1/Th2 response. These results highlight that the mammalian expressed M2e soluble proteins as a promising vaccine development platform.

Development of live attenuated influenza vaccine in collaboration with start-up industry: The team is supporting industry/start-ups in the development of novel live attenuated influenza vaccine for all age groups. They have established an Influenza cell culture system for growth and characterization of Influenza vaccine strains *in vitro*, which can be further evaluated *in vivo* model. The team has also developed fluorescent labelled Influenza viruses, a powerful tool for *in vitro* and *in vivo* studies.

A mouse model for Influenza research: Inbred BALB/c mouse strain were intranasally inoculated with mouse-adapted Influenza/A/PR8 /1934 strain and 2009 Influenza/A H1N1 pandemic strains to identify virulence signatures *in vivo*. They were observed for signs of infection. The mice demonstrated symptoms of acute viral disease and lethal infection. In another ongoing immunogenicity studies, mice were challenged with the virus and are monitored each day for clinical signs of disease for at least 10-14 days. The BALB/c mice are also used being used for immunogenicity assessment of novel vaccine candidates.

Way ahead: The Horizon 2020 DBT-EU next-generation Influenza vaccine program includes partners and multiple PIs across the globe (the US, Europe, and India). The five-year program aims to deliver affordable flu vaccine with an increase, or high efficacy at low cost and possible exploitation and further improved flu vaccine concept dealing with technological shortcomings. To achieve these goals, R&D institutes, SMEs in EU and institutes, and private industry partners from India, USA, and Europe with excellent, complementary expertise and know-how have joined forces. Under this program, THSTI will establish a ferret facility, the first one in the country. Besides, Prof. Gagandeep Kang will lead the development of a Controlled Human Infection Model (CHIM). This will advance novel vaccine candidates to Phase I and Phase IIa clinical trial in partnership with CMC, Vellore. One path forward would use a highly coordinated and iterative approach in which through clinical trials of novel vaccine candidates, correlates of protection and immune factors identified will further help in designing and optimizing new generation vaccine candidates.

Validation of Structurally Occluded conserved epitopes as the new addition towards universal Influenza vaccine development and implementation towards respiratory viruses: The emergence and re-emergence of novel respiratory viruses (nCoV-2019, SARS) along with influenza have underlined the necessity for the development of therapeutic and preventive strategies to combat viral infection. In recent times, the infectious disease has appeared and reappeared in a more virulent form or a new epidemiological setting. Influenza virus exhibits high antigenic diversity with antigenic drift, shift, and reassortment. Hence, an urgent need of universal influenza vaccine is being focused towards novel concept of targeting highly conserved structurally occluded sequence, which has been shown to elicit antibody response with full and cross-protection. Reports show that pandemic H1N1 isolates exist as monomers in solution. Recent serum antibodies repertoire analysis has shown the presence of antibodies that binds to highly conserved residues on monomers, which are occluded in trimeric HA formation. The proposed intra-monomer sequences are reported to be conserved in at least more than 4468 sequences studied from HA (H1 and H3) sequence database. To target and validate the concept of Influenza HA monomer displaying structurally occluded epitopes as an ideal target for universal vaccine development, the methodology for possible generation of structurally stabilized HA

monomer using H1N1 pandemic strain (H1N1) as starting model and further the immunogenicity of the designed candidate was standardized by Dr. Srivastava's team.

The antigenic potential of the purified trimers (pandemic and chimeric cH3/H1) and generated monomers were further characterized and validated using western blot, hemagglutination assay, ELISA, and bio-layer interferometry assays. The purified protein show recognition with anti-his and anti-HA antibodies in western blot analysis, HA assay's up 1:64 times dilution with 1% chicken RBCs, and exhibit picomolar binding affinity towards monoclonal antibody CR6261. Stabilized protein (by selectively structure-based mutation) upon thrombin cleavage generated a well-stabilized oligomer with no aggregation in solution. Monomeric subunit also exhibits the Hemagglutination assay, ELISA and shows nanomolar affinity with antibody CR6261 at bio-layer interferometry assays. The cH3/H1 construct prepared by replacing the globular head of the H1 subtype with H3 also recognizes and shows an affinity towards the CR6261 antibody, which often abolish in the H3 strain having Asn38 in place of His38 (H1 backbone). Asn38 gets glycosylated in H3 subtypes and abolish binding of broadly neutralizing antibody CR6261; however, designed cH3/H1 construct retains the head-based property of H3 subtype and stem domain-based characteristics of H1 (**Figure 1.17**).

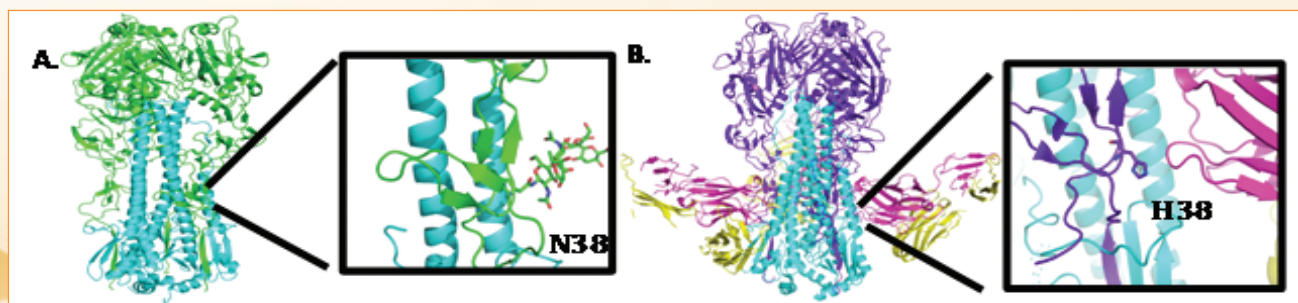


Figure 1.17: (A) Trimeric HA from H3 subtype displaying glycosylated Asparagine at 38 position abolishing Cr6261 reactivity (B) Probable model for cH3/H1 displaying conserved Histidine at 38th position from H1 stalk part

Once fully characterized, they tested these constructs for its immunogenic potential in BALB/c mice in prime-boost based immunization regimen. Three groups of animals with five animals each, and a control group which received PBS+adjuvant, were immunized with different proteins. Group one received trimeric HA protein from pandemic strain, and group 2 received monomeric protein, whereas group 3 received cH3/H1 and monomeric H1 in a 50-50 ratio. The pre-bleed was collected from all animals, including the control group, to check for any pre-existing immunity for the Influenza virus. Under the immunization regimen, they immunized the animal at 0 days and boosted the animal at 21 days. Fourteen days after each immunization, they collected the blood to check

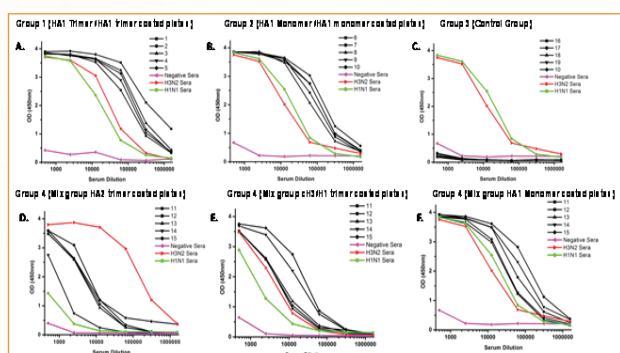


Figure 1.18: A) HA1 trimer and B) HA1 monomer specific antibody titers (IgG), evaluated using ELISA with protein immunogens coated plates followed by incubation with serial diluted sera, C) control group animal response showing no preexisting immunity, D, E and F) shows the IgG response of animals received cH3/H1 and monomeric H1 protein towards, H3N2 (HA2), chimeric trimer and monomer coated plates.

the antibody response. Each protein immunized group shows a very high antigenic titer upon a single boost. The response of group 2 animals, which were immunized with monomeric protein, was found to be similar to that of the trimeric group. Group 3, with a combination of the protein, shows the binding response towards H3, H1 as well as chimeric protein (**Figure 1.18**). The sera from protein boost in all the immunized groups were

further used to study the isotype class switching important for virus clearance and vaccine efficacy. The IgG isotype response was dominated by IgG1 and IgG2a antibodies in the sera from all the immunized groups.

Way ahead: The designed candidate, once established, will be evaluated for its potency for protection through a direct challenge or after passive immunization. The immune response in animals survived, recovered, or diseased after the challenge will be evaluated to designed study. Similar study design will be evaluated for designing better candidates with implementation and application towards other respiratory diseases.

ANTIMICROBIAL RESISTANCE IN BACTERIAL PATHOGENS

Antimicrobial resistance in bacterial pathogens and the human microbiome



Dr. Bhabatosh Das's

team is working on the gastrointestinal tract microbiome of the Indian population and investigating the role of gut microbiota in

(i) health and disease (including chronic and acute diarrhoea), and (ii) emergence of antimicrobial-resistant (AMR) enteric pathogens. The team is probing whole-genome sequences of bacterial pathogens to identify horizontally acquired genetic elements linked with AMR genes and understand the acquisition and dissemination mechanisms of antibiotic resistance traits. They are working to develop a strategy to reduce the rate of emergence of AMR pathogens.

Insights into the genome dynamics and interactions between core and acquired genome in enteric pathogens

Bacterial genomes are highly heterogeneous, ranging from 500 to 10,000 kb in size, composed of single or multiple circular or linear chromosomes (Chr) with or without extrachromosomal genetic elements. Almost all the bacterial species are hosts to horizontally acquired mobile genetic elements (MGEs), the agents of open source evolution. The bipartite genome of *Vibrio cholerae* harbours sporadic and conserved MGEs. The MGEs contribute to toxin production (CTXΦ),

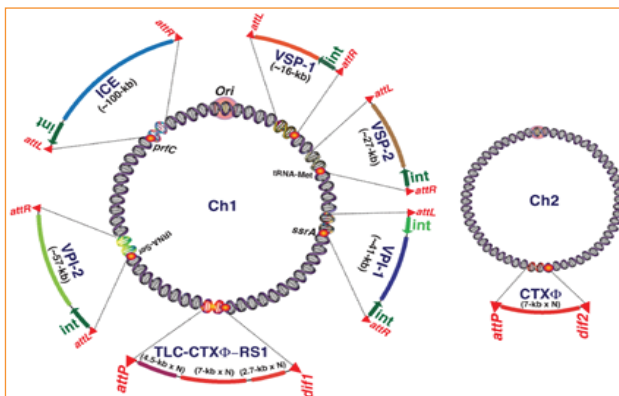


Figure 1.19

intestinal colonization (VPI-1), metabolic functions (VPI-2, VSP-1, VSP-2) and antibiotic resistance (ICEs) (Figure 1.19).

To understand the dynamics of MGEs in the bacterial genome, Dr. Das's team engineered the genome of *V. cholerae* to monitor *in vitro* and *in vivo* stability of different MGEs. These included genomic islands (GIs), integrative conjugative elements (ICEs), prophages, and cryptic genetic elements (Figure 1.20).

Recombinant vectors carrying the integration module of each of the GIs, ICE, and CTXΦ helped to understand the site-specific reversible and irreversible integrations of MGEs in the *V. cholerae* chromosome (Figure 1.21). They also investigated

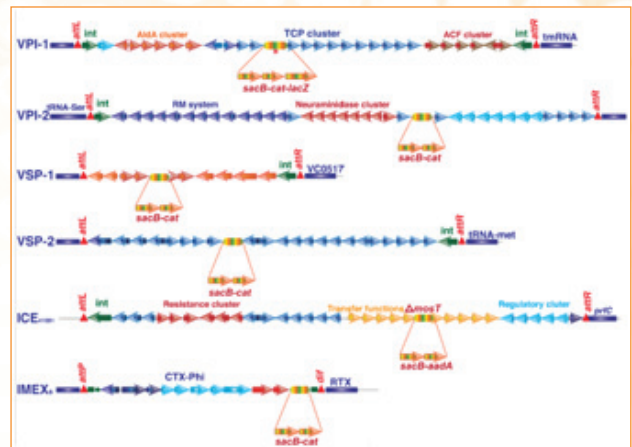


Figure 1.20

the cross-talk between MGEs and the core genome in the cholera pathogens. They deleted more than 250 acquired genes from six different loci in the *V. cholerae* chromosome. This revealed the contribution of CTX-prophage in the essentiality of SOS response master regulator LexA, which is otherwise not essential for viability in other related bacterial species, including *Escherichia coli*. In addition, they observed that the core genome encoded RecA helps CTXΦ to bypass the host bacterium immunity and allow it to replicate in the host cell in the presence of similar prophage in the *V. cholerae* chromosome. We observed that two GIs provide a competitive growth advantage to the O1 El Tor over the classical biotype in nutrient-limited growth conditions. Finally, multiomics data reveal the importance of MGEs in modulating the levels of cellular proteome and metabolome in *V. cholerae*. This study, for the first time, engineered the genome of *V. cholerae* to remove all the GIs, ICEs, and prophages from their genome and revealed new interactions between core and acquired genomes. The engineered strain could be a potential candidate for understanding the evolution of cholera pathogen and the development of new therapeutic interventions.

Way ahead: (i) Study the stability of mobile genetic elements linked with antimicrobial resistance genes in the genome of bacterial pathogens.

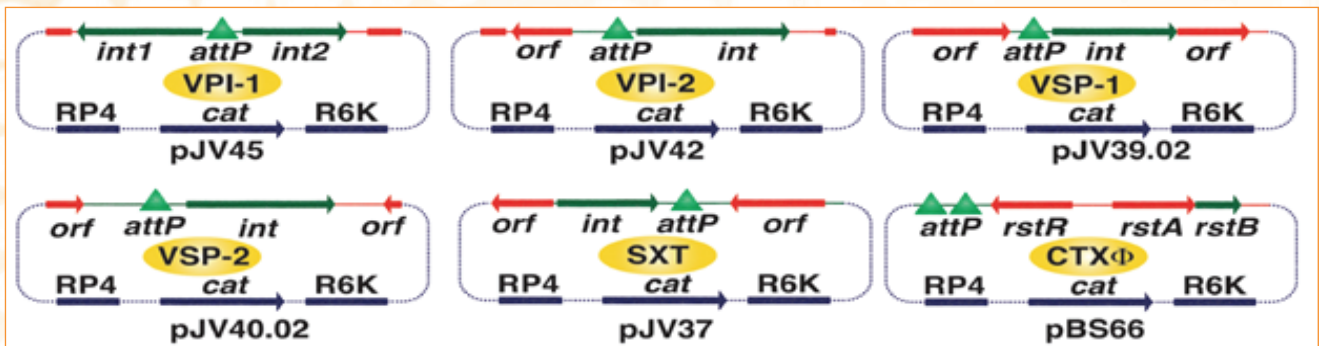


Figure 1.21

(ii) Identify the target gene(s) associated with the stability and vertical transmission of mobile genetic elements in the bacterial pathogens.

CHIKUNGUNYA



Dr. Rajesh Kumar's team is working on the isolation and characterization of monoclonal antibodies for Chikungunya virus (CHIKV) envelop glycoprotein and their

potential utility. The envelope glycoprotein is a trimer of heterodimers of E1 and E2. Both E1 and E2 participate complementarily in CHIKV cell entry. The E2 is mainly responsible for cell attachment and E1 helps in fusion and promotes viral membrane fusion within acidified endosomes to release CHIKV nucleocapsid into the host cell cytosol. The E2 glycoprotein

is found to be the major target of neutralizing antibodies in naturally acquired immunity in CHIKV infected patients with cleared viremia. The mature E2 protein forms 3 immunoglobulin like fold structure and is made up of three domains i.e. A, B & C. Recent studies have shown that CHIKV enters into the host cells via two different mechanisms i.e. one via glycosaminoglycans (GAGs) dependent via domain B and other GAG independent mechanism via domain A. Domain C does not play any role in virus entry. These studies give us a clue that domain B and A could be promising target for developing neutralizing antibodies-based therapeutics. They used domain A and B construct expressed in both bacterial and mammalian system. These purified proteins were used to immunize mice. The immunized sera from these mice were tested for their binding and neutralization potential. The best neutralizing animal will be scarified for mAb production by two different technology platforms (hybridoma and Phage display) (Figure 1.22). These studies are expected to help understand if domain B could be a promising target for developing neutralizing antibodies-based therapeutics.

Way ahead: The isolated mAbs will be further characterized for their neutralization potential against different CHIKV lineages and related alphavirus. The potential neutralizing mAbs will be further tested for their therapeutic potential in suitable animal models.

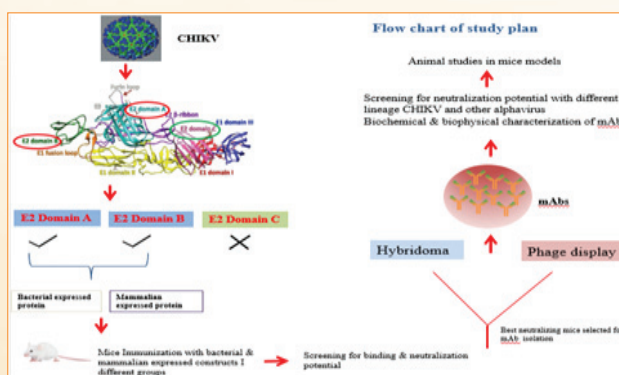


Figure 1.22







MATERNAL AND CHILD HEALTH

MATERNAL AND CHILD HEALTH

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

The focus of maternal and child health program is on discovery for developing tools for diseases that have public health significance, evaluating interventions for reducing early life morbidity, creating a novel infrastructure for research and developing capacity among young investigators. It is a platform for the maternal and child health interdisciplinary team to generate extramural grants and collaborate with hospitals to fulfil this mandate. The team aims to achieve the translational goals through two approaches:

- Observational studies that can utilize multidisciplinary contributions in discovery research to develop novel biomarkers and interventions that facilitate the translational pipeline.
- Randomized controlled trials (RCT) of interventions that will have a quick impact on improving childhood outcomes

GARBH-INI: Interdisciplinary Group for Advanced Research on Birth outcomes – DBT INdia Initiative:

Led by **Prof. Shinjini Bhatnagar** GARBH-INI is a THSTI flagship and a DBT Mission Programme. Close to 8000 pregnant mothers have been enrolled with documentation of 6000 pregnancy outcomes in a cohort established in Gurgaon



Civil Hospital to study preterm birth. GARBH-INI cohort had enrolled 7878 participants into the cohort and 6577 of them have completed their pregnancy as on

March 22, 2020. The enrolment of the cohort was affected due to the COVID-19 pandemic and the clinical activities were being resumed gradually when this report was drafted. The goal for the next few months is to complete enrolment of 8000 participants into the cohort and here are the specific objectives of the cohort:

- Development of population-specific gestational age assessment models using fetal biometry, metabolite features and ultrasonographic images
- Focused evaluation of specific risk factors (clinical and molecular) that may predispose to preterm birth (PTB), so as to identify novel preventive interventions
- Multi-omics approach for identification of molecular determinants of normal pregnancy & for risk stratification of preterm birth: Complete epigenomic, proteomic, microbiome analysis on 20 term and 20 preterm infants.
- Multi-Omics Signatures of Human Placenta: Evaluation of real time assessment of placental markers for prediction of PTB, placental function and development. Develop

biomarker panels to help stratify pregnancy phenotypes based on placental molecular markers to assist clinical management and provide timely intervention.

Key findings are summarized below:

- High Preterm birth (PTB) at 3%, with high rates of other adverse (stillbirth rate 2.3%, low birth rate 27%, small for gestational age 38%) birth outcomes
- First trimester dating formula (called Garbhini-1) developed from the biometry of the fetus from mothers enrolled in the GARBH-INI cohort found to be more sensitive and accurate than global formulae. Dating formulae for later trimesters are being developed; metabolite panels are being assessed for postnatal dating, particularly useful for pregnant women who visit hospitals for the first time in pregnancy with labour. These formulae and metabolic panels will contribute to accurate phenotyping of preterm birth, a critical requirement for optimal management of these babies.
- Initial univariate analyses have shown that maternal body mass index and biomass fuel use and/passive smoking has higher risk for PTB. In a more in-depth analysis, associations are being examined between dietary patterns, quality of dietary protein intake and pregnancy outcome to identify dietary patterns associated with optimum fetal growth and adequate gestation. The second important evaluation is to study the dose-response relationship and windows of vulnerability between air pollution exposure & birth outcomes.
- A multi-omics approach is being used for identification of molecular determinants of

pregnancy and for risk stratification of PTB. Specific salivary and high vaginal proteins, and vaginal microbiome have been documented to alter across normal pregnancy. As a next step multi-omics signatures for PTB are being identified, with evidence in variances in DNA methylation, vaginal microbiome and first trimester maternal metabolites in mothers with preterm.

More detailed integrated analyses with proteomic data for functional characterization of the microbiome changes is ongoing.

GARBH-INI platform to study other important adverse pregnancy outcomes

GARBH-INI cohort provides a unique opportunity to study fetal growth restriction (FGR) and stillbirth. This will be the first such systematic study of these two adverse pregnancy outcomes that have major public health implications. The team's initial analyses show a prevalence of 12% for small for gestational age as assessed by the ultrasound at 30-32 weeks of gestation. This increases to 38% at birth, indicating that late FGR is three-fold more prevalent than early FGR. Over the next year, they will identify specific risk factors of early and late FGR which will help design interventions to be tested by RCTs

Fetal growth restriction (FGR):

The primary goal of **Dr. Ramachandran** is to develop clinically translatable solutions for preterm birth and fetal growth restriction. His research



efforts are brought to fruition by building them upon the platform provided by GARBHINI program. Although gestational weight gain of

Indian women can be a simple surrogate marker of maternal nutrition and fetal growth, it has not been documented longitudinally in a large series in India till now. The team's data shows 75% women gain less total gestational weight when followed across their pregnancy; the 50th centile weight gain of these women at 40 weeks compares with the 10th centile of global standards. The study objectives include, (i) Description of the longitudinal trajectories of fetal growth and (ii) Evaluation of determinants of fetal growth & risk factors of early and late FGR.

Descriptive analysis of longitudinal fetal growth is complete. Prevalence of small for gestational age as assessed by the ultrasound at 30-32 weeks of gestation is 12%. This increases to nearly 38% at birth, indicating that late FGR is three folds more prevalent than early FGR in the study population. Evaluation of the risk factors of early and late FGR in the population will be done over the next year. Identification of risk factors of FGR will help design interventions to be tested by RCTs.

Gestational weight gain (GWG) & birth weight:

Dr. Ramachandran estimated the independent association between gestational weight gain and birth weight and assessed the longitudinal correlation of the gestational weight gain and fetal growth across pregnancy. Around 27% of the women had iGWG at 18-20 weeks & 43.3% at the time of delivery. First trimester BMI, parity, and social factors like type of family and religion are independently associated with GWG. There are 6% lesser odds of delivering a small-for-gestational age (SGA) baby with every kilogram increase in GWG after adjusting for confounders. Over the next year, the team will complete the GWG trajectories analysis and assess their relationship

with fetal growth. Identification of risk factors of inadequate GWG will help design interventions to be tested by RCTs.

*Predicting small-for-gestational age delivery (SGA):
(In collaboration with University of Bergen, Norway)*

Objectives:

- To evaluate accuracy of globally published predictive models for SGA in our population
- To calibrate published models using Indian population data
- To develop population-specific predictive models for small for gestational age

Evaluation of seven models is complete and calibration underway. Development of population-specific models will be done over the next year. Accurate prediction models for SGA will enable triaging and early referral to appropriate care.

Accurate estimation of gestational age:

Objectives:

- To compare different methods of estimation of gestation age
- To develop population specific dating models for different trimesters of pregnancy

Mean difference between USG-Hadlock and LMP-based dating at the time of enrolment is -0.44 ± 2.02 weeks; LMP-based method overestimates GA by nearly three days. At an individual level, only 50% agreement on those as to who were labelled as preterm. The first trimester dating formula (called Garbhini-1) developed from the biometry of the fetus from mothers enrolled in the GARBH-INI cohort was found to be more sensitive and accurate than global formulae.

Way ahead: Dating formulae for later trimesters are being developed. Development of a pregnancy dating model for second and third trimester are both in progress. Better pregnancy dating models will ensure accurate timing of clinical care

Modifiable risk factors associated with pregnancy outcomes:

Dr. Ramachandran's team is studying specific risk factors of PTB including ambient and household air pollution and its relationship with pregnancy outcomes.

Objectives:

- To estimate the association between ambient and household air pollution and (i) PTB, (ii) FGR
- To standardize the exposure assessment methods for ambient and household air pollution

Ambient air pollution monitoring was done for 350/550 sq.km for a period of six months. Geo-coding of participant addresses was completed. Around 200 participants enrolled and 85 followed up for exposure assessment. Study has been halted temporarily due to difficulties in field work and participant household visits. Efforts made to move towards increasing the satellite component of the ambient air pollution monitoring.

Way ahead: Personal exposure monitoring for 100 participants will be done over the next year. Enrolment and follow-up of 800 participants will be done to document their exposure.

Maternal nutrition:

Maternal micronutrients (Selenium) and associations with pregnancy outcomes: There are significant differences in levels of specific micronutrient between mothers who deliver term

vs those who deliver preterm. Selenium assays have now been standardized on the ICP-MS by using Tellurium (10ppb) as an internal standard.

Way ahead: Over the next year selenium estimation for 2000 participants and Vitamin D, Zinc, Chromium and Iron in at least 500 participants at 3 time points at enrolment will be done.



Maternal Stress:

Dr. Nitya Wadhwa's team is assessing stress outcomes on pregnancy, fetal growth and birth weight. The goal is the development of methods

to identify mothers at risk of PTB and intrauterine growth restriction resulting from maternal stress.

Objectives:

- To complete the follow up of the stress study cohort of pregnant women
- To publish the results after completion of biological analysis for telomere length, hair shaft cortisol levels and biochemical measurement of oxidative stress by plasma carbonyl and proteomic quantification of carbonylated proteome

Recruitment of participants was concluded in December 2018 with a cohort size of 2164 women. Dr. Wadhwa's team was able to administer the psychological instrument for assessing stress for the second time (at 26-28 weeks) and also document a pregnancy outcome in only 1,330 of the 2,164 women enrolled. Six hundred fifty-seven women out of the 2,164 missed their second follow up at 26-28 weeks. In the remaining 177 women, they were unable to document the pregnancy outcome although they did come for their 26-28 week follow up.

- Of the 1,330 women with complete follow up, 14 women had intrauterine deaths. The remaining 1,316 women delivered live babies. 154 were preterm and 1,162 term births.
- Biochemical, genomic and proteomic analysis is ongoing. Hair shaft cortisol estimation of enrolled women at 18-20 and 26-28 weeks is complete. The telomere length and plasma protein estimation are ongoing. An integrated time series analysis to identify clinical and/or biological markers of stress that can be used to predict women who will go on to have an adverse pregnancy outcome is planned.

Way ahead: Complete biologic analysis of 'stress study' and complete integrated analysis for the project.

Multi-omics signatures of human placenta and PTB



The major focus of **Dr. Pallavi Kshetrapal's** team is to study the role of placenta in adverse pregnancy outcomes, primarily PTB. Placenta is a highly specialized

transient organ of pregnancy that facilitates the exchange of nutrients and waste between the mother and her developing fetus. Placental insufficiency has been implicated in a range of serious pregnancy complications such as pre-eclampsia, intrauterine growth restriction (IUGR), recurrent spontaneous abortions, etc. As the placenta is difficult to obtain during pregnancy, surrogate markers of placenta in maternal circulation are ideal tools to study placental functions in real time. With these goals the targets for 2019-2020 were:

- Isolation and characterization of the placental enriched extracellular vesicles
- Profiling the protein cargo of the placental EVs isolated from maternal blood of term and preterm delivering mothers

Development of human placental research initiated on the GARBH-INI platform as a multidisciplinary interinstitutional program and apply for dedicated funding.

Placenta as a fetomaternal organ governs the outcome of pregnancy by functioning as an immunotolerant zone from implantation to growth of the semi-allogenic fetus. It prepares the mother's body by sending out signals in the form of hormones secreted by the placenta or messages encapsulated in the extracellular vesicles (EVs) carried as mRNA, proteins and lipids, into the mother's circulation to enhance growth and optimal development of the fetus. These EVs of endosomal origin reflect the contents of their parental cells, in this case the placenta.

To profile the protein cargo of the circulating placental extracellular vesicles from mothers delivering term and preterm births:

Dr. Kshetrapal's team conducted a nested case control within the longitudinal study design of GARBH-INI to determine the protein cargo present in circulating placental EVs in maternal plasma of term and PTB across gestation (i.e. first, second and third trimester). Placental-derived EVs were enriched from the total EV population based on their expression of membrane-bound placental alkaline phosphatase (PLAP). The analysis revealed that there was no change in characteristics (i.e. size, shape and markers) for the PLAP+EVs between the two birth outcomes. However, there were differences in numbers across gestation with low levels observed in PTB. A comparison

between the PLAP+EV proteome from term and PTB revealed 96 proteins differing significantly ($p < 0.05$, False Discovery Rate 1%) across gestation. Bioinformatics analysis of differentially expressed proteins revealed consistent upregulation of inflammatory pathways, upregulation of epithelial mesenchymal transition pathways at term and down regulation of coagulation/complement activation in preterm. They had collaborated with domain experts globally, to gain expertise in the field of exosome isolation and purification and have been successful in reproducing and improvising the protocols at our lab for future experiments.

Way ahead: The plan is to develop in vitro cell culture systems, explants and/ or primary placental culture as molecular biology tools to study the role of these candidate genes in context of placental development and function.

To decipher the role of placental EVs as vehicles of immunomodulation during human pregnancy: Human pregnancy has been reported as a period of immune inertness or as a “Th2 phenomenon” since T helper type 2 (Th2) cells are classically considered to be involved in anti-inflammatory responses. The balance between the Th1 and Th2 state in pregnancy is an important factor to decide on the outcomes of pregnancy. Recent research has demonstrated that tumor cells can produce or promote the production of immune-modulating exosomes, and how the contents of these exosomes support cancer progression and immune escape. Similarly, placental EVs (pla-EVs) have been reported to impart immune privilege to fetus due to presence of immunosuppressive cargo, that maintains an immune tolerance zone at the maternal fetal interface of the placenta. The objective is to

understand the immunomodulatory properties of these exosomes on the maternal peripheral immune system.

Dr. Kshetrapal could optimize the isolation of placental released EVs from maternal plasma and their subsequent characterization. These have been further standardized for yield of particle number and protein content. Initial experiments optimizing the co-culture of pla-EVs with the PBMCs derived from healthy individuals reveals lowered activation of anti-CD3 and anti-CD28 stimulated CD4+ T cells.

Way ahead: She plans to investigate the phenomenon of fetal tolerance-promoting modulations in the maternal immune system by the placental released EVs. These will be studied by checking for T cell priming and functionality at the level of proliferation, apoptosis and activation using flow cytometric assays, post T cell co-stimulation in the presence of preterm and term pla-EVs.

GARBH-INI has been invited to join the Multi Omics of Maternal & Infants Global Consortium (MOMI) where multi omics signatures will be identified in over 1500 mother infant dyads pooled across five global cohorts. Interesting questions are being addressed within the cohort framework using AI based applications for developing prediction models based on clinical & epi factors, image features from fetal biometric & transvaginal cervical images and doppler parameters. Work in progress is for two exciting applications; predicting preterm birth using ultrasound images and Computer-Assisted Low-cost Point-of-care Ultrasound based on specific sweep videos which can be used as a triaging tool in smaller hospitals.

Vaginal Microbiome:

Dr. Bhabatosh Das's group is studying the vaginal microbiome of Indian women and examining (i) role of vaginal microbiota in the birth outcomes (ii) specific microbial genomic signatures positively or negatively associated with PTB. His team is isolating different *Lactobacillus* species from the vaginal milieu of Indian women, decoding their genome sequences and looking for functions associated with PTB.

Insights into the vaginal microbiome and genome of *Lactobacillus* species isolated from the Indian women:

Microbial species present in the vaginal milieu of reproductive age women showed a large personal component and varies widely in different ethnic groups at the taxa, genomic and functional levels. Vaginal microbiota of the reproductive age women plays an important role in resistance against colonization of nonindigenous microbiota in the reproductive tract and prevention of urogenital diseases including bacterial vaginosis, sexually transmitted infections and fungal and viral infections in the urinary tract. Additionally, vaginal microbiota of the biological mother is a major determinant of the composition of gut microbiota of vaginally delivered new borns. We currently lack an understanding of the baseline vaginal microbiota of reproductive age Indian women, the extent of taxonomic and functional variations of vaginal microbiota between individuals, and the genomic repertoires of the dominant vaginal microbiota.

In their study, Dr. Das's team analyzed the metagenome of high vaginal swab (HVS) samples collected from 40 pregnant Indian women enrolled in the GARBH-INI cohort. Composition and abundance of bacterial species was

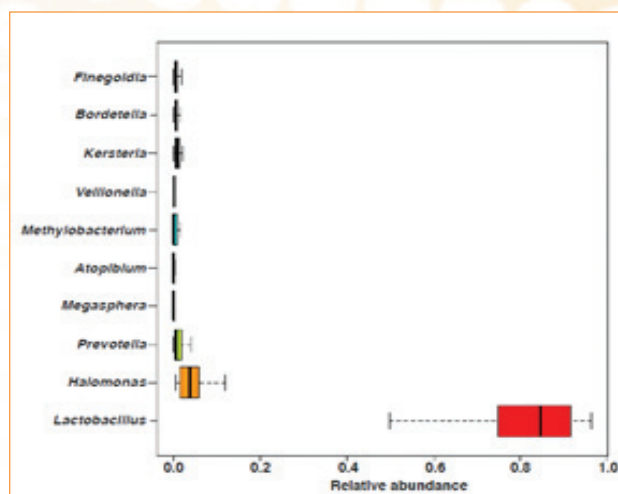


Figure 2.1: Boxplots showing abundant bacterial taxa in the high vaginal swab (HVS) samples (n=40). Bacterial taxa were identified based on the 16S rRNA gene sequence homology. Relative abundance was measured based on the number of sequencing reads. *Lactobacillus* is the most abundant metagenomically identified bacterial genus in the HVS samples. Boxes correspond to interquartile range. The central line indicates median relative abundance. Lines extending vertically from the boxes presenting variability.

characterized by pyrosequencing 16S rRNA gene. The sequencing depth employed revealed a total of 123 bacterial genera mostly belonging to 4 different phyla Firmicutes (85%), Proteobacteria (9%), Bacteroidetes (3%) and Actinobacteria (2%). Three bacterial genera (*Lactobacillus*, *Halomonas* and *Achromobacter*) were detected in all the women (n=40) sampled, while 7 bacterial genera (*Bordetella*, *Kersteria*, *Prevotella* and *Pigmentiphage*) were detected in 90% (n=36) women. Ten most abundant bacteria in the 40 HVS samples are shown in **Figure 2.1**.

Comparative analyses of the relative abundance in all the subjects revealed that the vaginal microbiomes of our population are mainly dominated by the genus *Lactobacillus*. The ten prominent species of *Lactobacillus* (*L. iners*, *L. crispatus*, *L. gasseri*, *L. johnsonii*, *L. jensenii*, *L. vaginalis*, *L. fornicalis*, *L. acidophilus*, *L. coleohominis* and *L. pontis*) with relative abundance >0.001 are shown in **Figure 2.2**.

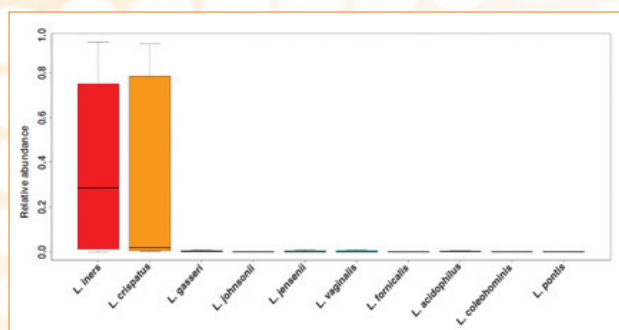


Figure 2.2: Relative abundance of different *Lactobacillus* species in the high vaginal swab (HVS) samples (n=40). Bacterial taxa were identified adopting metagenomic approach. *L. iners* and *L. crispatus* are two most dominated *Lactobacillus* species. Lines extending vertically from the boxes presenting variability.

Several species of *Lactobacilli* were clustered into three Community State Types (CSTs) (**Figure 2.3**). *L. iners*, *L. crispatus*, *L. gasseri* and *L. jensenii* are most frequently detected *Lactobacillus* species in the vaginal environment of Indian women. Other than *Lactobacillus*, several species of *Halomonas* were also identified in the vaginal environment of most of the women sampled. To gain genomic and functional insights, we isolated several *Lactobacilli* species from the HVS samples and explored their

whole genome sequences. We analyzed the genome of four dominant *Lactobacillus* species, *L. iners*, *L. crispatus*, *L. gasseri* and *L. paragasseri* to represent the CSTs and identify functions that may influence the composition of complex vaginal microbial ecology (Table 2.1). This study reports for the first time the vaginal microbial ecology and genomic insights into *L. iners*, *L. crispatus*, *L. gasseri* and *L. paragasseri* isolated from Indian women.

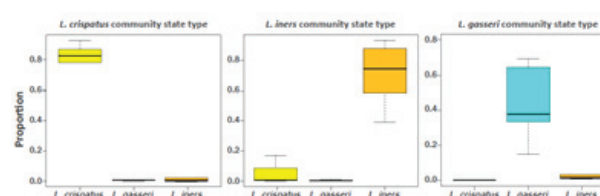


Figure 2.3: Bacterial community state types (CSTs) observed in the 40 pregnant women. The CSTs were inferred based on the Silhouette index. The CST dominated by *L. iners* is most prevalent (n=20) followed by *L. crispatus* (n=14) and *L. gasseri* (n=6), respectively.

Way ahead: Sequence and analyze the genome of *Lactobacillus* species isolated from the vaginal milieu of reproductive age Indian women.

Table 2.1. Relevant whole genome sequencing information of different *Lactobacillus* species isolated in the present study

Characteristics	<i>L. iners</i> Indica	<i>L. gasseri</i> Indica	<i>L. crispatus</i> Indica
Date of Isolation			
Sequence size (Mb)	1.33	2.09	1.64
No. of scaffolds	1	5	59
Median sequence size (kb)	1331.11	22.42	21.28
GC content (%)	33.2	34.9	37.3
N50	-	1845454	33495
L50	1	1	17
Longest scaffold size (bp)	1331119	1845454	87794
Number of sub-systems	162	266	172
Total number of ORF	1237	2331	1733
Number of RNAs	88	57	26
ORFs encode virulence, disease and defense	17	50	20
Phage, Tn, Plasmid related ORFs	4	33	6

Maternal Infection and Inflammation:



Dr. Shailaja Sopory's group focuses on infections and inflammation during pregnancy and in neonates with consequences on growth and development.

Objectives:

- Establish a profile for expression of pro- and anti-inflammatory cytokines across normal pregnancy.
- Extend the pregnancy cohort into a birth cohort, to look for association between antenatal exposure on the infant's growth, neurodevelopmental and metabolic outcomes.

As a delicate immune balance is maintained during pregnancy, any untimely or sudden onset of abnormal inflammatory events can disrupt this balance leading to FGR and SGA. Her team has started by identifying self-recorded cases of infections during pregnancy and their association with FGR/SGA. Initial analysis showed bacterial vaginosis (Odds Ratio=1.26, $p=0.02$), gastroenteritis (Odds Ratio=1.49, $p=0.007$) and cough (Odds Ratio=1.32, $p=0.0005$) at any time during pregnancy as risk factors for SGA and cough (Odds Ratio=1.37, $p=0.002$) at any time during pregnancy as risk factors for FGR. To look at inflammation across pregnancy, the approach is to use a multiplex bead-based assay to simultaneously look at multiple cytokines across normal pregnancy and then in pregnancies with outcomes of SGA/FGR. Her team standardized their assays on serum samples collected cross-sectionally during pregnancy. Of the 20 cytokines analyzed, 4 were not detected in any of the

samples and will be excluded in the future assays. Data from 6500 participants has been screened for eligibility criteria to be included for analysis on normal pregnancies and for association between SGA and FGR outcomes and both analyses are in progress.

Way ahead: (i) Complete assays and analysis on inflammatory markers in across pregnancy, (ii) Study differences in expression of various analytes (which include C-reactive protein, Alpha-1 glycoprotein, VEGF, sFlt to name a few) between women delivering term vs preterm (collaboration with the MOMI consortium), (iii) Study associations between antenatal inflammation and infant growth profiles, various developmental and neurodevelopmental outcomes in the second phase of GARBH-INI (starting from December 2020).

Multidimensional omics for predictors of PTB

Metabolomic analysis for clinical biomarker discovery and prediction models: Metabolomics is closely linked to the functional phenotype, since the metabolites mirror the dynamic processes that occur in the cell and are the end-products of the biochemical pathways. Dr. Kshetrapal's team is using both targeted and untargeted platforms for biomarker discovery of PTB and development and validation of prediction models for postnatal age assessment.

- Sera Metabolomics for PTB:** The team has acquired data as a discovery cohort on the metabolites (using LC-MSMS; untargeted approach) on maternal sera collected in the first trimester of pregnancy (<20 weeks) from mothers delivering term ($n=61$) and preterm ($n=61$). Data is being analysed on the Metaboanalyst 4.0 (online, R-based GUI platform) using a defined plan of analysis.

b) **Validation of a metabolite panel for postnatal assessment of gestational age (GA) on cord blood and neonate heel dried blood spot in low and middle-income resource settings in India:** They collected 1031 paired cord blood and neonatal blood taken as heel prick. They measured endocrine markers (TSH and 17-OHP) using PerkinElmer AutoDELFIA Immunoassays; the amino acids and acylcarnitines (a panel of 4 acyl-carnitines (C5, C16, C18:2, C5DC), three amino acids (alanine, leucine, tyrosine) using the NeoBase Non-derivatized MSMS kit on the Ab/Sciex 3200 MDQTRAP MS/MS platform. Multivariate models are being developed using GA (estimated by dating ultrasound) as gold standard and these analytes with relevant clinical covariates as predictors/independent variables. The data will be split into a training (70%) and testing (30%) data set and regression models will be applied to measure the performance of the models to predict postnatal gestational age. These metabolic panels will contribute to accurate phenotyping of preterm birth, a critical requirement for optimal management of these babies.

Way ahead: Integrated analysis of these data sets (clinical and molecular) will help identify molecular biomarker/s and development of models to predict preterm birth.

Augmenting research platforms

The GARBH-INI programme has helped to augment research platforms such as the data science hub for big data using global standards, and nutrition biochemistry labs for conducting micronutrient assays. 21CFR part II compliant Biorepository for GARBHINI, has expanded to host 850,000 different

types of maternal and neonatal biospecimens. Ultrasound image repository has longitudinally collected ~ 4 lakh Images and 400 videos. Both repositories are envisaged as national resources.

Pregnancy and COVID-19 pandemic

With the CoVID-19 epidemic, there is an urgent need to guide perinatal clinical management and the team has used this opportunity to study the epidemiology of COVID-19 in pregnant women and their babies.

MCH with a focus on early life morbidity and mortality and host immune response

Intervention for improving outcomes in early life infections:

Zinc as an adjunct for treatment of clinical severe infection in infants younger than 2 months: health gain, financial risk protection, and cost-effectiveness analysis

Objectives:

- To enroll 840 young infants with severe clinical sepsis
- Publication on “descriptive characteristics of participant population, across sites”
- Publication on “assessment of cost for hospitalization and its predictors in young Nepalese infants with sepsis

Till 30th April 2020, the study had enrolled a total of 2,464 infants, and 653 young infants in the one-year reporting period. There were 94 deaths (3.8%) during hospitalization for clinical sepsis. In the 12-week study period 188 deaths (7.6%) were documented. Overall treatment failure rate is 15% and severe illness requiring rehospitalization of the enrolled infants in the 12-

week follow up period is 9.3% (222/ 2,397). The overall lost to follow-up is <3%. Following closure of Kanti Children and Kasturba hospital sites, Dr. Wadhwa identified and approached Kalawati Saran Children's hospital and after necessary approvals and site set up, initiated the site in July 2019. With the concurrence of the DSMB they have engaged with an independent biostatistician who will make an assessment and recommendation to CISMAT (and the project management team) to find out whether it is worthwhile to continue the trial or it should be closed. Over the past year a grant was awarded by CISMAT (Centre for Intervention Science in Maternal and Child Health) and the site prepared for initiation of this sub study. Manpower recruited were trained in data collection and analytics. Data collection was initiated after Ethics Committee (EC) approvals of all participating hospitals.

Impact of COVID-19: Temporary halt of active screening and recruitment for both the main trial and this sub-study from 23rd March for about 2 months; However, the follow up of all infants already enrolled in the study was continued. Activities have resumed at all sites except MAMC which has been designated as a COVID-19 only hospital by the government.

Evaluation of interventions for early life morbidity in large Randomized Controlled Trials:

Evaluate efficacy of Vit D supplementation (vs placebo) given to 900 infants from birth to 24 weeks on host immune responses to OPV and BCG and postnatal growth:

Objectives:

- a) Complete the assays to estimate immune responses to OPV in all 900 enrolled infants.
- b) Measure markers of neutrophil activity and inflammation in a subset of 200 infants.

Efficacy of Vit D supplementation (vs placebo) was given to 900 infants from birth to 24 weeks was evaluated on host immune responses to OPV and BCG and postnatal linear growth. Almost all infants were Vitamin D deficient, 40% were neutropenic at 6 weeks. Markers of inflammation rose rapidly after birth and reached levels above those seen in developed countries. Univariate associations have been found between CXL13 levels (a marker of germinal centre activation) at 6 weeks and immune response to Hepatitis B vaccine at 24 weeks. Small but statistically significant associations were also found between markers of neutrophil activity and growth at some time points.

Evaluate efficacy of oral zinc in young infant sepsis for reducing case fatality and subsequent 12-week mortality across 7 hospitals:

Objectives:

- a) Enrolment of 840 young infants with severe clinical sepsis to accomplish the target sample size
- b) Initiate the extended cost effectiveness sub study within the ongoing RCT

Around 2500 infants have been enrolled across 7 hospitals in the ongoing RCT evaluating efficacy of oral zinc in young infant sepsis for reducing case fatality and subsequent 12-week mortality; 12-week overall risk of death is 8%. Intracellular zinc levels are lower in neutrophils & eosinophils in recovered patients. We have initiated equity studies on health gain with zinc intervention within the ongoing trial

Associations between the frequency and number of immune cells and intracellular zinc levels with recovery in infants with sepsis. Dr. Sopory's team has the hypothesis that changes in intracellular free zinc levels of immune cells on zinc

supplementation may alter the proliferation and activation status of various immune cells. Hundred more infants are to be enrolled in this sub study to complete the immunophenotyping. This study is embedded in a randomized double-blind placebo-controlled zinc supplementation trial of infants aged 3 days up to 2 months hospitalized with clinical severe infection in reducing case fatality during hospitalization. Immunophenotyping is being carried out to document the numbers and frequencies of different immune cells and the distribution of intracellular zinc at admission (V1), 48-72 h post supplementation (V2) and at discharge (V3).

Till date 297 participants have been enrolled in the study, 76 were added in the last year. FACS analysis was carried out on 566 samples for neutrophils and eosinophils and on 83 samples for monocytes, T and B cells, NK cells, NKT cells and dendritic cells. As it is a double-blind study the initial plan of analysis is to conduct a univariate analysis with the immune parameters as independent continuous variables and the time to recovery as a dependent (continuous) variable to estimate associations between immune parameters and the outcome variable. They are also storing samples from a subset of infants enrolled in the parent study for DNA and RNA extraction to understand host gene expression and identify etiology of sepsis.

Way ahead: The enrolments will continue till the proposed sample size is achieved and more robust analysis is possible. More data will be obtained on Monocytes, B, T, NK, NKT cells and dendritic cells besides neutrophils. The team plans to look into deep RNA sequencing for getting gene expression information on both host and pathogens.

Low birth weight:

Dr. Wadhwa's research is focused on advancing knowledge and contributing to generating evidence for prevention or treatment in early life infections and illnesses with a focus on micronutrients, smallness at birth, host response to infections and illnesses.

Evaluate the impact of continuous Kangaroo Mother Care (KMC) initiated immediately after birth compared to KMC initiated after stabilization in newborns with birth weight 1.0 to <1.8 kg on their survival in low-resource settings:

Objectives:

- Completion of the target sample size of 1,680 for the India site
- Initiate 24-month follow-up for neurodevelopment assessment of enrolled infants

Immediate KMC was evaluated in VLBW new borns in an RCT for improving survival in the first month of life in 4000 babies enrolled across five countries. Significant reduction in neonatal death, risk of hypothermia and sepsis has been seen in iKMC group. Neurodevelopmental impairment assessment at 24 months is ongoing.

Intervention for improving outcomes in neonates with low birth weight: A multi-country randomized clinical trial to evaluate the impact of continuous KMC initiated immediately after birth compared to KMC initiated after stabilization in newborns with birth weight 1.0 to <1.8 kg on their survival in low-resource settings. A multi-country randomized clinical trial to evaluate the impact of continuous KMC initiated immediately after birth compared to KMC initiated after stabilization in newborns with birth weight 1.0 to <1.8 kg on their survival in low-resource settings

To complete the target sample size of 1,680 for the India site and be aligned to the target of completing the overall estimated sample size of 4200 mother-infant dyads.

The team estimated that a total of 4200 infants would need to be enrolled to detect a 20% relative mortality reduction. The DSMB conducted two interim analyses, at 50% and 75% enrolment. After the second interim analysis in December 2019, the DSMB recommended stopping further enrolment in the trial because of clear benefit in neonatal mortality reduction. The project team has recruited 3,211 infants across all 5 sites; 1,377 (43% of total recruitments) from India. Analysis and manuscript writing 4-day workshop held. Neonatal mortality was a significant 24% lower in those who received KMC compared with those who received KMC after clinical stabilization (RR 0.76; 95% CI: 0.64–0.90; $p=0.001$). For the secondary outcomes, there was a significantly lower proportion of infants with suspected sepsis (22.9% vs 27.8%; adjusted RR 0.82, CI: 0.73–0.93; $p=0.001$) and hypothermia (5.6% vs 8.4%; adjusted RR of 0.65, CI: 0.52–0.83; $p<0.001$) in the iKMC compared with the control group.

Follow-up study of the main iKMC clinical trial:

The follow-up study to evaluate the impact of continuous KMC initiated immediately after birth compared to KMC initiated after stabilization in newborns with birth weight 1.0 to <1.8 kg on their neurodevelopmental outcomes in low-resource settings.

Site preparedness completed, manpower recruited and a training workshop was conducted on neurodevelopment outcome assessment in India and Tanzania. Data collection was initiated after Ethics Committee (EC) approvals of all the participating hospitals in addition to WHO ERC. The

India site had recruited 1,377 infants in the main iKMC study; 1,207 infants are the study population for the follow up study. The team enrolled 810 infants till March 2020; thereafter enrolments and follow up had to be temporarily halted.

Impact of COVID-19: Temporary halt of active screening and recruitment iKMC ND study from 23rd March for about 2 months; Activities resumed in June 2020.

Way ahead:

- Submission of and publishing the manuscript on main results for iKMC study
- Complete site close out activities with data archiving for iKMC study

Reduction of sepsis burden among neonates:

Sepsis is an uncontrolled/dysregulated host immune response to either an infectious or a non-infectious agent that the host recognizes as foreign. One of the major causes of sepsis is septicemia triggered by *Acinetobacter baumannii* (Acb) and other ESKAPE pathogens. It is unclear how Acb triggers sepsis in neonates and adults. Dr. Krishnamohan Atmakuri's group explores Acb-mediated pathogenesis as a model system to understand, diagnose, treat and alleviate sepsis.

Design, construction and utilization of recombinant Methionine tRNA ligase for exploitation of click chemistry strategies to understand Acb-mediated pathogenesis:

Prominent septicemia-causing organisms include ESKAPE pathogens. Often these being opportunistic, sepsis is thought to be due to host's dysregulated immune response. Several groups hypothesize that LPS or teichoic acid (TA) of ESKAPEs are key triggers for dysregulation. However, several clinical trials on LPS- and TA-specific inhibitors failed to see sepsis brought to

control. In contrast, administering appropriate antibiotics within first 48-72 hours of sepsis suspicion significantly reduces its progression. Case fatality rate among neonates with MDR- and XDR-ESKAPes is extremely high (~50%) primarily because of lack of accurate resistome profile. Since antibiotics resistance has no direct correlation with virulence, Dr. Atmakuri's group hypothesize that identifying and stopping pathogen secreted virulent functions will influence a positive outcome from sepsis. These secreted proteins of the pathogen are thought to derail the immune system to their advantage and induce the dysregulated immune response. Identifying these that may be present in minute levels in host blood is very challenging. Even direct mass spectrometry of host blood fails to detect pathogen proteins.

To address this challenge, Dr. Atmakuri's group employed click chemistry approach that involves metabolic labelling of pathogen proteome with specific amino acid derivatives for downstream enrichment and identification in the hay stack of host proteome. His group first aligned the crystal structures of Met tRNA ligase of *E. coli* and Acb. Though the primary sequence identity between these two proteins is <23%, their active

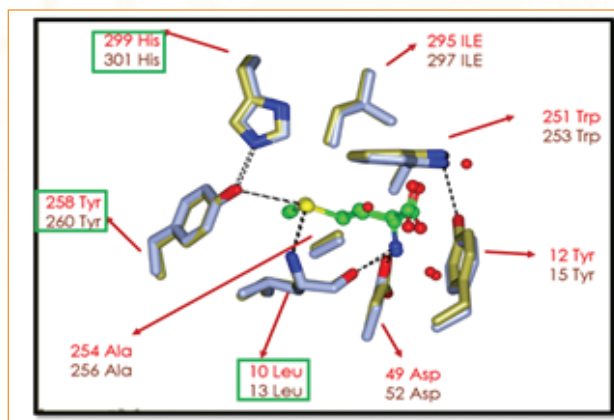


Figure 2.4: Active site of Methionine tRNA ligase of *E. coli* and Acb are conserved. Red numbers, Acb amino acids; Brown numbers, *E. coli* amino acids; Gold, *E. coli* Met tRNA ligase active site; Grey, Acb's Met tRNA ligase active site. Boxed in green; amino acids to be mutated to incorporate modified methionine.

sites are almost 100% conserved (Figure 2.4). Since it is prior established that altering three amino acids (boxed in green in Figure 2.4) in the active site of *E. coli* Met tRNA ligase to a different combination mutates the protein sufficiently enough to incorporate modified Methionine into *E. coli* proteome, this group too mutated the corresponding three amino acids of Acb Met tRNA ligase using site-directed mutagenesis.

The mutated Met tRNA ligase was then moved into a clinical isolate of Acb and its ability to incorporate modified Methionine into its proteome tested (Figure 2.5A). To enhance the incorporation of

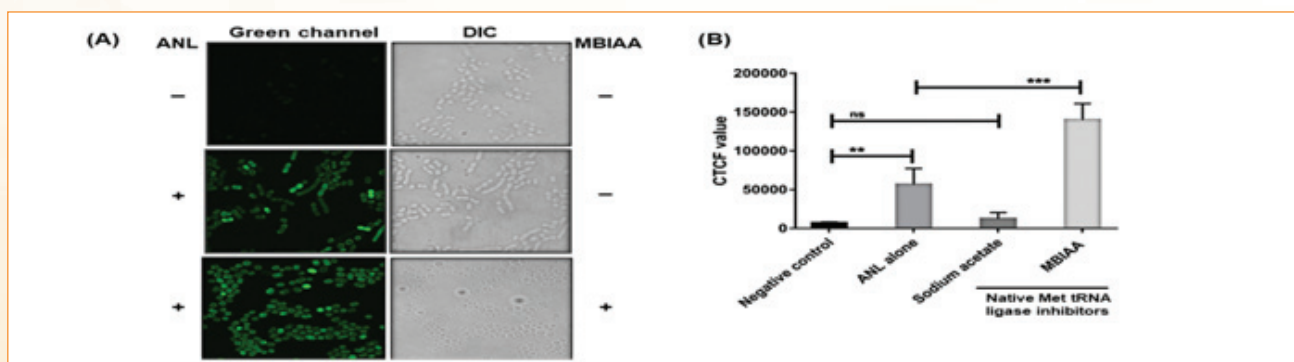


Figure 2.5: The recombinant Met tRNA ligase of Acb efficiently incorporates modified methionine (ANL). Recombinant Acb (mutant met tRNA ligase under an inducible (arabinose) promoter) grown to 0.1 OD (A600 nm), washed in 1X PBS, pH 7.4, divided into four aliquots and induced 10 hours with 1% arabinose. Except for negative control, rest added with 1mM of ANL for 14 hours incorporation. Third aliquot also received sodium acetate to 10 mM, 4th received 100µg/ml cocktail of amino acids except Met (MBIAA). After 14 hours of ANL incorporation, cells washed in 1X PBS, cell fixed for 15 minutes with 4% PFA and exposed with 25µM DIBO Alexa 488 alkyne for 1 hour, washed again to remove excess alkyne and mounted on slides and visualized on green channel (A) and correlated total cell fluorescence quantified (B) for comparative analyses, ** $p \leq 0.005$ (unpaired T-test). Since sodium acetate inhibited incorporation of ANL, the images are not shown in A. They behaved similar to negative control.

the modified Methionine and simultaneously reduce the incorporation of naturally available Methionine by native Met tRNA ligase, they further standardized conditions to inhibit function of native Met tRNA ligase (**Figure 2.5**). Their fluorescent microscopy analyses indicated that indeed the mutated Met tRNA ligase incorporates the modified Methionine into cells and their proteome (**Figure 2.5**) and inhibiting native Met tRNA ligase with a cocktail of other amino acids (at 100 ug/ml each) (but not sodium acetate (10 mM) significantly enhanced ANL incorporation (**Figure 2.5**).

Way ahead: Their immediate plan is to employ the click chemistry approach to identify secreted virulent proteins from three Acb clinical isolates in in vitro infections studies with alveolar macrophages, nasopharyngeal and lung epithelial cells. They intend to then shortlist those secreted proteins that are exhibited by all three Acb isolates in all three cell lines. Antibodies will then be generated against them and an array designed to detect Acb secreted proteins in blood of sepsis patients who are blood culture positive for Acb. Those Acb proteins that also get detected in sepsis patients will then shortlisted and knock outs generated in one clinical isolate. These recombinant Acb strains will then be tested for their potency to causes sepsis in a sepsis mice model. This will help them to identify the right virulent protein targets of the Acb that might be the key triggers for derailing sepsis.

Maternal nutrients and child health

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.

Dr. Gopinath's 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. At the date of project completion on 11th February 2020, a repository of 17 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). The second most predominant vitamin deficiency was observed with vitamin B12, where at least 70% of the women displayed levels less

than 200 pg/ml. 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. The team has identified at least 347 differentially expressed genes (DEGs) that are altered in MSCs that were treated with vitamin D and differentiated into the myogenic lineage for a week. Of these 347 genes, 130 genes belonging to the Human Skeletal Muscle Proteome are differentially regulated in vitamin D treated MSCs. Functional analysis of these genes indicated that the Gene Ontology terms corresponding to the upregulated genes are “peptide biosynthetic processes”, the “mammalian Target of Rapamycin (mTOR) pathway, and “Cellular responses to stress”. Of the downregulated processes, they

found “catabolic processes”, “cellular senescence”, “mitotic cell cycle check points”, and “Hedgehog signaling” to be the top Gene Ontology terms. Currently, validation studies are in progress to confirm these results.

Way ahead: The group intends to assess myogenic differentiation potential and proliferation characteristics of the 17 MSC lines. Future experiments are aimed at trying to understand the significance of these genes in fetal myogenesis and the interactions between vitamin D receptor (VDR) and chromatin modulators in these genes to define the epigenomic landscape during vitamin D signaling. The group wants to address whether women with severe vitamin D deficiency display dysregulation in the candidate genes from the RNA sequencing analysis have that might perturb myogenic progression in the fetus.





MULTIDISCIPLINARY CLINICAL AND TRANSLATIONAL RESEARCH

MULTIDISCIPLINARY CLINICAL AND 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. Diagnostics development also demands multidisciplinary efforts to identify unmet needs and develop diagnostics customized to needs in India. The team at THSTI has focused on innovation in diagnostics for infectious diseases. Aptamer-based assays are under development to detect snake bites and pesticide poisoning. The team also focuses on the development of technology platforms for bioprocess improvement. Last year, the team began working on implantable medical devices and 3-D organoid models.

DIAGNOSTICS OF INFECTIOUS DISEASES

Blood-borne infections



Dr. Gaurav Batra's team is working on developing a high sensitivity multiplex point-of-care test (POCT) system for the detection of blood-borne infections. The performance of commercially available rapid POCTs for HCV and HBV is inferior compared to central laboratory tests. Moreover, there is no available multiplexed POCT for the simultaneous detection of antigen and antibody markers for HIV, HBV, and HCV. A

high-performance multiplexed POCT covering both the antigen and antibody markers is of enormous value for resource-poor settings. The team uses a lateral flow assay (LFA) format, which is the most widely used POCT format because it is easy to use, rapid, affordable, and scalable.

Nevertheless, traditional LFAs often suffer from problems, e.g., poor-sensitivity. To improve the performance of LFA format, the team has replaced the colloidal gold (used for signal generation in traditional LFA) with upconverting phosphor nanoparticles (UCNPs) as a tracer, with optimized flow properties. The team has developed high-performance UCNPs based POCTs for HBsAg, and anti-HCV-antibodies. The long-term goal is to combine these assays in one cartridge.

Last year, the team reported the development of whole blood compatible UCNP-based LFA for the detection of HBsAg and anti-HCV antibodies. On HBsAg assay, further work was done to increase the stability of the test at 37°C. The improved version is stable at 37°C for at least 50 days. When compared to a commercial conventional visually read LFA for HBsAg, the developed UCNP-LFA had a Limit of Detection (LoD) of 0.1 IU HBsAg/ml in spiked serum. In contrast, the LoD of the conventional LFA was 3.2 IU HBsAg/ml. The developed UCNP-LFA fulfills the WHO criterion for

blood screening (LoD ≤ 0.13 IU HBsAg/ml) in terms of analytical sensitivity. They evaluated the UCNP-LFA and conventional LFA with well-characterized sample panels. In comparison to a central laboratory test, UCNP-LFA showed 95.4% [95% CI: 89.5% - 98.5%] sensitivity whereas sensitivity of the conventional LFA was 87.7% [95%CI: 79.9–93.3%]. These results indicate that the UCNP-LFA is superior in terms of analytical sensitivity as well as clinical sensitivity compared to the commercial LFA for HBsAg.

The team further worked on the anti-HCV antibody UCNP-LFA to improve the stability of this test. The improved test is stable for at least six months at room temperature (study ongoing). They evaluated UCNP-LFA with well-characterized sample panels. In comparison to a central laboratory test, UCNP-LFA for anti-HCV Ab showed 98.4% [95% CI: 94.3% to 99.8%] sensitivity and 98.0% [95% CI: 95.4% to 99.4%] specificity.

Way ahead: The team is still trying to improve the UCNP-LFA for HIV p24-Ag to bring the performance close to central lab tests. They will incorporate the above assays in a multiplexed cassette design for the simultaneous detection of HIV, HCV, and HBV infections.

Tropical fevers

Acute febrile illness (AFI) is common in the tropics/subtropics and caused by very diverse pathogens. The availability of a reliable point-of-care test (POCT) that can quickly identify a pathogen from a group of pathogens causing fever, is of paramount importance for patient treatment, surveillance, and antimicrobial resistance prevention. Infectious diseases that cause a significant burden of AFI in tropics and subtropics include Malaria, Dengue, Chikungunya, Typhoid, Scrub Typhus, among others.

Despite the strong need, commercially available POCTs for these infections are of poor quality, and there is a strong need to develop high-quality POCTs for tropical febrile infections. Simultaneous detection of multiple etiologies of tropical fevers requires multiplexed POCTs. Successful development and implementation of singleplex and multiplexed POCTs for AFI will help in choosing an appropriate treatment option for the patient and control antimicrobial resistance through the 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.

Highly sensitive POCT for the detection of Plasmodium lactate dehydrogenase (pLDH) antigen:

The commercially available POCTs targeting Pan-*plasmodium* antigen, pLDH, lack sensitivity, and better POCTs are required to detect low parasite density infection and differentiation between *P. vivax* and *P. falciparum* infections. One of the reasons for the poor-sensitivity of commercial tests is the unavailability of high-affinity antibodies that are truly pan-epitope specific (efficiently binding to the LDH of all the five human *Plasmodium* species) or targeting specific epitope on pLDH (for differentiation of *P. vivax* and *P. falciparum*). Dr. Batra's team has generated monoclonal antibodies targeting Pan, Pf, and Pv-specific epitopes on pLDH. They used pan-pLDH antibodies for the development of proof-of-concept UCNP based dipstick assay for panLDH detection.

Second-generation dengue NS1 antigen detection assays for routine diagnosis and surveillance:

Dr. Batra's team has used in-house anti-NS1 monoclonal antibodies to generate dengue NS1 dipstick assay. The assay has an analytical

sensitivity at least three times higher than the commercial POCTs (two commercial POCTs were compared) for each DENV serotype. Moreover, the assay showed no serotypes preference in NS1 detection, unlike commercial assay with clear serotypes preference. The assay is being converted to dry LFA from dipstick format. The serotype-specific LFA is also under development.

Way ahead: Their long-term plan is first to develop high-performance POCTs for the detection of major tropical fevers and then develop a multiplexed POCT for the simultaneous detection. In the upcoming year, they will finalize the NS1 assays and scrub typhus antibody detection assay.

TB Diagnostics



India continues to grapple with TB burden, one of the main reasons for which is a lack of proper diagnostic.

Dr. Tarun Kumar Sharma's group partnered with Prof. Jaya S. Tyagi to

develop aptamer-based diagnostics assays for TB and meet this exigency of an affordable and reliable TB (both pulmonary and meningitis) diagnostic.

Dr. Sharma's team had developed aptamers against HspX and GlcB antigens of *Mycobacterium tuberculosis*. They validated these aptamers for their ability to detect pulmonary and extrapulmonary TB. To achieve a higher sensitivity by detecting more than one antigen at a time, they have also developed aptamers against the MPT-64 antigen, a useful biomarker for tuberculosis. The developed aptamer was optimized Post-SELEX by truncation. The truncated aptamer (7-1) candidate evinced high selectivity for the MPT-64 antigen. It does not evince any cross-reactivity with various

bacterial lysates (**Figure 3.1**). This aptamer was further adapted on an electrochemical sensing platform. This aptamer was able to detect as

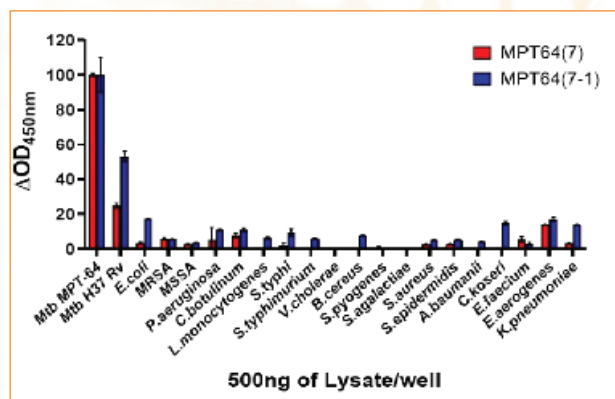


Figure 3.1: Evaluation for cross-reactivity of MPT-64(7) parent (red) and truncated aptamer (blue) for bacterial lysates from various bacterial pathogens.

low as 1 ng antigen (**Figure 3.2**). A pilot study on clinical specimens is planned.

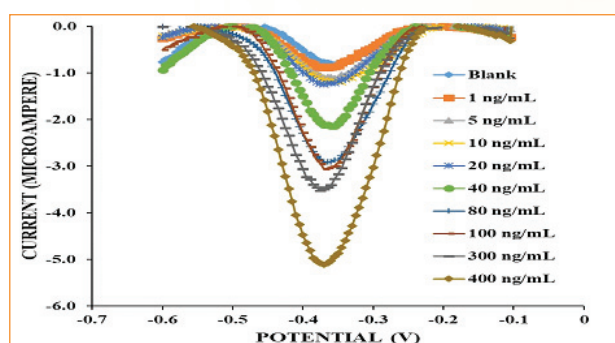


Figure 3.2: Sensor response (DPV) to target antigen, MPT-64.

Way ahead: The team has planned to explore the clinical utility of MPT-64 specific aptamers.

DETECTION OF SNAKE BITE

Diagnostics for differentiating between poisonous and dry bites and ascertaining the venomous species are not available. So, polyvalent antiserum is administered, which illicit severe immune response. Dr. Sharma's lab is developing point-of-care (POC) diagnostics to identify snake bite and the species. Following identification of the species, a bi- or mono-specific antivenom can be administered to reduce the immune response.

India reports an average of 58,000 snakebite deaths annually, supports the rationale of developing this diagnostic.

Dr. Sharma's team has developed a panel of aptamers against crude venom of *Bungarus caeruleus* (Indian Krait). Using a well-optimized SELEX strategy followed by next-generation sequencing, they identified a panel of aptamers with high-binding capacity for the venom of

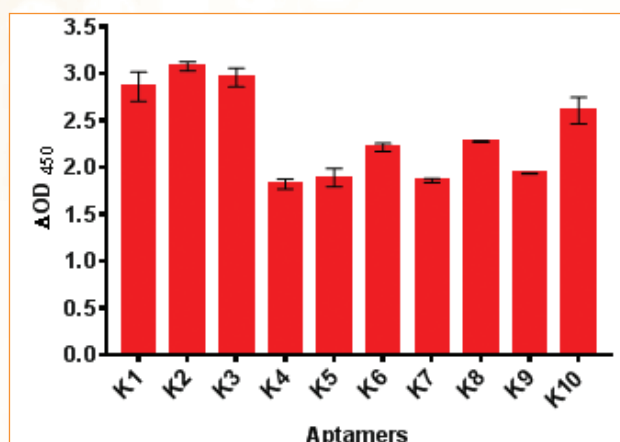


Figure 3.3: Evaluation of binding of selected aptamer against crude venoms of *Bungarus caeruleus* (Indian Krait).

Bungarus caeruleus (Figure 3.3). They further assessed the cross-reactivity of these aptamers. The best performing aptamer candidate K6 and K8 evinced high-selectivity for the venom of *Bungarus caeruleus* when compared to venom of *Naja naja*, *N. kaouthia*, *N. oxiana*, *Daboia russelii*,

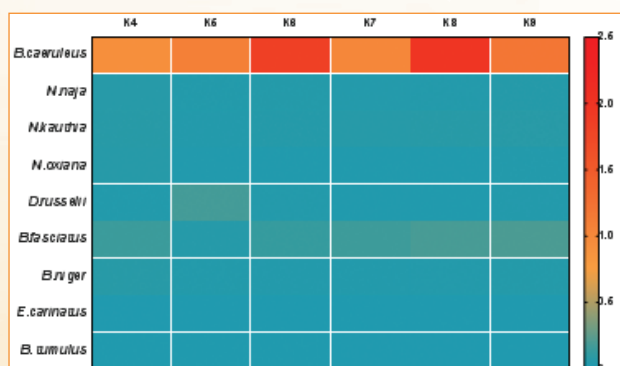


Figure 3.4: Heat-map representing selectivity of aptamers to *B. Caeruleus* venom. All aptamers bind selectively to *B. Caeruleus* and does not evinced significant cross-reactivity to the venom of other snake species namely *Naja naja*, *N. kaouthia*, *N. oxiana*, *Daboia russelii*, *B. fasciatus*, *B. niger*, and *Echis carinatus* and a red scorpion (*Buthus tumulus*) venom. Red color represent highest binding while cyan colour represent lowest binding.

B. fasciatus, *B. niger*, and *Echis carinatus* and a red scorpion (*Buthus tumulus*, Figure 3.4). They evaluated the ability of aptamer to detect snake

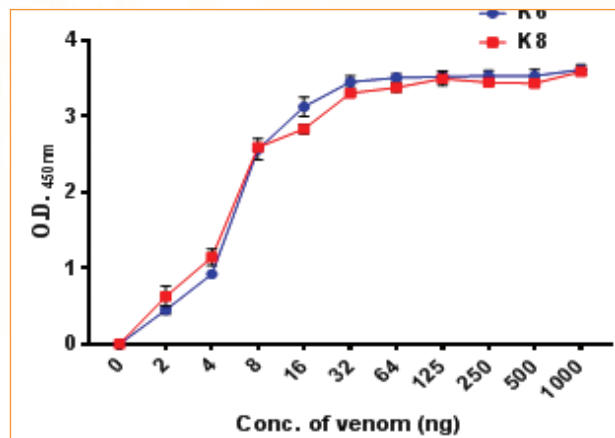


Figure 3.5: Low end detection limit of K6 and K8 aptamers in serum background.

venom in serum. It is evident from Figure 3.5 that both aptamers (K6 and K8) can detect as low as 2 ng venom in the serum background. Finally, using mass spectrometry, they found that the aptamers target ***β-bungarotoxin***, a lethal neurotoxin.



In addition to this, an alternative approach to developing a supporting diagnostic tool for snake bite, a monoclonal antibody-based approach was also used in collaboration with **Dr. Rajesh Kumar**. Preliminary studies were performed in mice to isolate and characterize monoclonal antibodies (mAbs) for snake venom using recombinant phage display technology. Preliminary data showed few mAbs binding specifically to big four snake venoms (Figure 3.6). They are now characterizing these mAbs for their epitope specificity, biochemical, and biophysical characterization. Isolation of mAbs to specifically bind to particular snake venom is underway.

Dr. Sharma's group has also identified peptide aptamers against the venom of the India Cobra

(*Naja naja*). The developed aptamers evinced good binding for 3-Finger toxin (3FTx) and venom of elapids (Cobra and Krait) that contain a decent amount of 3FTx. They didn't observe any significant cross-reactivity with the venom of Vipers (Russell's and Saw-scaled vipers, **Figure 3.7**)

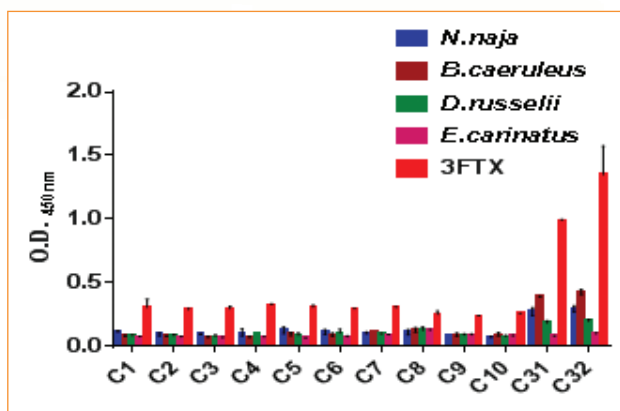


Figure 3.7: Binding of peptide aptamers for 3FTx and crude venom of Big Four

Way ahead: After the *in vitro* validation, the team aims to develop electrochemical sensors as POC diagnostics. They will evaluate the envisaged POC diagnostic against body fluids for the detection of snake bites. The isolated mAbs will be characterized for their diagnostic and therapeutic potentials.

BIOSENSING FOR ENVIRONMENTAL POLLUTANTS

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

The group has developed a novel SELEX strategy to enhance the sensitivity of aptamer-based NanoZyme (catalytic gold nanoparticles) 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 aptamer-NanoZyme assay (**Figure 3.8**) evinced visible color change (blue color) in the presence of pesticides. In

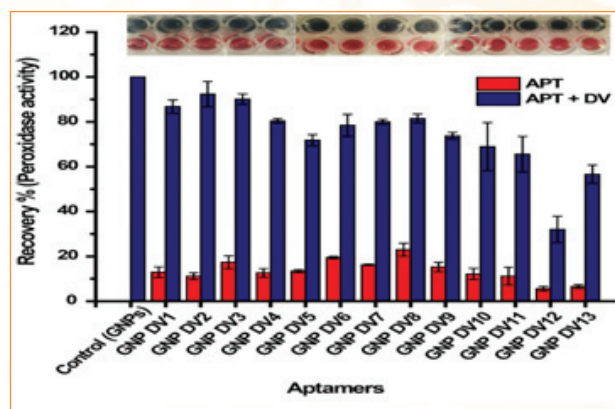


Figure 3.8: Recovery % of peroxidase activity after addition of pesticides (DV-Dichlorvos, Fip- Fipronil, IMD- Imidacloprid, MAL- Malathion, Thia-Thiamethoxam) to GNP and aptamer complex. The binding of aptamers on GNPs surface blocks their peroxidase activity. The aptamers upon binding to the pesticides 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. Big Four

contrast, no such change was observed in the absence of pesticides (color remained red). The best performing aptamer candidate (GNP DV2) was highly selective and did not display any cross-reactivity with other pesticides (from the same or different class) (**Figure 3.9**). The limit of detection of aptamer-NanoZyme assay has been established, and this sensor can detect as

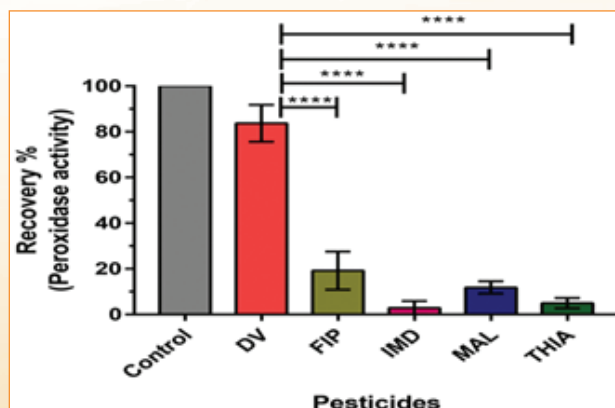


Figure 3.9: Specificity of the GNP DV2 towards the DV. The aptamer displayed a highly selective binding for DV with no significant cross-reactivity to other tested pesticide molecules. Here the level of significance is determined with one-way ANOVA representing the highly significant response of DV2 for DV**** $P < 0.0001$.

low as 15µM dichlorvos. They also evaluated the target-induced structural change in the aptamer using a CD. The affinity (Kd) of these aptamers for dichlorvos was determined using ITC. The aptamer GNP DV2 demonstrated its affinity in the nanomolar range.

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

Development of rapid diagnostics for antimicrobial resistance/susceptibility



The team jointly led by **Dr. Susmita Chaudhuri** and **Dr. Niraj Kumar** is focused on developing process & products that could enable pathogen identification and antimicrobial susceptibility testing within 90 minutes.

Rapid Pathogen capture and culture: They have accomplished the following: 1) established that 1-hour culture of pathogens can scale-up pathogen density to the detection limit of existing techniques for pathogen-identification (PCR) and the native biofluids are nutrient-enriched enough to support their growth for said duration; and 2) developed and tested two prototypes for rapid pathogen capture (first with whole-blood and second with urine) and observed that more iterations are required to achieve functional prototype.

Pathogen identification: They have developed an *in silico* work-flow for identification of pathogen-specific unique peptides/proteins. Briefly, a master database of all possible peptide fragments (window size: 8-35 amino acid) for ESKAPE pathogens was created and then used to identify peptides/proteins that are only present in any one pathogen. Of the identified *Staphylococcus aureus*-specific unique peptides/proteins, secretory proteins were identified using web-based localization prediction tools and validated for their pathogen-specificity using PCR-based method. They have also identified pathogen-specific unique peptides/proteins for remaining pathogens of ESKAPE group and are now validating the identified secretory targets.

Way ahead: (i) Development of **Minimally Viable Prototype (MVP)** for rapid pathogen capture and culture, (ii) Validation of pathogen-specific unique peptides/proteins for remaining pathogens of ESKAPE group and development of diagnostic-grade binders against at least two pathogens.

Development of a rapid antimicrobial susceptibility phenotypic assay for ESKAPE pathogens

The aim is to develop a solution for the unmet need of a rapid phenotypic Antimicrobial Susceptibility Test for sepsis and urinary tract infection. The team has shown a direct correlation of intracellular ATP levels with bacterial growth and conducted proof-of-concept studies to show that the relative ratio of bacterial ATP levels correlates indicates growth or death, in uropathogenic *Escherichia coli* and *Klebsiella pneumoniae*, with the initial batch of chemical sensors. They have developed a prototype kit for rapid AST profiling in physically separate concentration gradient of antibiotics having novel sensors that give a read-out as ATP changes due to cell growth, and performed

a detailed evaluation with clinical isolates. They have prepared a minimally viable prototype (MVP) with all reagents and completed evaluation with 280 clinical isolates with 98% concordance. This innovative diagnostic kit can ensure guided antibiotic prescription at the point-of-impact within 2 hours.

Way ahead: (i) Complete testing of the **Minimally Viable Prototype** with more clinical isolates (a total of ~500), (ii) Develop colorimetric sensor for the improved version of the assay.

Implantable medical device and 3-D organoid models

Biocompatible placenta-derived patches for soft tissue wound healing

The prospective of regenerative medicine relies on the practical development of medical devices that encourage new, functional tissue formation *in vivo*. Skin transplantation is a requisite technique in plastic surgery for the recovery of prevalent posttraumatic and chronic skin wounds. Soft tissue injuries involve impairment to muscles, tendons, and ligaments that result in pain and inflammation. Wound healing can be characterized as under healing, as in the setting of chronic wounds, or hypertrophic scar formation after burn injury. There is a need for products that facilitate rapid wound healing and are majorly used for chronic wounds such as diabetic and venous leg ulcers. Allogenic tissues such as human placental tissue such as amniotic membrane have been used without noteworthy restrictions. Therefore, it is vital to find superior, technologically-advanced alternatives such as minimally invasive procedures and rapid wound healing products.

Dr. Santosh Mathapati started his work last year and is focused on developing an appropriate experimental design and methods in the direction



of ease of regulatory approval of medical devices, i.e., making of final amniotic membrane, and placenta-derived tissue product.

Way ahead: The long-term goal of this project is to develop wound healing materials that can treat several types of wounds, including lower extremity diabetic ulcers, burn wounds, surgical wounds (e.g., post-laser surgery, donor sites, or grafts, post-podiatric procedures), combat, and traumatic wounds.

Processed placental tissue and 3-dimensional human hepatic organoid model

Cell-based assays have been an essential pillar of the drug-discovery process to provide a simple, fast, and cost-effective tool to avoid large-scale and cost-intensive animal testing. Hepatocyte-like cells (HLCs) generated from human pluripotent stem cells (hPSCs) such as embryonic stem cells (ESs) and induced pluripotent stem cells (iPSCs) have shown great promise to satisfy this need by providing an unlimited source of cells that emulate the genotype of the donor or primary hepatocytes. Dr. Mathapati's team is studying the development of 3-dimensional hepatic organoid by using placental tissue (amniotic membrane and cotyledon) and adult stem cells. Subsequently, long-term functional maintenance of hepatocytes will be studied comparing the 2-D vs. 3-D system. These human iPSC organoid models could fit in the broader system of iPSC disease modeling and drug discovery.

Way ahead: Plans include the development of surgical wound healing materials, cardiovascular implants (conduits and patches), orthopaedic implants, and 3-D organoid models for drug discovery and disease modeling.





NON-COMMUNICABLE DISEASES

NON-COMMUNICABLE DISEASES

INTERPLAY BETWEEN EFFECTOR AND REGULATORY T CELLS IN AUTOIMMUNE DISEASES



The immune-biology laboratory, headed by **Dr. Amit Awasthi**, is working to understand molecular pathways that define the generation and functions of effector and regulatory

T-cells in various autoimmune disease conditions. The laboratory is primarily delineating functions of the Th9 and Th17 cells in inflammatory bowel disease, asthma, and cancer immunity. Besides, the team is also establishing the experimental model of the tumor to decipher the anti-tumor functions of T cells and the role of checkpoint inhibitors in cancer immunotherapy.

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 nourish all living cells. The cells of the immune system have a very active metabolism during immunological reactions and are no exception. Aerobic metabolism of glucose (via oxidative phosphorylation) provides energy to resting naïve T-cells. Metabolic checkpoints are one of the critical regulators of T-cell responses. The activity of metabolic enzymes or concentration of a specific

metabolite were suggested to be important checkpoints in the immune response during 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 the intra and extracellular pathogens as well as inducing tissue inflammation in autoimmunity and allergic inflammation. On the contrary, regulatory subsets of CD4⁺ T-cells (inclusive of Foxp3⁺ regulatory T-cells (Tregs) and type 1 regulatory T (Tr1) cells) suppress effector T-cell functions and contribute to the resolution of tissue inflammation in autoimmune diseases.

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

The team identified, for the first time, that the extracellular ATP (eATP) induces the differentiation of human Th9 cells. They show 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. At the same time, exogenous NO could rescue the generation of Th9 cells even upon inhibition of purinergic receptor signaling. Moreover, they identified that ATP induces transcription factors, mTOR and HIF-1 α , essential for the induction of human Th9 cells. The findings thus identify that ATP-induced nitric

oxide potentiates HIF1 α -mediated metabolic pathway that leads to IL-9 induction in Th9 cells. They found that the ATP-NO-mTOR-HIF1 α axis is essential for the generation of human Th9 cells, and modulation of this axis may lead to the therapeutic intervention of Th9-associated disease conditions (**Figure 4.1**).

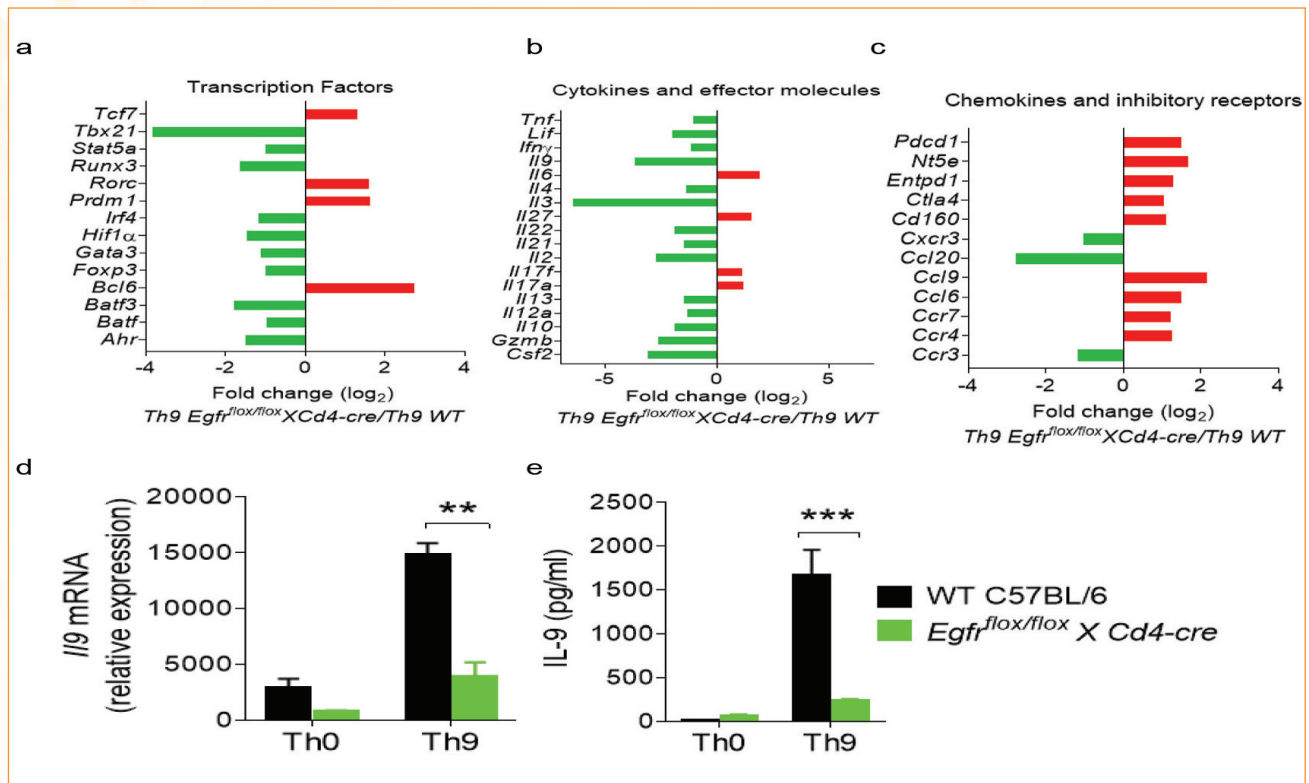


Figure 4.1 (a-e): Conditional deletion of EGFR in CD4 cells reduces the expression of IL-9 and Th9 associated genes.

Transcriptional and metabolomic regulation of Th9 cells:

Given the essential role of IL-9 and Th9 cells in mediating immunity against extracellular pathogens and tumor immunity, the team went on to study the molecular pathway that leads to the differentiation of Th9 cells. They employed whole-genome RNA sequencing (RNAseq) to unravel the molecular underpinning of the pathway. Global gene expression profiling analysis coupled with pathways analysis identified that Th9 cells substantially upregulate the expression of Epidermal Growth Factor Receptor (EGFR),

indicating the role of EGFR in the development and functions of Th9 cells. They confirmed the findings by qPCR analysis and found enhanced expression of EGFR in Th9 cells as compared to Th0 (**Figure 4.1a**). They further tested the functional role of EGFR in Th9 cells by blocking EGFR by Gefitinib, an EGFR specific inhibitor. They found that Gefitinib could significantly suppress the development and functions of Th9 cells (**Figure 4.1b, c**).

OT-II-TcR transgenic T-cells differentiated into Th9 cells in the presence or absence of Gefitinib to block EGFR signaling. They found that Th9 cells regress tumor growth. In contrast, Gefitinib-

treated Th9 cells failed to control tumor growth, implicating that EGFR signaling is crucial in inducing anti-tumor functions of Th9 cells (**Figure 4.1d**). Taken together, **the team identified a new pathway that is essential for *in vivo* functions of Th9 cells.**

wEGFR-Areg signaling is essential for Th9 cell differentiation

Further substantiating the findings, they used *Egfr^{fllox/flox}XCd4-cre* mice with *Egfr* gene conditionally deleted in CD4⁺ T-cells, and performed the nanostring analysis of gene expression. Nanostring analysis revealed that when compared to WT mice, Th9 cells from *Egfr^{fllox/flox}XCd4-cre* mice showed downregulation of key transcription factors - cytokines and chemokines associated

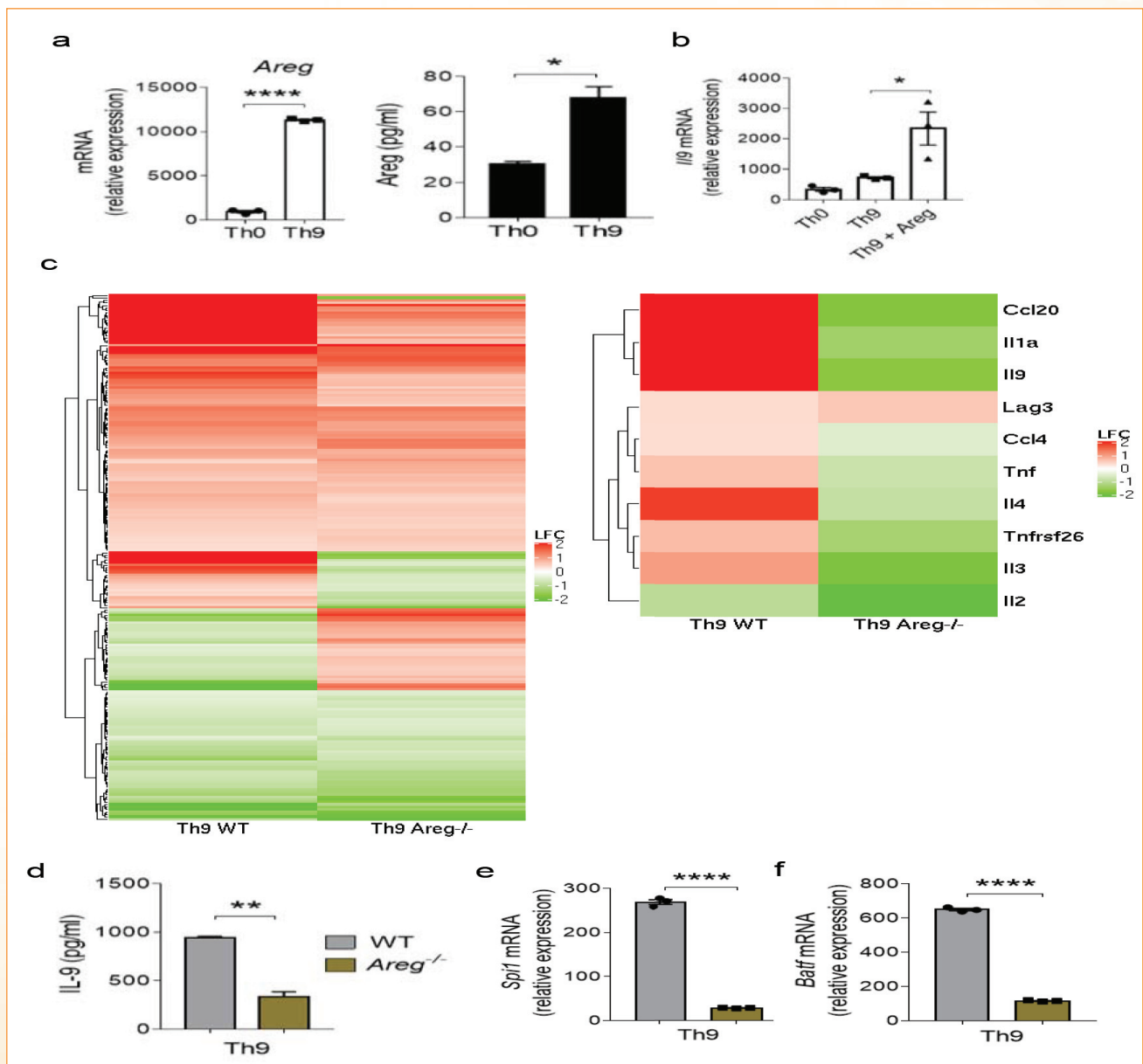


Figure 4.2 (a-f): Expression of EGFR ligands in Th9 cell differentiation.

with Th9 cells and an upregulation of inhibitory receptors suggesting EGFR is essential for the developmental programming of Th9 cells (**Figure 4.2a-c**). There was a consistent reduction in the IL-9 production and *Il9* expression in Th9 cells from *Egfr^{flox/flox}XCd4-cre* mice.

Since EGFR activation requires the binding of a ligand, the team next tested the expression of EGFR ligands in Th9 cell differentiation. They found an upregulation of expression of *Areg* in Th9 cells (**Figure 4.2a**). However, other EGFR ligands such as *Tgfa*, *Egf*, and *Begf*, were not enriched in Th9 cells as compared to Th0, suggesting an exclusive role of *Areg* in Th9 cell development. They also observed *Areg* in the culture supernatants of Th9 cells as opposed to Th0 cells. Supplementation of r*Areg* enhanced Th9 cell differentiation (**Figure 4.2b**). Further on, they used T-cells from *Areg*^{-/-} mice to understand the role of *Areg* in Th9 cell differentiation. They differentiated naïve CD4⁺ T-cells isolated from WT and *Areg*^{-/-} mice into Th9 cells and performed RNA-Seq. Transcriptomics profiling identified differentially expressed genes in *Areg*^{-/-} Th9 cells as compared to WT Th9 cells. They noted *Il9* among the top downregulated genes in *Areg*^{-/-} Th9 cells as compared to WT Th9 cells (**Figure 4.2c**). They validated the RNA-Seq data by performing *in vitro* assays. *Areg*^{-/-} Th9 cells showed a decreased expression of *Il9*, *Spi1*, and *Batf* in Th9 cells (**Figure 4.2d-f**). Also, *Areg*^{-/-} Th9 cells showed a reduction in *Egfr* expression, suggesting that *Areg* is essential for EGFR-mediated Th9 cell differentiation. These data demonstrated that *Areg*-mediated EGFR activation amplifies IL-9 induction in Th9 cells.

NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

Team Members: Madhu Dikshit, Samrat Chatterjee, Dinesh Mahajan, Sanjay K Banerjee, Ruchi Tandon, Shailendra Asthana, Amit Yadav, Ajay Kumar, Renu Goel, Yashwant Kumar

Excessive accumulation of lipids in the liver of non-alcoholic fatty liver disease (NAFLD) patients induces metabolic imbalance leading to oxidative stress, inflammation, lipotoxicity, and apoptosis. The global prevalence of NAFLD is around 30%. Obesity and Type II diabetes further enhance predisposition to non-alcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocarcinoma. Currently, no drug is available in the market for the treatment of NASH; however, several drug candidates are under various phases of clinical trials. NASH is in the focus of many pharma, biotech companies, and research institutes alike. Moreover, due to slow progression, there is no stage-specific diagnostic test, except repeated liver biopsy, which also has ~30% variability for the confirmed diagnosis. NASH, therefore, poses a dual challenge – (i) discovery of new effective drugs, (ii) identification of stage-specific biomarkers to facilitate accurate diagnosis, and also for the assessment of disease progression or regression, in response to treatment. The economic burden for NAFLD/NASH is more than multi-billion USD for developed nations and developing nations like India. There is a high socio-economic need to develop affordable therapies to address this epidemic in India.

The NCD program has a multidisciplinary team with complementary skills and expertise provides a unique opportunity to meet the unmet needs in this area. Team of scientists at THSTI is working in tandem to achieve the following objectives:

- Standardization and validation of *in vitro* and *in vivo* models of non-alcoholic fatty liver disease (NAFLD), to evaluate new chemical entities and plant-based extracts to identify therapeutic leads
- Identification of stage-specific molecular signature for diagnosis/ prognosis of dyslipidemia, inflammation and fibrosis associated with NAFLD

1 A. Standardization and validation of *in vitro* and *in vivo* models of non-alcoholic fatty liver disease (NAFLD)

(Madhu Dikshit, Ajay Kumar, Ruchi Tandon, Sameena Khan, and Sanjay K Banerjee)

It is essential to establish reliable test models for the assessment of the efficacy of test molecules and herbal extracts for a drug discovery program. NCD program scientists are establishing and validating target, cell, and animal-based screening models, to create a robust screening platform for identifying modulators of NAFLD. The idea is to set up miniaturized, high throughput amenable assays that can be transferred to a robotic platform and screen a large number of compounds with precision, robustness within a short period.

a) *In vitro* Protein Assays

- ◀ **ASK1 enzyme assay:** Ask-1 catalytic domain was expressed, purified, and the protein was used to set up the kinase assay using ADP Glo technology. Selenosertib was used as a positive control, and 16

in-house compounds evaluated. Two compounds were identified with IC_{50} values in the range of 1 μ M. The assay can be transferred to a high-throughput platform.

◀ **Expression of FxR ligand-binding domain and FXR agonistic activity:**

Initial efforts to express and purify the FxR ligand-binding domain are in progress and an *in vitro* assay is being set up to identify FxR agonists.

b) Cell Assays

Models to study metabolic and mechanistic processes integral to the pathogenesis of human NAFLD/NASH have shortcomings and, therefore, not very conclusive. Hepatic cell lines are a reliable substitute over primary culture as 1) hepatic cell lines grow steadily, 2) have almost an unlimited lifespan, and 3) have

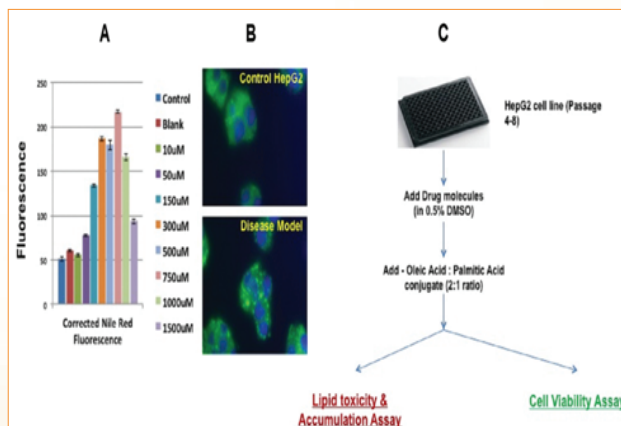


Figure 4.3: HepG2 screening assay for the lipid accumulation and lipotoxicity. (A) Bar graph depicts the effect of a gradient of FFA conjugate concentrations on Nile red fluorescence in HepG2 cells. (B) Fluorescent microscope images of the accumulated intracellular lipids as large fluorescent droplets in the presence of FFA conjugate (700-800 μ M in this case). (C) Flowchart of the experimental protocol for HepG2 cell screening assay.

a stable phenotype. Moreover, cell lines are readily available and easy to handle among different laboratories. **Dr. Ajay Kumar's** team has established phenotypic *in vitro* cell-based



assays for NAFLD based on the three key hallmarks of the disease - Steatosis (lipotoxicity), inflammation, and fibrosis using hepatic cells, i.e., HepG2, THP1, and LX2 respectively.

- **Lipid accumulation model:** Efficacy of shortlisted extracts/compounds was evaluated against lipid accumulation in the human HepG2 cell line. This was done with a pre-determined FFA conjugate concentration (400 μ M) to obtain maximum lipid accumulation (determined by Nile red/DAPI staining). Another measure to shortlist the FFA conjugate concentration of choice was the monitoring of significant changes in cell health (if any) in response to the varying FFA concentrations. Fatty acid (Nile Red staining readout /cell number (DAPI staining readout) ratio using the standard small molecules such as Saroglitazar and Selonsertib and herbal extract. This assay will be used to conduct the screening of herbal extracts and in-house molecules using a high throughput platform.
- **Hepato-protection model:** Human HepG2 cells were subjected to an FFA conjugate (700 μ M), which resulted in lipid-induced toxicity leading to >20% reduction in cell viability (determined by MTT assay).
- **THP model:** Compounds showing no toxicity and $\geq$ 20% reduction in accumulated lipids in HepG2 cells were

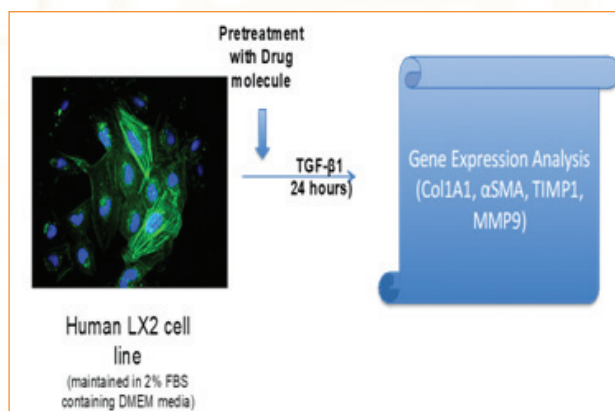


Figure 4.4: Schematic representation of anti-fibrotic screening using LX2 cell line-based assay.

investigated for inflammatory markers in PMA differentiated THP1 cell line.

- **LXII model:** Almost 25-30% of NASH patients exhibit fibrotic phenotype. LXII cells mimic the hepatic stellate cells will be used to study the efficacy of different herbal formulations/compounds to resolve fibrotic response.

c) **Diet-induced Animal Models of Fatty Liver Disease**

Male C57BL6/J mice (20-25 grams), and Male Sprague Dawley rats (180-200 g) were used to establish fatty liver disease models. Animals were maintained at the small animal facility of THSTI as per IAEC's approval and institute animal usage guidelines. The animals were housed in the ventilated cages with controlled airflow, light/dark cycle, temperature (25 $\pm$ 2°C), and relative humidity. They were acclimatized in the above condition for at least one week before starting the experiment. Access to standard chow, special diet(s), and water was provided to them *ad libitum*.

◀ Mice model:

C57BL6/J mice were randomized into control (1324 P, Altromin International, Germany), high-fat high fructose (HFHF) (D16030909, Research Diet Inc., USA) and drug/extract treated groups (8 mice in each group). After the confirmation of insulin resistance at 4 weeks, the extracts or Saroglitazar were administered (p.o.) in 0.5% carboxymethylcellulose per day. After 8 weeks, body weight changes, tail and body length measurement, blood collection, fasting blood glucose, intraperitoneal glucose tolerance tests were performed. Mice from all groups were euthanized, and liver, kidney, heart, skeletal muscle, epididymal and adipose tissue collected and weighed. The plasma samples and collected tissues were stored at -80°C. Gene expression profiling of select genes involved in metabolism, inflammation, and fibrosis, as well as histological analysis of liver tissues, were performed.

Biochemical Analysis: Plasma and hepatic triglyceride (TG), serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), total cholesterol, and insulin were measured in the plasma.

Intraperitoneal Glucose Tolerance Test (IPGTT): At the end of eight weeks, six hours of fasted mice were administered a glucose dose of 2gm/kg b. w. i. p. and blood glucose concentration was measured at 0 (immediately after), 15, 30, 60, 120 minutes after glucose administration, using a commercially available glucometer (Accuchek Active, Roche).

◀ Rat model:

The rats were divided into four groups.

- Control group - control diet (Rodent Diet with 10Kcal% Fat, Cat No. D12450B, Research Diet Inc., USA)
- CDHF group rats - choline-deficient high fat (CDHF) diet (Rodent Diet with 60Kcal% Fat without added choline, Cat No. D05010403, Research Diet Inc., USA) for eight weeks.
- Third and the fourth group - CDHF fed rats treated with test drug (HDFC+Test) and standard drug, Pioglitazone (HFCD+PIO), respectively, from 5th week to 8th week (four weeks).

Each group had 6-8 rats. At the end of eight weeks' measurements performed were: body weight changes, tail length, fasting blood glucose, intraperitoneal glucose tolerance. Rats from all the groups were sacrificed. The serum samples and liver tissues were collected and stored at -80°C. Serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and triglyceride (TG) levels were measured in the serum using Siemen's autoanalyzer as per the vendor's instruction. Intraperitoneal glucose tolerance test (IPGTT) and fasting blood glucose levels were monitored. Liver tissue samples tested for hepatic TG levels. Liver sections stained with haematoxylin and eosin (H&E), Masson's trichrome (MT) and Oil Red O stains, and histological examinations performed. Gene expression profiling of select genes involved in inflammation and fibrosis were also performed.

Way forward:

- ◀ **Cell-based nuclear receptor assay:** Luciferase reporter gene assay to identify potential FxR agonists.

- **THP model:** Gene expression of M1 and M2 lineage-specific markers, including NOS2, Arginase, and others and cytokine levels in the presence and absence of test substance.
- **LXII model:** Gene expression levels of fibrotic markers targeted using qRT-PCR.
- **3D liver organoid model:** To screen the lead molecule(s) and extract(s) on lipid accumulation and lipotoxicity.
- **Co-culture model of hepG2 cells with differentiated macrophages and Stellate cells:** *In vitro* co-culture models to decipher the interplay between hepatocytes, and Kupffer cells (hepatic macrophages) or differentiated THP-1 cells (macrophages) to test the effect of lead compound(s) and herbal extract(s).
- **Diet-induced advanced fatty liver disease model:** Mice or rat model exhibiting NASH like features and fibrosis will be established.

1 B. To evaluate new chemical entities and herbal extracts to identify therapeutic leads



(Dinesh Mahajan, Shailendra Asthana, Ajay Kumar, Ruchi Tandon, Sanjay K Banerjee, and Madhu Dikshit)

NCD program focuses on the preclinical drug discovery with significant emphasis on computational biology, medicinal chemistry, and DMPK research. The team's approach is to identify and develop new drug leads based on small molecules as well as phytopharmaceutical extracts of Indian herbs documented in traditional literature for their therapeutic applications in non-communicable (NAFLD and NASH). **Dr. Dinesh Mahajan's** research team aims to identify an optimized new

drug lead with proof-of-concept studies in the above-mentioned experimental models.

Identification of hits through computational biophysics and computer-aided drug discovery



Dr. Shailendra Asthana and his team are working to identify the small molecule and peptide-based inhibitors by using the computational biophysical, molecular

dynamics simulation techniques with structural bioinformatics and computer-aided drug discovery approaches (**Figure 4.5**). The team is working on two targets Farnesoid X Receptor (FXR) and Apoptosis signal-regulating kinase1 (ASK-1) for the discovery of small molecules against NAFLD.

The FXR (bile acid-sensing transcription factor) regulates multiple metabolic processes. Modulation of FXR might overcome several

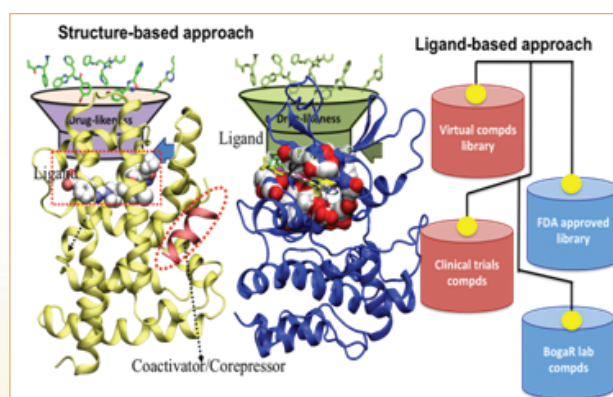


Figure 4.5: The virtual screening components

metabolic pathologies. Still, mechanism-based side effects have plagued the pharmacological administration of full FXR agonists. FXR structures are dynamic and flexible, reported crystals/co-crystals structures, with the understanding of molecular-level changes such as internal residue

wiring of different states and their transition states is a prerequisite to block the biological function of the target protein.

Extensive computational modelling helped understand the dynamic change in the pattern after the virtual binding of different compounds. They observed that both the agonist, either natural or synthetic molecule stabilize the formation of an extended helix $\alpha 11$ and the $\alpha 11$ - $\alpha 12$ loop upon binding. This binding strengthens a network of hydrogen bonds, repositions helix $\alpha 12$, and enables co-activator recruitment. OCA, which has recently been approved by the FDA for PBC treatment, was taken as control and is currently investigated in late-stage clinical development for NAFLD/NASH. Clinical trials of OCA, report that the over-activation of FXR is responsible for adverse effects such as elevated cholesterol levels. Full FXR activation also blocks bile acid biosynthesis and hinders with metabolic cholesterol degradation. Recent reports have shown that the synthetic partial agonist induces conformational states capable of recruiting both co-repressors and co-activators, leading to an equilibrium of co-activator and co-repressor binding. Partial FXR activation, therefore, appears to be a suitable strategy to avoid mechanism-based side effects of FXR targeting. Preclinical studies and phase I clinical trial findings with a partial FXR agonist confirmed the success of this approach. However, the molecular mode of action by which partial agonism is established and its structural determinants in terms of ligand binding and conformational changes in the FXR ligand-binding domain (LBD) have remained elusive.

For FXR, both the ligand- and structure-based approaches are used since the clinically most advanced anti-FXR molecule i.e., obeticholic acid

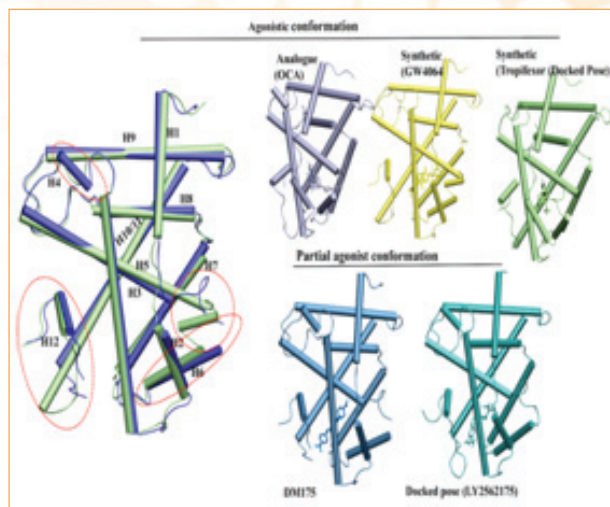


Figure 4.6: The FXR proteins with their different binding partners. The key zone of FXR are shown by dotted circles in red color.

and its binding site is known. The ligand-binding site in FXR is defined by α -helices (from 2 to 12) (**Figure 4.6**).

The agonistic ligands CDCA, OCA, and GW4064 occupy the same binding pocket but show differences in their residue-specific interaction modes. The dramatic conformational change was observed from different co-crystals in FXR's with three different types of binding that is agonist, partial agonist, and antagonist. The structural plasticity of FXR from the different analysis is perhaps not surprising given the numerous precedents for the flexibility of LBDs in response to the binding of different ligands. The team is working to understand the FXR's static (crystals) and dynamic behavior (extensive molecular dynamic simulations), its binding site, and some critical zones like co-activator and co-repressor and their impact on FXR modulation. Computational biophysical methods will help to identify the molecular recognition patterns and the key determinants so that the identified residue level information can be used for small molecule designing.

Three different approaches to screen small molecules are executed: A1 screening of virtual

compound library, A2 in-house synthesized compounds from Dr. Mahajan's Lab, A3 compounds obtained from BogaR (Industry collaborator).

ASK-1, Another focus of the group is to identify the anti-ASK-1 molecules. Apoptosis signal-regulating kinase 1 (ASK1, also known as MAP3K5), a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family, activates the p38 mitogen-activated protein kinase and the c-Jun N-terminal kinase (JNK) signaling cascades in response to various stressors. ASK1 activity is tightly regulated through phosphorylation and interaction with various binding partners. Here, they are trying

to identify the anti-ASK-1 molecule by using two different approaches:

- Mimicking the active lead Selenosertib by directly targeting the ATP binding site of ASK-1: the dynamic pocket of ASK-1 is targeted to screen the virtual compound library, along with our in-house and compounds from the industry collaboration. From the multi-step virtual screening top, 25 compounds have been screened. Based on another drug-like analysis, the top 5 compounds were selected for molecular dynamics simulation studies. Based on comparative analysis with Selenosertib, the promising candidates are shown in **Figures 4.7 and 4.8**.

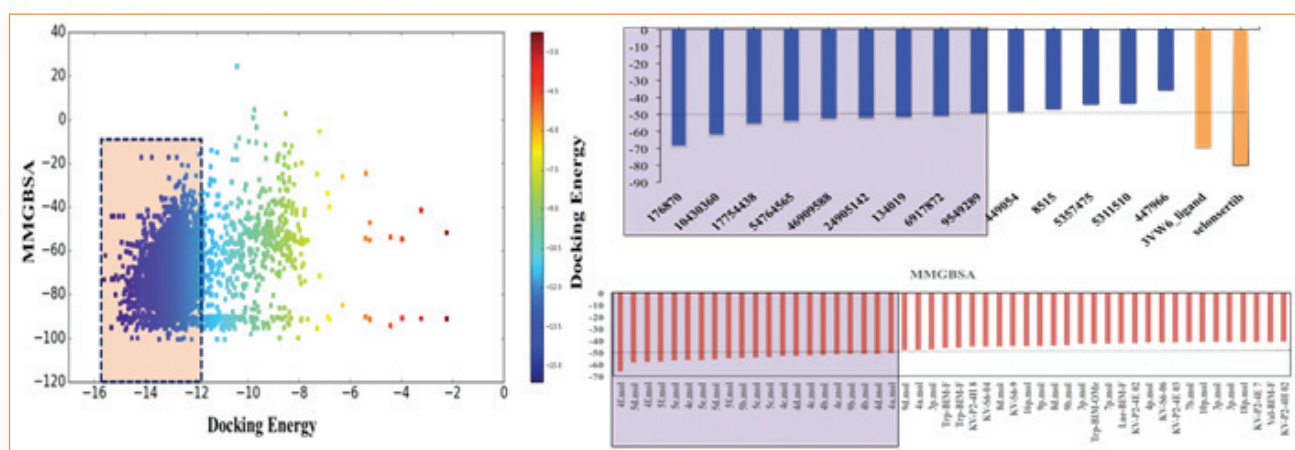


Figure 4.7: Post-processing analysis of screened compounds. Based on cut-offs the top compounds has been selected for further analysis.

- Protein-protein interaction approach by using the key interactors of ASK-1 which are reported to be involved critically in ON/OFF modulation. These key interactors interaction interface site will be used to design the active peptide and small molecule. At present, the ASK-1 and Trx interaction studies were performed to identify the most likely binding site. Furthermore, after the validation of the identified interaction interface, the hotspot residues were identified, which allow us to design the peptides.

Way forward:

- FXR and ASK1, computational hits have been identified by using the different in-house curated chemical compound libraries. The biophysical characterization is currently under progress.
- From BogaR lab (700 compounds) 6 potential hits have been Identified. Currently, *in vitro* validation studies are in progress.
- ASK-1 inhibition: based on the knowledge of interacting proteins, a protein-protein

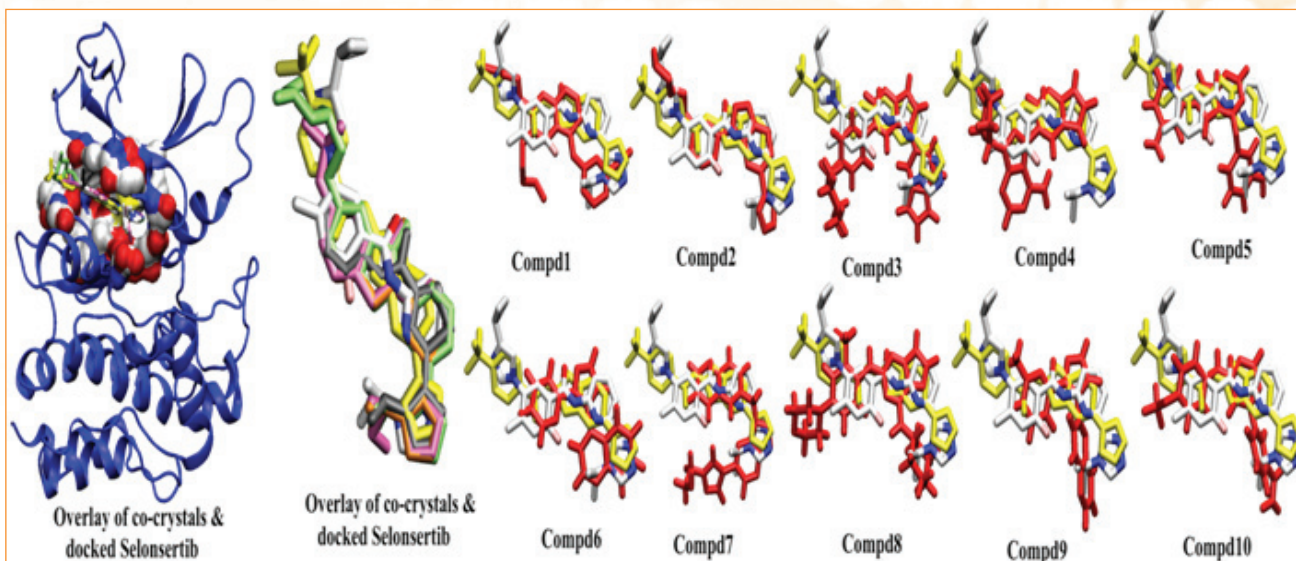


Figure 4.8: The superimposition of identified compounds against ASK-1. The overlaid structure of identified compounds with its control has been shown to highlight that the identified compounds not only occupying the same residues of the binding site but their mode of interaction is also similar to actives.

interaction approach is being applied to identify the active peptides from the interface sites. The computationally identified peptides will undergo protein-peptide docking, and their thermodynamic characterization to design the small molecule.

New drug discovery

For small molecules-based drug discovery, Dr. Mahajan's group is using two different approaches for drug lead identification.

- Repositioning approved drugs, or drug leads evaluated in phase II/III trials exploiting cell-based functional assays mimicking the disease phenotypes (e.g., Steatosis and inflammation).
- Identification and development of New Chemical Entities (NCEs) focused either on a specific molecular pathway or a drug target.

His team has identified a novel series of small molecules and a drug lead based on *in vitro* screening assays by exploiting autophagy induction as a possible therapeutic approach for NAFLD. The newly identified compounds were potent autophagy inducers, restricted the

triglyceride load, and improved overall cellular health parameters in cell-based assays. Using medicinal chemistry optimization studies, a potent and orally bio-available drug lead (DR62) with a significant therapeutic effect in an animal model was found. The lead reduced the triglyceride levels in rat liver when administered orally for four weeks in studies based on Choline Deficient High Fat (CDHF) induced NAFLD model (**Figure 4.9**).

In a target-specific approach for drug discovery, the team designed, synthesized, and identified a new hit (IC₅₀=138 nM based on enzymatic assays; **Figure 4.10**) based on a novel scaffold as an ASK-1 inhibitor. ASK-1 inhibition is known to have a therapeutic effect on liver fibrosis.

Way forward: The team has invested in understanding the mode of action and pharmacology of the newly identified drug lead based on autophagy induction. Dr. Mahajan's medicinal chemistry team is also working to generate more analogs around this lead to understand Structure-Activity Relationship (SAR). The SAR study aims to develop more efficacious drug lead, key pharmacophore understanding,

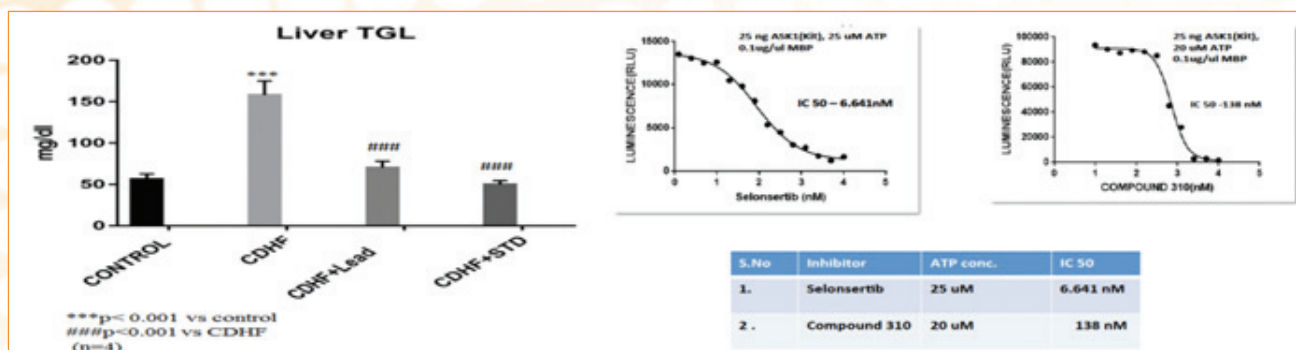


Figure 4.9: Reduction in triglyceride accumulation in rat liver post 4 week treatment with drug lead in Choline Deficient High Fat (CDHF) diet based NAFLD model.

Figure 4.10: IC50 value for ASK1 inhibition; Selenosertib is a clinical lead and compound 310 is the new identified hit

and broaden the patent space for intellectual protection.

THSTI-Dabur project

(Madhu Dikshit, Ajay Kumar, and Dinesh Mahajan)

The project with Dabur has embarked on developing a therapeutic formulation for NAFLD. In-depth deliberations resulted in shortlisting of herbal extracts/compounds with hepatoprotective action. The team at THSTI established and validated an *in vitro* model that mimics lipid accumulation in hepatocytes (steatosis phenotype) in the human HepG2 cell line. Standardized extracts and standards (Picroside-II, Silibinin, Hepano, *Silybum marianum*, *Picrorhiza kurroa*, *Cyperus rotundus*, *Phyllanthus niruri*, *Swertia chirayita*, *Terminalia chebula*, *Piper longum*, *Tinospora cordifolia*, *Tephrosia purpurea*, *Curcuma longa*, *Andrographis paniculata*) from Dabur were evaluated for their effect on human HepG2 cell line for their hepatoprotective effect and their efficacy against lipid-induced cytotoxicity.

The results of this preliminary screen have been shared with Dabur. Six of the total fourteen extracts have been shortlisted for further investigations in THP cell line, LXII cells, and also in high fat-fed C57BL/6 mice. Conclusions would be drawn only after the completion of the ongoing studies, and analysis of the collected data.

Way forward: Development of AYUSH and phytopharmaceutical-based drugs with continued active collaboration between THSTI and Dabur India.

2. Identification of stage-specific molecular signature for diagnosis/ prognosis of dyslipidemia, inflammation and fibrosis associated with NAFLD.

(Madhu Dikshit, Yashwant Kumar, Renu Goel, Amit Yadav, Sanjay K Banerjee, and Samrat Chatterjee)

A. Monitoring of circulating lipoproteins during the progression of NAFLD

Modulation in the lipoproteins levels is seen in patients of diabetes, cardiovascular disorders and non-alcoholic fatty liver disease. Lipoproteins, such as chylomicrons (CM), very low-density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high-density lipoprotein (HDL) are complex of lipids and apolipoproteins. CM has the highest amount of triglycerides as compared to VLDL, IDL, LDL, and HDL. While HDL has higher phospholipid content as compared to LDL, IDL, VLDL, and CM. The team is currently isolating the lipoproteins from the plasma by density gradient ultracentrifugation, and intend to assess the level of lipids and apolipoproteins, interacting proteins and PTMs which might vary during the progression of

NAFLD. To undertake these studies, method for the separation of plasma lipoproteins has been established using standard isopycnic potassium bromide density gradient centrifugation. Six distinct fractions of lipoproteins have been analyzed for lipid content using commercial kits and LCMS (Figure 4.11).

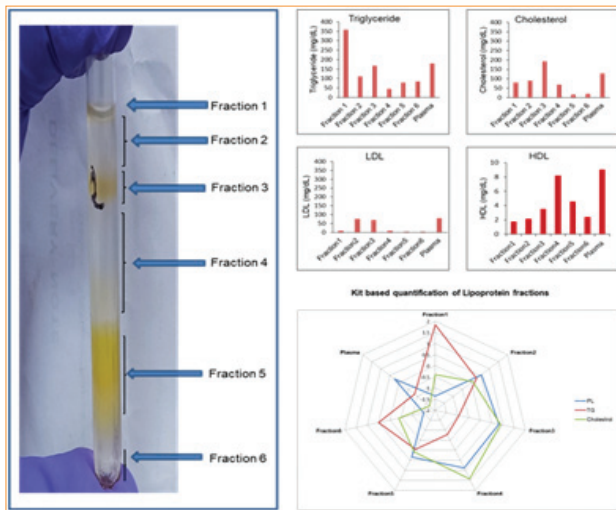


Figure 4.11: A. Plasma fractionation; B. Lipid content in various fractions and plasma; and C. LCMS based quantification of Lipoprotein fractions,

Way forward: Analysis of samples from healthy, and NAFLD patients; analysis of the data for differences and identification of particular lipids and proteins.

B. High throughput lipidomics during NAFLD in HFHF fed mice

Lipids play a central role in the development of different metabolic diseases, including NAFLD. Using high-resolution mass spectrometer and data analysis pipeline, Dr. Yashwant Kumar with his team has established a state of art lipidomics workflow that could identify approximately 1000 lipids species in plasma and liver. During this period, liver tissues from chow-fed and high fat-high fructose-fed mice, and also mice treated with standard drug Saroglitazar (SG) were analyzed by the lipidomics workflow. The principal component

analysis showed good separation with well-clustered data (Figure 4.12).

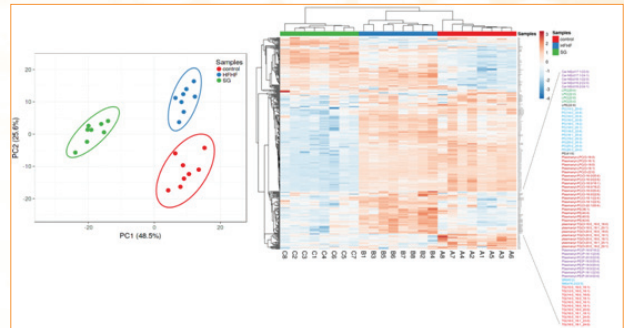


Figure 4.12: A. Principal component analysis (PCA) of Control, HFHF and SG mouse. B. Clustering of identified lipids

Further, investigation of the data demonstrated changes in one cluster during disease condition, and it was reversed in the Saroglitazar treated group. Lipids in this cluster are ceramides, lysophosphatidyl cholines, ether lipids, sphingomyelins and triglyceride lipids. Increased amount of ceramide and sphingomyelins have been linked with inflammation, insulin resistance, and oxidative stress. Other lipids of phospholipid class were also represented in this cluster. Free fatty acids derived phosphatidylcholine (PC), and lysophosphatidylcholine induce lipotoxicity. Saroglitazar treated and control mice showed a similar level of PC and LCP. High levels of PC and LCP were seen in the liver of HFHF fed mice. The highlighted cluster had different ether lipids associated with inflammatory response and antioxidant effect towards different reactive species.

Additionally, low levels of lipid species of triglycerides were seen in Saroglitazar treated group as compared to the HFHF fed mouse. Surprisingly, few of TG with lower carbon number were enriched in the highlighted cluster. The importance of lipids with a lower number of carbon and saturation needs to be investigated in NAFLD.

Way forward: Confirmation of these findings in another group of ongoing mice experiment. Similar studies would also in human liver biopsies from the patients of NASH and no-NASH.

C. Effect of Medicinal plants from the Northeastern India on serum metabolomics

An ongoing study is evaluating the efficacy of medicinal plants from the Northeastern region (NER) of India by examining insulin resistance and related parameters in rats. Serum samples from seven groups at 0, 3, 10 and 14 weeks were analyzed for metabolomics - Control (C), High Fat-High Fructose (HF), Group 3 (T1), Group 4 (T2), Group 5 (T3), Group 6 (T4), Group 7 (T5). Principal component analysis (PCA) and partial least square discriminant analysis (PLSDA) distinguished clusters in the control rat serum metabolome from other groups at all the time points. Comparison between classes showed that the metabolite profile of the rats from T3, T4, and T5 group, which were also protected against HFHF induced insulin resistance, was distinct from the remaining groups. Select metabolites, as seen in the heat map with significant changes (**Figure 4.13A**)

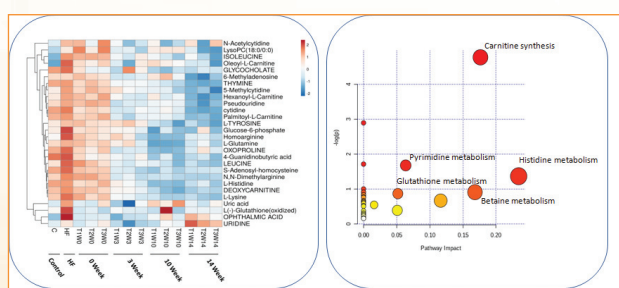


Figure 4.13: A. Expression pattern of metabolites at different time and comparison group; B. Enriched pathway identified from pathway analysis.

were also analyzed using ANOVA and a post-hoc test. Pathway analysis revealed dysregulation in carnitine synthesis, histidine metabolism, betaine metabolism, and glutathione metabolism (**Figure 4.13B**). Increased level of metabolites from the

carnitine synthesis pathway indicates that in HFHF mouse, there is an increased demand of energy through fatty acid oxidation. Mouse model treated with different plant extracts showed metabolites of carnitine biosynthesis with similar expression patterns to control mice. Increased expression of carnitine biosynthesis pathway was observed during enhanced carbohydrate utilization. Histidine metabolism-related metabolites have a scavenging effect on reactive oxygen and nitrogen species. As expected, increased expression of histidine metabolism has been observed in HFHF mouse, and this could be due to more production of reactive oxygen species in the obesity condition. They also observed dysregulation in the glutathione metabolism pathway; this could be because of increased insulin, free fatty acid, and reactive oxygen species in the HFHF mouse model. The other enriched pathway observed was betaine metabolism. Betaine has a primary role as methyl group donor and is very important for liver detoxification reactions. Increased expression of this metabolite in HFHF mouse model points to increased detoxification reaction in the liver. This study suggested that HFHF mouse model shows increased expression of metabolites involved in beta-oxidation, detoxification reaction, and reactive oxygen species. Using these herbal formulations could restore the diseases specific perturbed pathways. Select metabolites, as seen in the heat map with significant changes, were also analyzed using ANOVA and post-hoc test.

D. Bile acids and NAFLD

Bile acids regulate lipid absorption, distribution, and also metabolic processes by activating the FXR receptor. Excessive accumulation of bile acids in the liver causes liver damage, mitochondrial stress, generation of reactive oxygen species,



endoplasmic reticulum stress, and inflammatory response. Despite having a diverse effect on the liver, the primary, secondary, and conjugated bile acids have not evaluated for their

role in NAFLD progression. One study aimed to identify different bile acids and their modification during the progression of NAFLD. **Dr. Yashwant Kumar** developed a comprehensive library of bile acids with accurate mass, retention time, and fragmentation pattern. He first standardized the optimal LCMS setting to identify different types of bile acids in plasma, urine, and stool samples (**Figure 4.14**). Conjugated bile acids were more

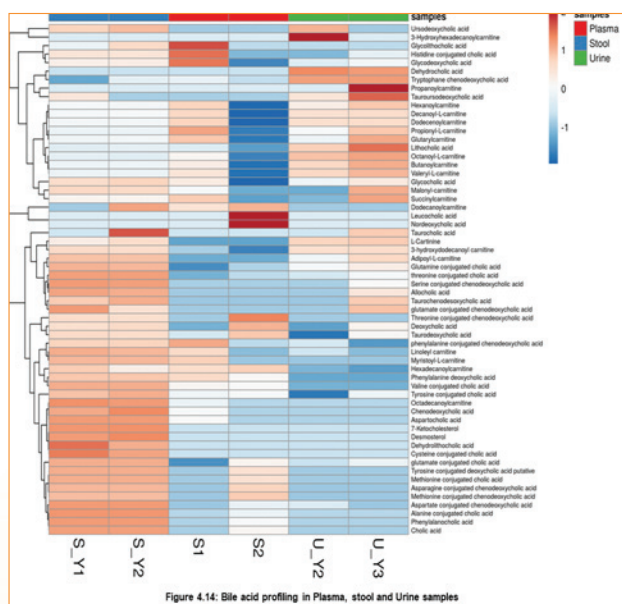


Figure 4.14: Bile acid profiling in Plasma, stool and Urine samples

abundant in the stool sample as compared to plasma and urine; primary and secondary bile acids more in plasma and urine samples. In this study, bile acids would be measured in plasma, urine, and stool samples of the human subjects as well as in mouse model to assess their correlation with disease occurrence and severity. The idea was that bile acid profiling in different stages of

NAFLD will help in identifying distinct changes during the progression of NAFLD.

Way forward: Untargeted and targeted profiling of various bile acids in NASH and No-NASH condition and different stages of NAFLD in the mice model of fatty liver disease.

E. **Proteomics in NAFLD animal models and patients:**



Dr. Renu Goel built a rat spectral library by performing quantitative proteomics analysis of liver from chow, and HFHF diet-fed SD rats, after digesting the tissue proteins with

trypsin to peptides and injected separately in a data-dependent acquisition (DDA) mode. The library is the most extensive catalog of rat proteome with ~8000 proteins, 1,63,537 distinct peptides, and 13,52,520 spectra (**Figure 4.15**). The spectral library built from the DDA runs was opened in PeakView and MarkerView to extract the peptide and the quantification information in each of the SWATH runs (**Figure 4.16**).

- Peptidyl-prolyl cis-trans isomerase, Dihydropyrimidinase, eukaryotic translation elongation factor 2 and anionic trypsin-1 upregulated in HFHF group
- Novel proteins such as agmatinase mitochondrial, Glutamine synthetase, cytochrome P450 3A2, isocitrate dehydrogenase mitochondrial, synaptic vesicle membrane protein, and others reported
- Pathway analysis showed enrichment of oxidative phosphorylation, citrate cycle, glyoxylate and dicarboxylate, and fatty acid

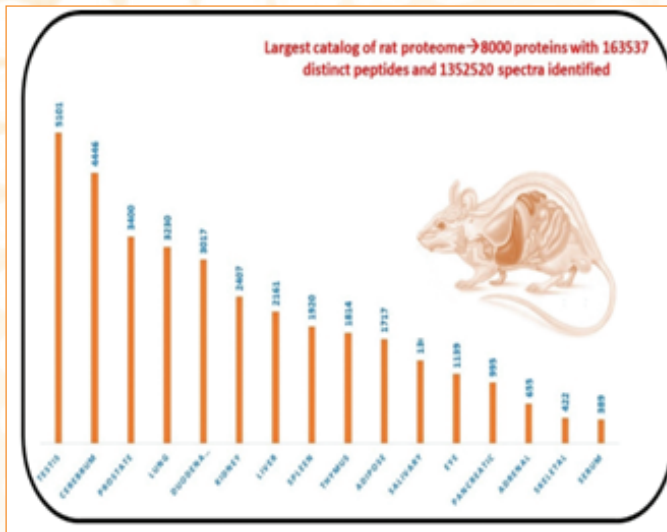


Figure 4.15: Proteome analysis of rat tissues – Generation of peptide library

degradation pathway (**Figure 4.17**). This study provides critical guidance for understanding about NAFLD pathogenesis.

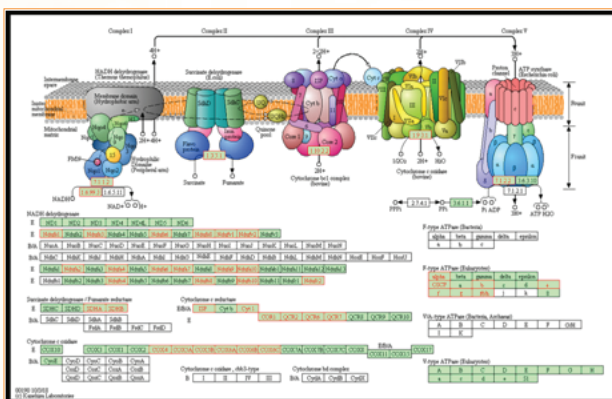


Figure 4.17: Oxidative phosphorylation pathway

Way forward: Plasma, apolipoproteins, and mice/human liver samples to find out the molecular signatures of NAFLD progression. It might also help in differentiating NASH and no-NASH.

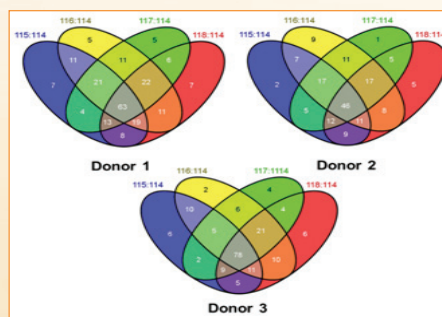


Figure 4.18: Overlap of differentially expressed proteins showing reproducibility within runs

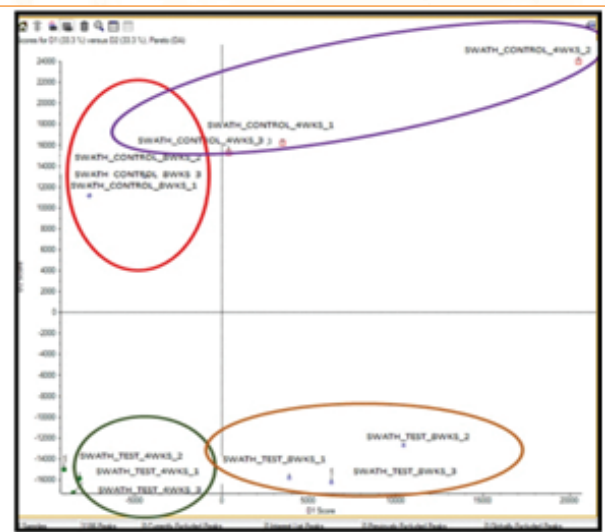


Figure 4.16: PCA analysis

In a collaborative work with ILBS, proteomics study on plasma samples from the subjects undergoing hepatectomy seems to be an ideal model, as it includes healthy individuals (donors) with normal liver function and portal hypertensive subjects (diseased model). Dr. Goel's team used plasma proteomics to understand the temporal expression of proteins after living donor liver transplantation during the process of liver regeneration. Plasma from the living liver donation (LLD) patients was collected before and after surgery on days 1, 3, 5, and 7. Samples were trypsinized and peptides labeled with 114, 115, 116, 117 and 118 Isobaric tags for relative and absolute quantitation. Quantification with LC-MS/MS helped identify 303 proteins; ~95 differentially regulated in all the categories (**Figure 4.18, 4.19**).

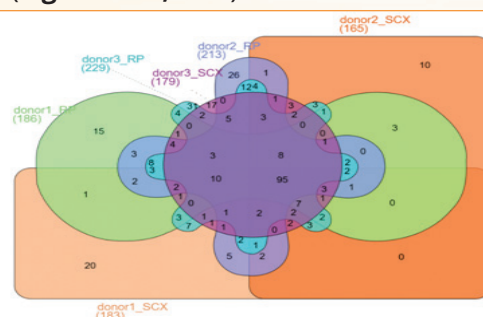


Figure 4.19: Reproducibility of identified proteins between triplicate runs. Total proteins by SCX 257; Total proteins by bRPLC 304; Total proteins = 356; ~ 25 differential regulated proteins found in all the replicates

Temporal changes were noted in the expression of C-reactive proteins, serum amyloid A1, leucine-rich alpha-2-glycoprotein, alpha-1-antichymotrypsin, alpha-1-antitrypsin, and retinal dehydrogenase among all the groups (**Figure 4.20**). Ingenuity

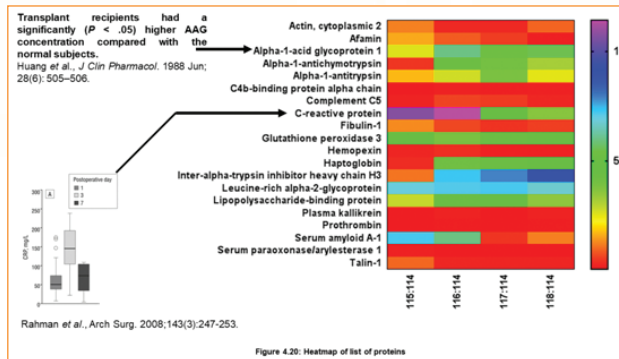


Figure 4.20: Heatmap of list of proteins

pathway analysis (IPA) showed activation of acute-phase signaling and the complement system in day 7 plasma sample. Actin cytoskeleton signaling and LPS/IL-1 mediated inhibition of RXR function were inhibited at day 7 post-operation. Elevated fibrinogen level was seen until 5th day after the transplant, which reverted to the therapeutic level on day 7.

Way forward: Biomarker discovery in plasma samples can lead to development of better and less invasive screening methods to assess the process of liver regeneration. This study presents a time-based expression of proteins after LLD at different stages of liver regeneration. The efforts will focus on specific goals to achieve faster and better liver regeneration.

F. **Computational Mass Spectrometry, Translational Bioinformatics, Metabolic diseases**

Posttranslational modifications and Big-data bioinformatics

Dr. Amit Kumar Yadav's team develops statistical tools, algorithms, software pipelines, and next-



generation visualization applications for high throughput analyses and interpretation of shotgun proteomics data for metabolic disorders.

They integrate big data obtained from omics studies and databases for PTM analysis in metabolic and liver diseases. Big data analysis from public data resources, as well as high-resolution shotgun proteomics data, is used for searching posttranslational modifications (PTM), their quantitation, and crosstalk. PTMs play a crucial role in mediating biological functions through structural binding, aiding enzyme activity, protein-protein interactions, subcellular localization etc. PTMs and their crosstalk drive disease development and progression.

NAFLD and NASH studies from omics and data mining: Genes associated with liver diseases were searched and analyzed using public databases. NASH and NAFLD genes, SNPs, and variants were fetched and analyzed for their associated pathways. Pathway mining of 499 genes led to the discovery of the importance of PTMs and immunomodulatory pathways (cytokines, PPAR- α , interleukins). The pathways related to lipid metabolism, transport and assimilation, β -oxidation, transcription factors, and kinase signaling were the top priority pathways for omics level studies to find newer drug targets and biomarkers. While fatty acid metabolism and signaling molecules were more important in initial stages of liver disorders (NAFL), other pathways like cell death and late immune responses, necroptosis, prostaglandins, secondary metabolism, RIPK-1 signaling, ubiquitination etc. become more important in later NASH stages.

An in-depth study on interactors of lipidated proteins with first neighbor propagation (**Figure 4. 21**) found mostly apolipoproteins (being

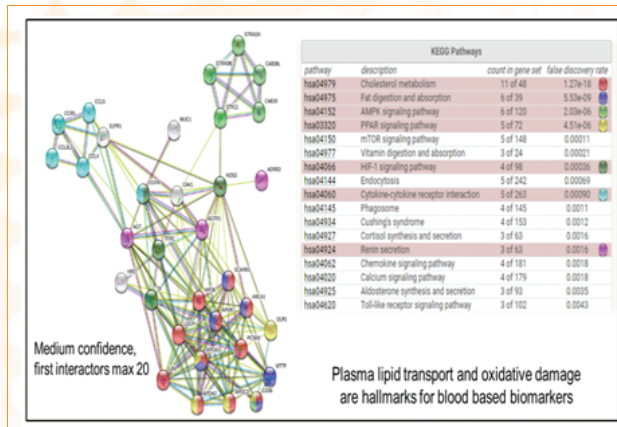


Figure 4.21: First interactors of proteins with lipidated sites reveal important KEGG pathways for liver diseases

studied further), kinases and cytokines involved in lipid transport, AMPK signaling. The team's findings suggest that early prediction of fibrotic markers from omics level data integration is a viable strategy for clinical studies.

Sirtuin interactors and PTMs: Sirtuins are protein deacetylases that play a protective role in metabolic and cardiovascular diseases. The PTMs are known to crosstalk among each other to bring about complex phenotypic outcomes. The team also integrated protein-protein interactions and PTMs from several databases to integrate information on 1251 sirtuin interacting proteins, with ~100,000 PTM sites (**Figure 4. 22**).

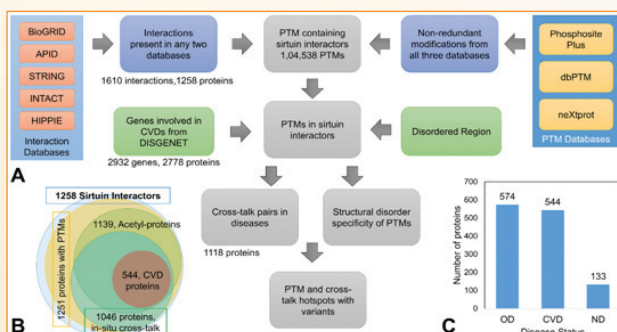


Figure 4.22: Integration of protein-protein interactions and PTMs for SIRTUIN interacting proteins for studying PTM and crosstalk hotspots

Key findings:

- Frequency of PTM site (83 per protein) and PTM types (5 per protein) higher than the global average for the human proteome
- ~60-70% PTM sites fall into ordered regions
- About 83% of the sirtuin interactors contained at least one competitive crosstalk (*in situ*) site with half of the sites among CVD associated proteins
- Identified 614 proteins containing PTM hotspots (≥ 5 PTM sites) and 133 proteins containing crosstalk hotspots (≥ 3 crosstalk sites)
- Large proportion of disease-associated sequence variants found in PTM motifs of CVD proteins. We identified seven proteins (TP53, LMNA, MAPT, ATP2A2, NCL, APEX1, and HIST1H3A) containing disease-associated variants in PTM and crosstalk hotspots (**Figure 4. 23**)

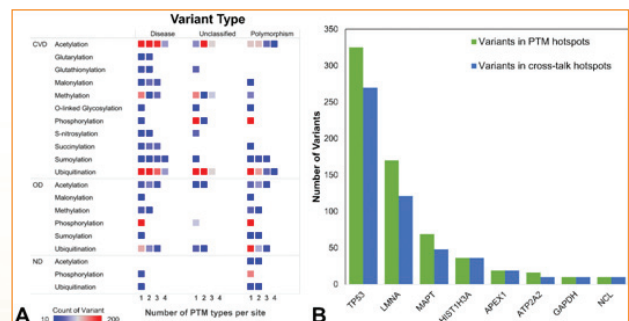


Figure 4.23: (A) The distribution of PTM-motif associated variants (MAVs) with respect to PTM types per site. (B) Proteins with ≥ 10 MAVs in PTM and crosstalk hotspot regions.

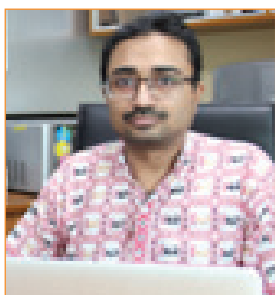
This study forms a platform for generating hypotheses that can be tested for a deeper mechanistic understanding gained or derived from big-data analytics.

PTMs have not been described in detail for their role in disease progression despite their importance

due to technical challenges. The findings lay out a foundation for targeted data acquisition and analysis towards a hunt for biomarkers in NAFLD.

Way forward: The team is working with AIIMS, Delhi, for biomarker identification using omics level analysis employing high-resolution mass spectrometry approaches to identify and validate the potential candidate biomarkers /panel of biomarkers for NASH. Specific PTM profiling work will be planned after the candidate PTM markers discovered in the pilot study.

1. Development of mathematical modeling and systems biology tools



Dr. Samrat Chatterjee's

'complex analysis group' is focused on solving biological problems using mathematical modeling and systems biology tools.

This group builds a model

based on a system of differential equations (ordinary, delay, and stochastic) to address diverse biological problems. They also analyze big data to identify significant molecules that might be responsible for diseases by using tools like clustering algorithms, graph theory, flux balance analysis etc to capture the regulatory proteins and/ or metabolites.

A. Effect of delay in transportation of extracellular glucose into cardiomyocytes under diabetic condition

Diabetic patients are at risk of cardiovascular diseases. To maintain a healthy cardiac function, systematic plasma glucose transportation into cardiomyocytes and in other cells is essential. Calcium is a crucial element in maintaining physiological oscillations (PO) in cardiomyocytes.

In contrast, insulin, its receptors, and other glucose transporters, like GLUT4, maintain glucose transport, to maintain normal cardiac function. Dr. Chatterjee's team built and analyzed a four-dimensional delay-induced model to understand the interaction among plasma glucose, plasma insulin, intracellular glucose, and cytoplasmic calcium of a cardiomyocyte under different parametric perturbations (**Figure 4.24**). A set of conditions was prescribed for the existence of

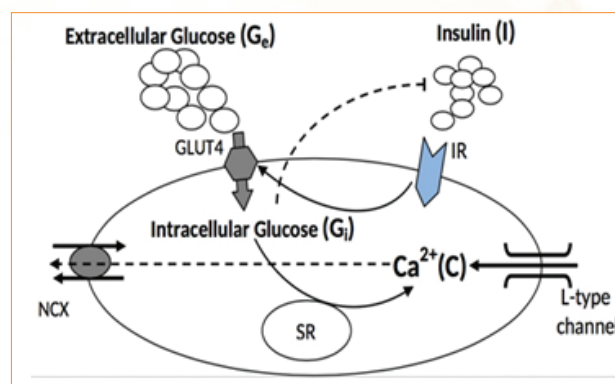


Figure 4.24: Schematic diagram of insulin dependent glucose-calcium interaction in a cardiomyocyte. Variables considered for the model are given in bracket.

Hopf-bifurcation, leading to periodic oscillations of the system around its interior equilibrium point. They looked for situations that would help to maintain normal calcium oscillations within cardiac cells along with a normal concentration of glucose and insulin in the blood. One of the main aims of the present work was to investigate the role of time delay associated with the transportation of extracellular glucose into the cardiomyocyte through GLUT4.

Key observations:

- Uptake rate of extracellular glucose through GLUT4 and the time required for the activities between insulin receptor and GLUT4 activation vital to maintaining regular calcium oscillation
- Time required to transport glucose from blood plasma to cellular cytoplasm has a possible

therapeutic value, and its regulation could restore normalcy in case of the diabetes-like condition

- Regulated glucose input into blood plasma would help to maintain normal oscillations of calcium in cardiomyocytes.

Way forward: Pancreatic beta cells and cardiomyocytes will be examined by using different models to capture the role of calcium in other cellular functions and relate them to diabetic conditions. Biological and clinical validation will follow.

B. Exploring methods to identify potential drug targets using genome-scale metabolic models

Gene knockout studies in cancer cell-lines are used to study the effect of an existing cancer drug or to develop a new drug. Another approach is the phenotypic screening of drugs in cancer cell-lines to find its effect on cell growth - Both costly and time-consuming. A computational method, in contrast, like metabolic networks, could be a good alternative to find drugs with selective ability in killing cancerous cells. Dr. Chatterjee's study is exploring methods that apply genome-scale metabolic models (GSMMs) to find possible targets and mechanisms related to cancer disease. The model was used to study the effect of single and multiple genes knockout on the growth rate of the cancer cell and compare the results with an online experimental database. They also captured the underlying mechanisms associated with the observed growth reduction rate due to gene knockout. They further analyzed top-ranked genes to get potential targets that were then experimentally validated.

Key findings:

- 143 gene knockouts reduce the cell growth across all the metabolic models of the NCI-60 panel and 1488 genes whose knockout does not show any effect on the growth rate of any cancer model.
- Reduction in the growth rate of cancer cells in 143 genes; biomass reduction score (BRS) of those genes is much higher than those of 1488 genes (**Figure 4.25**).

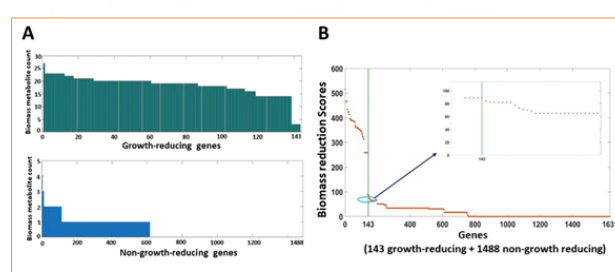


Figure 4.25: Effect of metabolic genes on biomass function. (A) The upper and lower panels indicate the histogram plot of the number of biomass metabolites influenced by each of the 143 growth reducing genes and 1488 non-growth reducing genes, respectively. (B) Biomass reduction scores (BRS) of genes following synergistic effect. BRS is high for first 143 growth-reducing genes and it is low for 1488 non-growth reducing genes.

- 143 genes used to identify potential drug targets, which reduce the growth rate of cancer cells but had no effect on the normal cells; the result validated experimentally.
- Gene ranking results compared with existing shRNA screening data. The rank-correlation results for most of the cell-lines were not satisfactory for the single-gene knockout. Still, it played a significant role in deciding the activity of the drug against cell proliferation.
- Multiple gene knockout analyses and the results showed a better correlation with the experimental observations.

Way forward: The tool will be used to study diseases like NAFLD and results validated by biologists.

C. Studying Input-Output relation in signaling networks under random perturbation

Therapeutic strategies that target crucial molecules have not fulfilled expected promises for most common malignancies. Major difficulties include the incomplete understanding and validation of these targets in patients and the use of single-pathway targeted approaches that are proving not very effective therapies for human malignancies. Signaling pathways are not linear pathways, but it includes molecular crosstalk. So, it is vital to understand the molecular crosstalk among crucial signaling pathways and how targeted agents may alter them. To achieve this, we need to consider and analyze the system as a complex network of interacting components. Dr. Chatterjee studied the Input-Output relation in signaling networks under random perturbation. They started with a simple general model for a circuit with two nodes A and B, where node A receives the input signal that affects the output node B (see **Figure 4.26**). A key objective was to develop an analytical

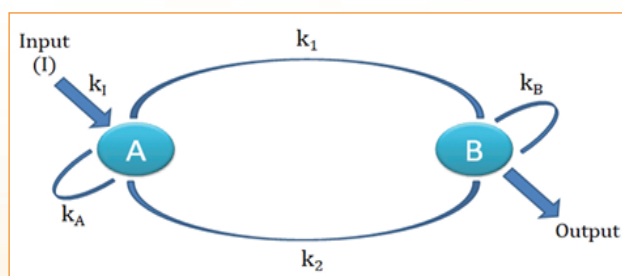


Figure 4.26: Schematic diagram for two-node motif showing all possible interactions between those two nodes.

formula derived by solving mathematical models (using a stochastic differential equation, SDE). The formula classifies and ranks motifs depending on their sensitivity profile and, in turn, rank nodes

depending on the motifs they are present in a vast network. This rank can identify sensitive nodes in a network with potential candidates for drug targets. The strategy developed could be useful in diseases like cancer, diabetes, and obesity, where the role of cell signaling is crucial.

Dr. Chatterjee's team built models for different two-node motif structures using SDEs and compared their stability under random perturbations. They observed that the nature of the signal output depends on the structure of the motif. Some motif structures showed bistability in the absence of noise. In other words, with the same parameter values, the output

signal showed two different steady-state values depending upon the starting concentration of the nodes. It is vital to understand the underlying phenomena of cellular functioning, such as decision-making processes in cell cycle progression, cellular differentiation, and apoptosis. They further observed that the bistable points merge into a monostable point under the influence of noise (**Figure 4.27**). The obtained

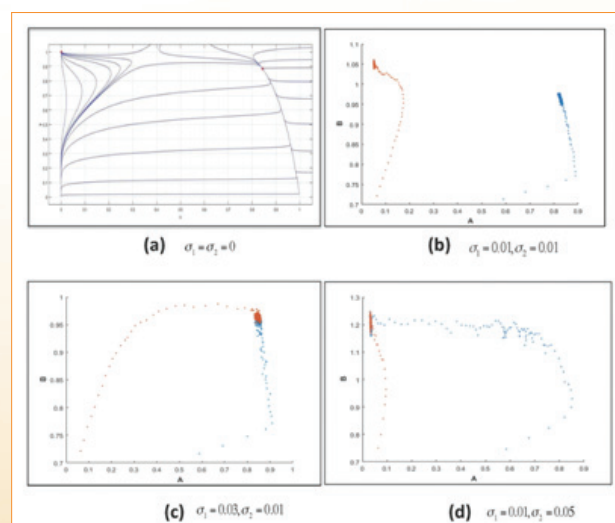


Figure 4.27: Stability properties. (A) Existence of bistability in the absence of noise. (B) Preservation of bistability under low noise. (c)-(d) Merging of bistable clouds into a monostable cloud due to increase in the intensity of noise.

information on the stability properties can help to rank the motif structure based on its sensitivity towards the random perturbations. The proposed method to rank sensitive nodes can be applied to screen potential candidates for drug targets. The method is especially applicable to diseases caused due to perturbations in signaling networks.

Way forward: To understand the reason for merging bistability under random perturbation. They are also looking for a suitable biological system for the application and validation of this tool.

4. Nitric Oxide - mediated pyroptosis and apoptosis of myelocytic cells

Dr. Madhu Dikshit's lab and others have demonstrated the role of exogenous nitric oxide (NO), in myelocytic cell survival and differentiation. Moreover, it is unequivocally accepted that mature



neutrophils express both iNOS and nNOS. By using K562 cells, Dr. Dikshit's team assessed the differences and similarities in cell survival and death

following over-expression of both NOS isoforms. Results obtained demonstrate RONS/JNK/Bax and caspase-3/-9 mediated apoptosis/pyroptosis in both NOS isoforms over-expressing K562 cells, suggesting that induction in the intracellular NO generation by distinct mechanisms might be useful in killing leukemic cells. This study also identified the subtle yet differential cell death mechanisms in K562^{iNOS} and K562^{nNOS} cells, warranting studies for better understanding of NO-mediated pyroptosis and apoptosis of myelocytic cells.





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Prof. Kiyoshi Takeda, Osaka University, Japan

Prof. Oluf Pedersen, University of Copenhagen, Denmark

Dr. Ankur Mutreja, University of Cambridge, United Kingdom

Ramandeep Singh

Drs. Nisheeth Agarwal, Dinesh Mahajan, Tarun K. Sharma, THSTI

Dr. Diwan S. Rawat, Delhi University

Dr. Ravi Datta Sharma, Amity University, Manesar

Dr. Krishan Gopal, CSIR-Institute of Microbial Technology, Chandigarh

Drs. Deepak Saini, N. Srinivasan, Raghavan Varadarajan, Indian Institute of Science, Bangalore

Dr. Harsha Gowda, Institute of Bioinformatics, Bangalore

Amit Pandey

Drs. Amit Awasthi, Tarun K. Sharma, Shailendra Asthana, Renu Goyal, Amit K. Yadav, THSTI

Dr. Vengadesan Krishnan, RCB, Faridabad

Dr. Amit Singhal, ASTAR, Singapore

Dr. Debasish Dash, CSIR-Institute of Genomics and Integrative Biology, Delhi

Dr. Niti Kumar, CSIR-Central Drug Research Institute, Lucknow

Prof. Parmod Mehta, Maharshi Dayanand University, Rohtak

Krishnamohan Atmakuri

Drs. Ramandeep Singh, Amit K. Yadav, Tarun K. Sharma, THSTI

Dr. Sivaram Mylavarapu, RCB, Faridabad

Dr. Anmol Chande, Emory partner center, ICGEB, Delhi

Dr. Tenzin, ICGEB, Delhi

Dr. Sarvanan Kumar, Thermo Fisher Scientific, Bengaluru

Nisheeth Agarwal

Dr. Ramandeep Singh, THSTI

Drs. Bichitra Biswal, Vinay Nandicoori, National Institute of Immunology, India

Dr. Jaya Tyagi, AIIMS, Delhi

Dr. Claudia Jessen-Trefzer, University of Freiburg, Germany

Dr. Preeti Saxena, South Asian University, India

Dr. Yogendra Singh, Delhi University, Delhi

Dr. Gobardhan Das, Jawaharlal Nehru University, India

Dr. Alka Rao, CSIR-Institute of Microbial Technology, India

Dr. Chaaya Iyengar, National Institute of Pharmaceutical Education and Research, Mohali

Dr. Varadharajan Sundaramurthy, National Center for Biological Sciences (NCBS), India

Guruprasad Medigeschi

Prof. Rakesh Lodha, AIIMS, Delhi

Prof. S. K. Kabra, AIIMS, Delhi

Dr. Anmol Chandele, Emory Vaccine Centre, ICGEB, Delhi

Jayanta Bhattacharya

Dr Ashish, CSIR-IMTECH, Chandigarh

Dr Vainav Patel, ICMR-National Institute for Research in Reproductive Health, Mumbai

Dr K. G. Murugavel, YRG Center for AIDS Research & Care, Chennai

Prof Lynn Morris, National Institute for Communicable Diseases, Johannesburg, South Africa

Dr Devin Sok, IAVI-Neutralizing Antibody Center, The Scripps Research Institute, La Jolla, California, USA

Dr. Jan Terje Andersen, University of Oslo, Norway.

Dr Stian Foss, University of Oslo, Norway.

Prof Andrew Ward, The Scripps Research Institute, La Jolla, California, USA

Milan Surjit

Drs. Sankar Bhattacharya, Tripti Shrivastava,

Shubbir Ahmad, Shailendra Mani, THSTI

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Dr. G. Arun Kumar, MIT, Manipal

Dr. Asha Abraham, Christian Medical College, Vellore

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MATERNAL AND CHILD HEALTH

Suchitra Gopinath

Dr. Aneeshkumar Arimbassery, NII

Dr. Niraj Kumar, THSTI

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Dr. Tarun K. Sharma, THSTI

Dr. K. Vengadesan, RCB, Faridabad

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Dr. Rajni Gaiind, Safdarjung Hospital, Delhi

MULTIDISCIPLINARY CLINICAL AND TRANSLATIONAL RESEARCH

Gaurav Batra

Dr. Navin Khanna, ICGEB, Delhi

Prof. Rakesh Lodha, AIIMS, New Delhi

Prof. John Antony Jude Prakash, Christian Medical College, Vellore

Dr. Urpo Lamminmäki, Prof. Kim Pettersson, University of Turku, Finland

Tarun Kumar Sharma

Dr. Rajesh Kumar, THSTI

Dr. Amit Kumar, IIT, Indore

Dr. Robin Doley, Tezpur University-Assam

Dr. B. N. Mohapatra, SCB Medical College, Cuttack

Dr. Anita Malhotra, Bangor University, UK

Dr. Jaideep C. Menon, Sree Narayana Institute of Medical Sciences and Amrita Institute of Medical Sciences (AIMS), Kerala

Dr. Anand Zachariah, Christian Medical College, Vellore

Prof. Jaya S. Tyagi (Department of Biotechnology, AIIMS New Delhi.

Prof. Neera Sharma (Department of Biochemistry, Dr. RML Hospital Delhi)

Dr. V.P. Myneedu (NITRD-New Delhi)

Dr. Amit Kumar (IIT-Indore)

Dr. D.J. Christopher (CMC-Vellore)

Santosh Mathapati

Dr Shinjini Bhatnagar, Translational Health Science and Technology Institute, Faridabad

Dr Dinesh Kalyanasundaram, Indian Institute of Technology, New Delhi.

Dr Jagadish Chandra Sharma, ESIC Medical College and Hospital, Faridabad.

NON-COMMUNICABLE DISEASES

Amit Kumar Yadav

Dr. Shalimar, AIIMS, Delhi

Dr. N. C. Talukdar, IASST

Dr. Debasis Dash, CSIR-IGIB

Drs. Niranjana Chakraborty, Subhra Chakraborty, NIPGR, Delhi

Dinesh Mahajan

Drs. Madhu Dikshit, Ajay Kumar, Sanjay Banerjee, Ruchi Tandon, Shailendra Asthana THSTI

Drs. N. C. Talukdar, Jagat Bora, IASST, Guwahati

Madhu Dikshit

Dr. Shalimar, AIIMS, Delhi

Drs JLN Sastry, A. Gupta and V. Sasibhushan (Dabur Research & Development Centre, Dabur India Limited)

Renu Goel

Dr. Anupam Kumar, Institute of Liver and Biliary Sciences, Delhi

Ruchi Tandon

Dr. Shalimar, AIIMS, Delhi

Dr. Pankaj Seth, NBRC, Manesar

Samrat Chatterjee

Dr. Milan Surjit, Sanjay Kr. Banerjee, THSTI

Prof. Nandadulal Bairagi, Jadavpur University, Kolkata

Prof. Joydev Chattopadhyay, ISI, Kolkata

Dr. Subhradip Karmakar, AIIMS, Delhi

Dr. Ramray Bhat, IISc, Bengaluru

Dr. Arup Banerjee, RCB, Faridabad

Prof. Ezio Venturino, Turino University, Italy

Prof. Anna Gambin, Warsaw University, Poland

Dr. Salman Azhar, Stanford University, USA

Dr. Dina Kondratieva, Tomsk National Research Medical Center, Russia

Yashwant Kumar

Dr. Shalimar, AIIMS, Delhi

Dr. Chiranjay Yajnik, KEM hospital, Pune



TRANSLATIONAL RESEARCH PROGRAM (TRP)

TRANSLATIONAL RESEARCH PROGRAM

BIOASSAY LABORATORY

The THSTI Bioassay laboratory (BL) has been established as a platform for the clinical development of vaccines and biologicals. It aims to be one of the cGLP laboratories, maintaining global standards for clinical assay services for vaccine-preventable pathogens. BL has partnered with both national and international agencies such as IVI, Korea; AFRIMS, Thailand; WHO reference lab, Australia; ICGB, New Delhi; IISc, Bangalore; CMC, Vellore; and Manipal Virus Research Centre. We are also an ICMR-recognized COVID-19 testing facility, the first one in the Faridabad-Palwal region. The team at BL has processed more than 15,000 clinical samples for diagnosis (April-June 2020) from several districts to date.

The establishment of BL was initiated in January 2019, with qualified human resources hired and trained as per ISO17025:2017 standards by April 2019. The technical and equipment Standard Operating Procedures (SOP) together with Quality System Manual (QSM), Laboratory Safety Manual (LSM), and Quality System Procedures (QSP) were completed by December 2019 with procedures for pre-validation of various assays commencing during this period. Following this, method validation was accomplished by interlaboratory comparisons and proficiency testing by March 2020. Application for NABL ISO 17025:2017 accreditations was submitted for seven assays in the last week of March 2020.

The scope of the work for accreditation at BL is divided into two phases. Phase I involved establishing assays for chikungunya and dengue serology and dengue viremia and serotyping. In this regard, 23 Quality System Procedures (QSP), 21 Standard Operating Procedures (SOP), and 20 Equipment Operating Procedures (EOP) were released. Forty of the existing equipment are under calibration and annual maintenance contract by NABL-accredited or by Original Equipment Manufacturers (OEMs). The procurement of seven additional new equipment is underway. Standardization protocols for all the seven assays involved standardization for RNA isolation from serum, PCR optimization, RNA lot variability, precision, and accuracy. In-house serum panels have been prepared for all the ELISAs, and variables such as specificity, sensitivity, and reproducibility and scalability have been assessed. The BL has received service enquiries from renowned companies/institutes, namely IIL Hyderabad, Panacea Biotech, Zydus Cadila, INCLEN, and Dr. Dang's laboratory. It is indeed a proud achievement for the nascent facility to have generated an income amounting to about INR 7,00,000.00 by providing assay services for the year 2019-20.

Phase II scope of work primarily focusses on establishing plaque reduction neutralization assays (PRNT) for Dengue, Chikungunya, Japanese encephalitis, and West Nile virus. BL

is entrusted with assays for Influenza virus detection, Hemagglutination inhibition assay, and Microneutralization assay for the extramural project awarded by the DBT-EU consortia to THSTI. The BL is also being considered by the Coalition for Epidemic Preparedness Innovations (CEPI) as a centralized laboratory. The BL will be required to provide assays for SARS-CoV2 PRNT as well as for two in-house developed assays examining namely the IgG and IgM ELISA and SARS-CoV-2 pseudovirus neutralization assay. BL is enrolled in a proficiency testing program run by Controllab, Brazil, for Dengue, Chikungunya, and SARS-CoV2 serology and molecular assays.

BL responded to the COVID-19 pandemic by proactively partnering with ESIC hospital, Faridabad, to initiate diagnostic testing in Faridabad. BL has also contributed to training human resources and establishing COVID-19 testing labs in several hospitals in the region. BL has also been chosen to offer validation services for diagnostic kits by The Foundation for Innovative New Diagnostics (FIND) and the Indian Council for Medical Research. CEPI is in the process of selecting BL as one of the centralized laboratories for SARS-CoV-2 vaccine development and some of the Indian vaccine companies have also expressed interest in working with BL for SARS-CoV-2 vaccine trials. Work is also underway to establish assays for a wide range of viral pathogens and also to support oral cholera vaccine studies (funded by BIRAC). A model for revenue-generation and self-sustainability is being planned and will be presented to the DBT for approval.

SMALL ANIMAL FACILITY (SAF)

THSTI's Small Animal Facility (SAF) is responsible for the breeding and maintenance of quality laboratory animals. The facility caters to the

research groups requiring lab animals in the NCR Biotech Science Cluster. Currently, it is extending researchers at THSTI and the Regional Centre for Biotechnology (RCB). SAF was established in compliance with the guidelines of the **Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)**, Ministry of Fisheries, Animal Husbandry and Dairying, Department of Animal Husbandry and Dairying, 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 of the Institutional Animal Ethics Committee (IAEC).

Progress made this year:

- Nine mouse strains added in the breeding colony.
- The animal import permit received for importing mouse strain from overseas animal suppliers.
- The acidification of drinking water was initiated to maintain the quality of drinking water of animals.
- The required testing kits were procured to implement molecular-based diagnostic screening for a few critical pathogens.
- A separate animal room was developed inside SAF to carry out animal experiments of infectious nature, which does not require the ABSL-III and above level of containment.
- As a part of the expansion plan, additional biosafety cabinets and individually ventilated caging systems for mice were procured and installed in the facility to increase the housing capacity.

- Breeding and maintenance of mouse models required for dengue research were initiated in the facility so that this can be used for testing the vaccine, antiviral agents, or potential monoclonal antibodies.
- The tender was published, and the process was initiated to implement the CCTV surveillance monitoring and access control system in the facility.
- To implement the up-gradation plan, the selected firm will help reach the goal of achieving international standards required in animal research and animal facility management.
- THSTI initiated the process of establishing a ferret holding and experimentation facility in the existing un-utilized primate facility building with required modifications or up-gradation needed as per the requirements. This will support research on respiratory viruses like influenza.
- Infectious Disease Research Facility (IDRF) was made functional as a specialized animal biosafety level III containment facility for TB and HIV research. The mandatory training required for working in ABSL-III facility was conducted. Animal research work in guinea pig and mouse models for infectious diseases such as tuberculosis were initiated in the specialized ABSL-III.

Way ahead:

THSTI is in the process of upgrading this facility to achieve necessary national and international standards for animal research and animal facility management. The goal is to make SAF a national resource and nodal center for collaborative work required in the field of small laboratory

animals within India. Currently, only one floor is operational. SAF is in the process of procuring more strains/stocks of required lab animals. 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.

BIOREPOSITORY

The Biorepository was established in 2015 as a component of the DBT funded Interdisciplinary Group of Advanced Research Birth Outcomes, a DBT India Initiative (GARBH-INI) at THSTI. With its systematic plan and expansion, it recently got identified as a separate platform in the **Translational Research Platform** in 2019 with the mandate to expand to programs in **the NCR Biotech Science Cluster and beyond**. Currently, it houses more than nine lakh biospecimen. It is now upgraded to build on the infrastructure, trained research staff, structured database and in the process of obtaining global certifications from accredited regulatory bodies. In the past year, it has been registered with International Society for Biologicals and Environmental Repositories (ISBER). The core group personnel have undergone various training programs, like ISO 9001:2015, Internal Auditing, Good Clinical Lab Practices (GCLP) for management of inflow of different types of samples under various studies. The team has been involved in collection, processing, and storage of biospecimen, and have been recognized and credited as authorships and acknowledgements in 7 publications/thesis/reports. With the NABL certified equipment and the expertise of the team members, the biorepository is a self-sufficient unit to undertake responsibilities of supporting major programs.

S.No.	Name of the studies	Types of samples	No. of samples	No. of participants	No. of samples retrieved
1	Interdisciplinary Group for Advanced Research on Birth Outcomes-DBT India Initiatives (Garbh-INI)	Maternal serum, blood, plasma, DNA, blood as PAX gene tubes, saliva, urine, high vaginal swabs, cord blood, umbilical cord tissue, placental tissue punches, placental membranes, paternal saliva and neonatal heel prick venous blood	~ 8.5 lacs	~ 7930	13534
2	Pediatric renal biology program: Research on nephrotic syndrome	Serum, Plasma, DNA, Urine and Blood as PAX gene tubes	~25000	~ 1020	NIL
3	Gall bladder cancer registry	Bile fluid, Gall bladder biopsy	~80	~ 20	20
4	Peripheral blood mononuclear cells (PBMCs) derived from healthy individuals	PBMCs	~ 6000	2000	NIL
5	COVID-19 Consortia	Serum, Plasma, NP/OP swabs, PBMCs	~50000	~ 1800	~2200

Recently, during the current COVID-19 pandemic, the biorepository has been recognized as one of the networks of “**National Bio-Resource Centre for COVID-19**” by Department of Biotechnology, Government of India. As directed by NITI-AYOG and Department of Biotechnology, the team has participated in developing policy guidelines for sharing biospecimen and data for research related to COVID-19. With establishment of the DBT-COVID-19 Research Consortium, 2020, by THSTI, between DBT autonomous institutions and hospitals in the NCR and Delhi region, a study cohort comprising of two cohorts i.e. COVID testing and COVID positive cohort across the collaborating hospitals has been established. Samples are being collected from enrolled participants and are being processed for blood derivatives and stored as sera, plasma, and peripheral mononuclear cells and stored in the biorepository to be shared as COVID-19 Bioresources.

Way ahead: In the coming year, we plan to extend our services to the NCR cluster, and other Academic institutes to augment their research activities by providing standardized collection, processing, and storage services; residual samples as reference material after the primary objectives

of the study have been met, strengthen the in-house biorepository into a self-sustained management facility for clinical and translational studies and integration of biorepository data with clinical data management.

DATA MANAGEMENT CENTRE & ARYABHATTA DATA SCIENCE AND ARTIFICIAL INTELLIGENCE PROGRAM AT THSTI (ADAPT)

The **Data Management Centre** (DMC) of the Translational Research Program at THSTI has established infrastructure and robust systems/processes to provide data management support to clinical research projects within MCH and also THSTI. DMC has an interdisciplinary team of data scientists, clinician scientists, data managers, quality analysts, data entry operators and interns (from various backgrounds including biostatistics, engineering).

Establish robust systems/processes to ensure data management services to the highest standards of quality: DMC, since it began in 2010, has supported four completed studies and is actively supporting nine (7 within MCH, 2 outside MCH, and within THSTI) more studies. The team is also currently supporting three PhD thesis for data cleaning and statistical analysis. They have established the data management process for both paper-based data capture and electronic data capture using in-house clinical data management platform with good quality control measures. More recently, for the GARBH-Ini program, they adopted a commercial vendor supported electronic data capture platform. The

aim is to transition to a more independent and flexible approach of using open-source platforms such as Redcap over the next year.

Video data capture and management for an international collaborative study (CALOPUS):

DMC and ADAPT is an active participant in the Computer-Assisted Low-Cost UltraSound project with the University of Oxford. This project entails the collection, real-time remote transfer of sensitive antenatal ultrasound videos from Gurugram Civil Hospital to THSTI, from THSTI to multiple radiologists working from different places in NCR and then between Oxford and THSTI for quality control. DMC & ADAPT have enabled the high-fidelity, rapid transfer and remote processing of the videos.

Big data management, cutting-edge analytical approaches using artificial intelligence and machine learning:

DMC and ADAPT enables processing of microbiome, proteomics and metabolomics data from the GARBH-Ini program. Big data analysis involves repetition of similar steps, thousands of times in a pipeline. The programmatic automation by ADAPT has led to efficient, error-free and reproducible data analysis. One example of such an analytical pipeline is mixed-effects modelling of longitudinal expression of hundreds of proteins identified in proteomic analysis.

Skilled team for methodologically advanced data analysis for intramural and extramural studies:

To support advanced analytics including artificial intelligence and machine learning, the team has acquired a state-of-the-art computational server and four workstations. They have a technical team of 15 members who

actively participated in activities such as variable harmonisation and clinical research workshops. Apart from this, interns of ADAPT are an active and integral part of the automation and programming aspects of the data management. The centre is, thus, developing individuals in the team towards making this facility a state-of-the-art data management platform.

Other Achievements from 2019-2020

Variable harmonization for international collaborations on Multi-Omics for Mothers and Infants (MOMI):

DMC and ADAPT is participating in a global collaboration on preterm birth known as Multi-Omics for Mothers and Infants or MOMI for short. The clinical variables collected in six large international cohorts in Africa and Asia have been harmonized in order to enable pooling of data to address questions of global health significance.

Rising up to the COVID-19 pandemic challenge:

A decade of experience in clinical data management placed the DMC in the best position to take up the challenge of setting up clinical studies in response to COVID-19 pandemic. A program to establish a clinical data and biorepository as resources to accelerate tools to combat COVID-19 was started in late March 2020. This involved setting up clinical research activities in nine hospitals all across Delhi NCR. The setup of electronic data capture and database development for the program was completed by the data management team over a very short period of time. Real-time data monitoring, prompt analysis and sharing of data with multiple industry and academic partners is being done actively. This was enabled by the programming skills and computational power developed at DMC & ADAPT.



CLINICAL DEVELOPMENT SERVICES AGENCY (CDSA)



The Team at Clinical Development Services Agency, THSTI

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 of public health importance. It fills a unique niche in the publicly funded clinical research and product development landscape in India. The main objectives of CDSA are:

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

- Support for all stages of clinical trials including design, planning, initiation, conduct, data, and safety monitoring, analysis and reporting
 - Advise for regulatory approvals and pathway for product development
 - Quality management for clinical research activities
 - Monitoring of studies for compliance to New Clinical Trial Rules 2019, ICMR, CDSCO-GCP guidelines, study protocol and other requirements as applicable
1. To undertake human resource capacity building for clinical development and other related activities to strengthen health systems and clinical research in India
 2. 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 10. Also, 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 reports to the Executive Director of THSTI. Besides,

a Senior Professor from the Medical Research Council Clinical Trials Unit (MRC CTU) at University College London (UCL), UK, provides oversight for CDSA as the Strategy Lead.

During the past year, CDSA strengthened the institutional platform and the governance structure. The team developed a performance-based contract career path to attract talented professionals, recruited high-quality professionals, enhanced its Clinical Research Unit (CRU) capabilities, built robust clinical trials IT infrastructure, developed an ecosystem for training, did capacity-building in clinical research applied for grants as co-applicants and provided support to several ongoing projects.

Team science underpins high-quality health research. The various departments within CDSA, each with their distinct role and competence, work cohesively for the successful delivery of a high-quality program.

- Clinical Portfolio Management
- Training
- Medical Affairs (Clinical Science)
- IT and Data Science
- Regulatory Science and Policy Support
- Administration and Finance

Clinical Portfolio Management:

The Department of Clinical Portfolio Management (CPM) has supported and facilitated the delivery of high-quality clinical research across a variety of disease areas. We have capabilities and strengths in project planning, protocol development with special emphasis on operational aspects, project management, site feasibility, site set-up & initiation, support ethics / regulatory submissions, study conduct, quality, and safety monitoring,

tracking of milestones, risk identification, data management and report writing,

The CPM department, with support from the regulatory science and training departments, has successfully provided study support to more than 20 clinical studies. Although the focus and expertise is on large multicenter late-phase clinical trials, the team has provided support to all kinds of research projects. This includes academic clinical trials, regulatory trials, longitudinal cohort studies, and seroprevalence /sero surveillance studies.

In the last one year, the CPM department has expanded its portfolio to include trial design/ conduct and taken initiatives to improve the quality monitoring. It has adopted risk-based methodology for monitoring for some new studies, introduced 'process monitoring', and developed tools to quantify the quality in the process being monitored and established electronic platforms (clinical trial management system) for more efficient management, quality monitoring, and tracking of study deliverables.

The specific studies/trials and the role of CDSA in each are detailed in **Annexure 1**.

Training:

The training department has been at the forefront in exploring new avenues to engage all its stakeholders and clinical researchers with various opportunities to learn and interact with the experts. CDSA programs stand out as they adhere to the quality standard, are peer-reviewed, guided by national as well as international subject matter experts, and in collaboration with Indian drug regulators (CDSCO), BIRAC, ICMR. Every year, the team strives to strike a new chord; this year is no

different. CDSA launched its online courses over the HRD portal, SWAYAM.

In 2019-20, CDSA conducted 18 face-to-face programs across 10 different cities across India,

engaging various important stakeholders like CDSCO, DBT, BIRAC, ICMR imparting training to various biomedical researchers, clinicians, scientists, and ethics committee members, clinical trial team members, etc.

Table 6.1: Summary of training programs conducted by CDSA across India

Details	Year-wise									Total
	2009-12	2012-13	2013-14	2014-15	2015-16	2016-17	2017-18	2018-19	2019-20	
Number of Training sessions	3	10	14	17	21	29	17	19	18	148
Cities	2	5	10	10	9	15	12	14	10	87
Faculty	11	112	146	175	233	236	120	149	174	1356
Participants	41	436	894	1241	1906	1510	4476	1344	1851	13699
Institutions	10	117	222	428	536	391	409	418	638	3169

Some highlights of work accomplished:

i. Certificate courses - SWAYAM portal

CDSA launched two online courses on the SWAYAM portal (<https://swayam.gov.in>). SWAYAM is a free online education portal supported by the Ministry of HRD, GOI. These online courses were designed and developed by CDSA's training team, reviewed and recorded by CDSCO and CDSA. The course was developed in collaboration with NPTEL/IIT Madras.

Around 5000 learners were benefitted by this endeavor. The courses were interactive with a discussion forum, weekly assignments, LIVE sessions with faculty, proctored exam, which was optional, etc. Based on sound and systematic approach, these courses offered a platform to learn from the best in the comfort of home. The courses were inaugurated during the DBT-Global Bio Summit in November 2019 by Hon'ble DBT Secretary, Dr. Renu Swarup, and DCGI (CDSCO), Dr. V.G. Somani in the presence of Prof. Gagandeep Kang, ED, THSTI.



Online Course 1: Current regulatory requirements for conducting clinical trials in India for investigational new drug/new drug (Version 2.0) [https://swayam.gov.in/nd1_noc20_ge13/preview]

Version 2.0 of the course incorporates the New Drugs and Clinical trial Rules, 2019, and is enriched with a quiz, Q & A, at the end of each session. This online course is presently ongoing, with 2891 learners enrolled.

Online Course 2: Regulatory requirements for medical devices including in vitro diagnostics in India (Version 2.0) [https://swayam.gov.in/nd1_noc20_ge14/preview]

This online course is presently in progress, with 2053 learners enrolled. Version 2.0 of this course includes all new information related to the medical device regulations in India. This online course, too, is ongoing with 2053 learners enrolled and has a Q & A at the end of each session.

A face-to-face exam will also be conducted by NPTEL/IIT Madras at 140 cities across the nation. Many aspirants are expected to enroll for the certification exam.

ii. National workshop on regulatory compliance for accelerating innovations – DBT, BIRAC-NBM, CDSCO as per NITI Aayog recommendations

CDSA steered a six programs' national workshop series on regulatory compliance for accelerating innovations with senior regulators from CDSCO and State Licensing Authorities as faculty. Four programs were held in 2019 at C-CAMP (Bengaluru), NIPER (Hyderabad), NIPER (Guwahati), and MS University (Vadodara). These programs were attended by more than 250 participants selected based on pre-set criteria. Feedback from all participants was solicited via an online feedback capture tool. The series is now complete, and an impact assessment is being conducted by the Quality Council of India (QCI) to understand the impact of this endeavor among all the target participants and institutions. This series was funded by DBT through BIRAC-NBM.

iii. Interactive meet on New Drugs & Clinical trials Rules: It's understanding and impact

The New Drugs and Clinical Trials (NDCT) Rules were published on March 19, 2019, by CDSCO. To facilitate a better understanding of the new rules, CDSA-THSTI organized an interactive meet with the regulators (CDSCO) who drafted the rules. The meeting was live-streamed on May 17, 2019. 371 participants representing 175 institutions attended this program while thousands watched it live.



iv. Good Laboratory Practice (GLP), documentation and laboratory safety

A program was conducted for all THSTI scientific fraternity, which includes, but not limited to faculty, technicians, research fellows, Ph.D. students to enable them to understand GxP, especially GLP. The importance of documentation and laboratory safety was also emphasized during this hands-on interactive program. 45 participants attended this program on September 23, 2020, held at THSTI.

v. Delivering training to 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 augment research capacity beyond the boundaries of metro cities and bigger towns. This year CDSA conducted a training program in Jawaharlal Nehru Institute of Medical Sciences (JNIMS), Imphal, Manipur training 147 participants, mainly ethics committee members, faculty, investigators, clinicians among others representing 7 institutes including JNIMS.

vi. Face to face short term training programs

CDSA conducted a few short term training programs on GCP, GCLP, and current regulatory requirements. One program on GCP and

regulatory requirements was held at THSTI as part of the committed training programs. Three GCP programs, one each at ICMR headquarters, Safdarjung hospital, and NIRT, Chennai, were conducted as part of capacity building for the projects that we support. These programs were funded by ITRC, BMGF, and USAID, respectively. BHU, Varanasi invited CDSA to conduct a 2-day program on GCP, and Current regulatory and ethical requirements for conducting clinical trials/research in India in November 2019. This was attended by 109 participants representing 27 national institutes across India.

vii. Skill development workshops – National Biopharma Mission

Department of Biotechnology (DBT) invited CDSA to conduct a series of four national workshops on GCP, and GCLP. CDSA is the knowledge partner, whereas Biotech Consortium India Limited (BCIL) is the managing partner. Three out of the four workshops were conducted at CDSA (Faridabad) and Venture Center (Pune) in Jan-Feb 2020. We had international faculty from NIH (USA), Leidos Biomedical Research (USA), National Institutes of Health - University of Philippines (Philippines), Institute of Medicine (Nepal), apart from several national experts and CDSA faculty.





New initiatives to enhance the clinical research ecosystem in India

i. Academic Clinical Research Unit (A-CRU)

In the endeavor to enhance CRU capability and align with global standards, CDSA established the 'Academic Clinical Research Unit'. They started with building and establishing the infrastructure and resources. After having set up robust IT systems and processes, they will build multi-disciplinary expertise for the entire life-cycle of a trial/ study particularly large multicentre trials - from design and funding application through protocol development conduct, including monitoring and oversight to data analysis, interpretation, reporting, and dissemination of results.

CDSA is collaborating with MRC CTU, and in the pipeline are visits of different CDSA team members to MRC CTU. They have been engaging with investigators from the grant application stage and contributing to operational design, conduct including monitoring and oversight, data management, and are co-applicants on

many recently awarded grants. Some recent grants where CDSA has been a co-applicant are: Neosepsis program (DBT), INDIGO (EU-DBT grant), Translational Research Program for Dengue (NBM, BIRAC), DH study (BMGF), IMPART (seed funding CRUK-DBT grant on affordable cancer care). CDSA was awarded a seed funding grant from CRUK-DBT grant on affordable cancer care as a primary applicant along with AIIMS, New Delhi and MRC-CTU, London.

ii. Steps to improve quality management in trials:

- CDSA has introduced process monitoring as part of the quality management plan. The CPM team has developed tools to quantify the quality of the process being monitored. This will aid the quality monitoring team to show trends in quality and the corrective and preventive action
- Adopted risk-based monitoring for some new studies and are developing a protocol for a 'SWAT' to compare conventional with risk-based monitoring
- Developing standard IT-based tools for on-site/ remote quality monitoring. This will enable quantification of the observations, and generation of standardized reports

iii. Common research application process:

They are working in collaboration with the Forum for Ethics Review Committees in India (FERCI) and PATH on an integrated electronic research application platform for clinical research approvals. We have developed an online workflow management software that helps ethics committees track submissions, generate queries, assign reviewers, and ensure the security of study documents. The common EC application form has

been incorporated in the software for filling and submission by investigators.

iv. Master's program in Clinical Trials

Currently, CDSA is engaged with DBT, India, and the Medical Research Council Clinical Trials Unit (MRC CTU) at UCL to develop Master's Program in Clinical Trials. The program will be one of the first postgraduate degree in India wholly dedicated to clinical trials and is focused on attracting young physician-scientists and other researchers who wish to pursue an academic career in clinical research. Developing a core of trained young scientists and physicians in clinical research in the country was one of the critical mandates of CDSA.

v. Good Clinical Practice Professional Certification Scheme (GCPPCS)

There is an ever-growing demand for trained and certified Good Clinical Practice (GCP) professionals in the clinical research arena both in academia and in industry. Setting up a uniform system to achieve the desired competence standard using internationally accepted best practice for 'Assessment' and 'Certification' is the key to addressing this unmet need. To this end, a

Certification Scheme for GCP professionals based on the International' Personnel Certification' standard (ISO 17024:2012) is being developed by CDSA. The GCP professional certification scheme will have two aspects – a system of accreditation of Training Institutions to ensure standardized, high-quality GCP training and GCP Professionals Certification (by Third-Party Certification) to promote certification of professionals both within the country and across the globe.

CDSA is the Scheme Owner and has constituted the Steering Committee, Technical Committee, and Assessment Committee comprising multiple stakeholders for example subject and process experts with representatives from ministries, regulators, government agencies, industry, academia, accreditation body, certification body, training institutions, and civil society organizations. A task force was constituted to design and develop the minimum standard of competence following which the Assessment Committee will develop the standards for assessment of competence. Once all documents are developed, they will be published in the public domain, inviting comments for the public for one month followed by deliberations.

Annexure I: Summary of ongoing projects where CDSA is providing clinical study support

S. No	Project Title (Funding Agency)	Principal Investigator / Institute	Role of CDSA	Contribution
1.	GARBH-INI: 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.	Zinc as an adjunct for the treatment of clinical severe infection in infants younger than 2 months (Research Council of Norway through GLOBVAC and CISMAG)	Dr. N. Wadhwa & Prof Shinjini Bhatnagar, THSTI	- Study start-up support - Quality management	- GCP compliant study documents, ICD, CRF, SOPs and data collection tools - Study execution as per Protocol and GCP guidelines - GCP trained project team
3.	The follow-up study to evaluate the impact of continuous KMC initiated immediately after birth compared to KMC initiated after stabilization in newborns with birth weight 1.0 to <1.8 kg on their neurodevelopmental outcomes in low-resource settings (WHO/ BMGF)	Dr. H. Chellani, Safdarjung Hospital, Delhi and Dr N. Wadhwa, CDSA	- Co-applicant/ Co-PI - Study start-up support - Internal quality management	- Successfully supported TOT & anthropometric standardization workshop for all participating countries - GCP compliant study documents and trained study team - Study execution as per Protocol, GCP and WHO guidelines
4.	Investigation of Rheumatic Atrial Fibrillation Using Vit K Antagonists, Rivaroxaban or Aspirin (PHRI, Canada)	Dr. G. Karthikeyan, AIIMS, Delhi	- Study start-up support - Project management - Data monitoring - Safety reporting reconciliation	- GCP-compliant study documents, ICD, CRF, SOPs and data collection tools. - Study execution as per Protocol, GCP and CDSCO guidelines

S. No	Project Title (Funding Agency)	Principal Investigator / Institute	Role of CDSA	Contribution
5.	Accelerating the application of stem cell technology in human disease – ADBS Study (DBT)	Dr. S. Jain, NIMHANS, Bengaluru	- Study start-up support - Quality management	- GCP compliant study documents ICD, CRF, SOPs and data collection tools - Study execution as per Protocol, SOPs and GCP guidelines
6.	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 (ICMR)	Dr. Padma Priyadarsini, National Institute for Research in Tuberculosis (NIRT), Chennai (02 sites across India)	- Study start-up support - Safety and data monitoring - Medical writing support	- Regulatory compliance and approvals - GCP and CDSCO compliant study documents, ICD, CRF, SOPs and data collection tools. - Site set-up as per project requirements - GCP trained project team
7.	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. Padma Priyadarsini, National Institute for Research in Tuberculosis (NIRT), Chennai (05 sites across India)	- Regulatory advisory - Study start-up support - Safety and data monitoring	- 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 Protocol, GCP and CDSCO guidelines - GCP trained project team
8.	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. M. Singh, ICMR (15 sites across India)	- Study start-up support - GCP training - Project management support - Safety and data monitoring - Data management support	- Regulatory compliance approvals - GCP and CDSCO compliant study documents, ICD, CRF, SOPs and data collection tools. - GCP and GCLP trained project team - Study execution as per Protocol, GCP and CDSCO guidelines

S. No	Project Title (Funding Agency)	Principal Investigator / Institute	Role of CDSA	Contribution
9.	Burden of multidrug-resistant neonatal sepsis in district hospital settings in India	Dr. J. Sankar, Dr. R. Agrawal, AIIMS, New Delhi (05 sites across India)	- Project management - Data management - Quality management - Clinical site management	- GCP compliant study documents ICD, CRF, SOPs and data collection tools - Study execution as per Protocol, SOPs and GCP guidelines
10.	Translational Research Consortium For Establishing Platform Technologies To Support Prophylactic and Therapeutic Strategies for Dengue Discovery to Proof-of-Concept (NBM, BIRAC)	Dr. A Chandele, ICGBE; Dr. N. Wadhwa, CDSA (03 clinical sites across India; 4 research institutes)	- Program management support - Quality management - Data management	Study execution as per Protocol, GCP guidelines and SOPs
11.	Understanding human Covid-19 infections: a DBT India Consortium (THSTI)	Prof. Shinjini Bhatnagar, THSTI	- Clinical operations Support - Quality monitoring	Study conduct in compliance with Protocol, GCP guidelines
12.	A Phase II, Open Label, Randomized Controlled Trial to Assess the Safety and Efficacy of Convalescent Plasma to Limit COVID-19 Associated Complications in Moderate Disease (ICMR)	Dr. N. Sharma, ESIC, Faridabad; Dr N Wadhwa, CDSA	- Investigators - Site start-up support - Quality monitoring	- Study execution as per Protocol, GCP guidelines and SOPs - GCP trained team
13.	Digoxin in patients with rheumatic heart disease- a randomised placebo-controlled trial (ICMR)	Dr. G. Karthikeyan, AIIMS, Delhi	- Site start-up support - Quality management - IP Management	Study start-up ongoing as per ICMR requirements

S. No	Project Title (Funding Agency)	Principal Investigator / Institute	Role of CDSA	Contribution
14.	Suraksha: South-Asian Breast Cancer Risk Prediction, Genetic testing and Health Management (seed funding, CRUK DBT Affordable Approaches to Cancer)	Dr N. Wadhwa, CDSA; Prof Deo, AIIMS New Delhi; Prof U Menon, MRC CTU, UK; Prof R Manchanda, QMUL, UK	- Contribute to protocol development - Project management - Support submission of PPI objective proposal to site IECs - Contribute to systematic review of breast cancer risk factors in South Asian populations	- Project coordination - Protocol development for PPI objective - Supported clinical sites in translation of documents, EC submissions - Contribute to protocol development - A systematic review of breast cancer risk factors in South Asian populations - Develop a mixed-methods multicentre PPI
15.	IMPART: Immune Checkpoint Inhibition after Radical Treatment in the mucosal squamous cancers (seed funding, CRUK DBT Affordable Approaches to Cancer)	Dr G. Duncan, MRC CTU, UK; Dr Lalit Kumar, AIIMS, New Delhi	- Support regulatory and ethics submissions - Site feasibility, site preparedness - Collaborate on PPI objective of seed funding	- Contribute to designing site feasibility questionnaire - Support submission of PPI objective proposal to clinical site IECs - Collaborate on PPI work
16.	Sero-prevalence study for COVID 19 in Mumbai (BMGF)	Dr G Kang, THSTI & Dr U. Kolthur TIFR, Mumbai	Independent quality monitoring	Contribute to delivery of high-quality research

Consultancy Services

Project Title (Funding Agency)	Principal Investigator / Institute	CDSA Role
Clinical Trial Regulatory Advisory and Data Safety Consultancy Services	Dr. K. Singh, National Biopharma Mission, BIRAC	• 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

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BOOK CHAPTERS:

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INVITED TALKS

Name of the faculty member/ Scientist	Title of the talk	Title of the event	Date
Bhabatosh Das	Diet and the Gut Microbiome: Applications, Functions and Implications for Therapeutics	10 th India probiotic symposium on Cutting edge science and applications: Intestinal microbiota and Probiotics (The Surya, New Delhi)	29 th February - 1 st March 2020
	Insights into Antimicrobial Resistance in Human Associated Microbes (Keynote Speaker)	Seminar on "Current Challenges of antimicrobial Resistance and Potential Intervention by Bacteriophages" (Hyatt Regency, Kolkata. (ICMR, NICED))	16 th -17 th September 2019
	Role of vaginal microbiome in preterm birth	International India, EMBO Symposium on Human Microbiome (NIBMG, Kalyani)	9 th -12 th November 2019
	Next Generation Sequencing Technology and Application	National seminar on Next Generation DNA sequencing (Pondicherry University)	12 th -13 th December 2019
	Insights into antibiotic resistance in human associated gut microbiota	Faculty Seminar (NIPER, Kolkata)	7 th November 2019
	Understanding the role of human gut microbiome in the emergence of antibiotic resistant enteric bacterial pathogens	Symposium on the "Frontiers in Biological Sciences" (St. Xavier's College, Kolkata)	21 st - 22 nd September 2019

Name of the faculty member/ Scientist	Title of the talk	Title of the event	Date
Amit Kumar Pandey	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)	08 th -10 th March 2019
	Targeting "Persisters": A new paradigm for tuberculosis drug development	"Evolution and transmission of drug-resistance tuberculosis in Agartala, Kohima and Imphal population" (ICGEB), New Delhi	14 th March 2019
	Drug development in tuberculosis	AICTE sponsored Quality Improvement Programme (QIP) for attending faculty, academicians working in Pharmacy colleges throughout India ((DIPSAR), New Delhi)	13 th March 2019
	Cholesterol-induced persistence in mycobacteria	AICTE sponsored Quality Improvement Programme (QIP) for attending faculty, academicians working in Pharmacy colleges throughout India ((DIPSAR), New Delhi)	04 th April 2019
Krishnamohan Atmakuri	1. Culture negative sepsis 2. Host genetic factors behind sepsis 3. Delineating transmission dynamics behind sepsis	Brainstorming meeting to identify and prioritize research questions and methodology for projects under the proposed multi-site, multi-disciplinary neonatal sepsis study consortium (AIIMS)	30 th October - 1 st November 2019
	Pathogens tool kit – why, how and when – glimpses from TB- and sepsis-causing bacteria	Paradigm shift in Clinical Microbiology – UP – UK Microcon 2020 (SRMs, Bareilly, UP)	6 th -8 th February 2020
Nisheeth Agarwal	EMBO TB Conference	EMBO TB Conference (NII, Delhi)	11 th -14 th February 2020
	National Conference on Synthetic Biology	National Conference on Synthetic Biology (Indrashil University, Ahmedabad)	24 th -25 th January, 2020
	NCR Cluster Seminar Series, Theme: Genomic tools and applications in modern biology	NCR Cluster Seminar Series, Theme: Genomic tools and applications in modern biology (NIPGR, Delhi)	12 th April 2019

Name of the faculty member/ Scientist	Title of the talk	Title of the event	Date
Guruprasad Medigeshi		Discussion Forum on Platforms and Health Technologies for Infectious Diseases with Epidemic Potential	1 st August 2019
	Chikungunya Vaccine.	19 th International Vaccinology Course (IVI, South Korea)	2 nd -6 th September 2019
Jayanta Bhattacharya	Therapeutic advances in vaccines and immunotherapy	DHR-sponsored National Level Workshop on Clinical and Translational Research for Precision Medicine (Manipal College of Pharmaceutical Sciences & Kasturba Medical College, Manipal Academy of Higher Education (MAHE), Manipal, Karnataka)	5 th December 2019
	HIV Prevention Research Goals & Priorities	Scientific Workshop under the Cohorts for HIV Resistance and Progression in Indian Children and Adults (CoHPRICA) Program supported by DBT and ICMR	13 th November 2019
Milan Surjit	Understanding host-pathogen crosstalk during hepatitis E virus infection	International Symposium in Biomedicines and Biomaterials (Northwest Minzu University, Lanzhou, China)	August 2019
	Understanding host-pathogen crosstalk during hepatitis E virus infection	Invited seminar (Lanzhou University, Lanzhou, China)	August 2019
	Understanding host-pathogen crosstalk during hepatitis E virus infection	Invited seminar (South Asian University, New Delhi)	September 2019
Shubbir Ahmad	Sequence Diversity of HIV-1 Envelope Protein and Immunogen (Vaccine) Design	International conference on Genomics and Proteomics pertaining to biological science (Aligarh Muslim University (AMU), Aligarh, UP)	6 th -7 th November 2019
Ramandeep Singh	In pursuit of new metabolic drug targets against <i>Mycobacterium tuberculosis</i>	TUPMAS (National Institute of Technology, Rourkela)	February 2020
	Toxin antitoxin systems in mycobacterial pathogenesis	EMBO meeting	February 2020

Name of the faculty member/ Scientist	Title of the talk	Title of the event	Date
Sweety Samal	Representative of THSTI	Indian vaccine delegate to NL vaccine mission (Netherlands)	8 th -11 th July 2019
	Influenza research program at THSTI	Interactive meeting of Infectious Diseases team of King's College (SJRI)	10 th October 2019
	Development of mouse model to study latent virus reactivation in simulated microgravity environment	Life Sciences and Technology meet (ISRO HQ)	30 th November 2019
Tripti Srivastava	An alternative path to address viral vaccine development; the challenge and way forward	International Conference on genomics and Proteomics pertaining to Biological Science (Interdisciplinary Biotechnology Unit, Aligarh Muslim University, India)	5-7 th November 2019
	Structural Insights and Biomolecular Interactions	Biomolecular Interaction study technologies (Advance Technology Platform Center, Regional Center of Biotechnology)	8 th November
Pallavi Kshetrapal	The unexpected joys of traveling the tricky terrains in biomedical research	Hansraj College, New Delhi	16 th October 2019
	Biosample collection, long term storage and retrieval in cohort studies	Biobanking International Symposium 2020 (Institute of Liver & Biliary Sciences, New Delhi)	
Gaurav Batra	Diagnostics for Infectious Diseases	BIO INTERNATIONAL CONVENTION 2019 (Philadelphia, USA)	4 th June 2019
Niraj Kumar	Rapid pathogen identification and phenotypic antimicrobial susceptibility testing	National Conference on Nano/ Bio-Technology 2019 (NBT-2019), December 19-21, Jawaharlal Nehru University, New Delhi	19 th -21 st December 2019
	Rapid pathogen identification and phenotypic antimicrobial susceptibility testing	60 th Annual Conference of Association of Microbiologists of India (AMI-2019) (Central University of Haryana, Mahendergarh, India)	15 th -18 th November 2019
Tarun Kumar Sharma	TB Dx innovation	THSTI-ANDC Science Setu program (Acharya Narendra Dev College, Delhi University)	25 th February 2020

Name of the faculty member/ Scientist	Title of the talk	Title of the event	Date
Amit Kumar Yadav	Hitchhiker's Guide to Integrative Systems Biology	Aranyam- Annual Botanical Festival (Acharya Narendra Dev College, University of Delhi)	25 th February 2020
	Multiplexed quantitative proteomics data analysis and visualization - the next frontier is now	11th Annual Meeting of PSI & International Conference on Proteomics for System Integrated Bio-Omics, One Health and Food Safety (NDRI, Karnal)	3 rd December 2019
	Interrogating disease mechanisms and biomarkers using mass spectrometry-based proteomics	Medical Biotechnology Course (Miranda House College, University of Delhi, Delhi)	5 th September 2019
	Higher order multiplexing for quantitative proteomics	Recent Advances in Bioinformatics conference (CSIR-NEIST, Jorhat, Assam)	25 th May 2019
Renu Goel	Plasma proteomics approach to understand the temporal expression of proteins after living donor liver transplantation at different stages of liver regeneration	Proteomics Society of India	2 nd - 4 th December 2019
	SWATH mass spectrometry as a tool for quantitative profiling of the bone marrow plasma from alcoholic liver disease	3 rd international conference on mass spectrometry, proteomics and polymer chemistry	20 th -21 st May 2019
Ruchi Tandon	Urotensin II Receptor Antagonists - A Potential Therapeutic Approach for Chronic Heart Failure	International conference on Calcium Signalling: Regulatory mechanisms to impact on health and diseases (Regional Centre for Biotechnology, Delhi-NCR)	31 st January - 1 st February 2020
Samrat Chatterjee	Mathematical tools used to understand disease dynamics and drug target discovery	Faculty presentation series in Mathematics department (Amity University, Gurgaon)	8 th November 2019
	Studying Input-Output relation in signalling networks under random perturbation	International conference of Mathematical Sciences and applications (ICMSA 2020) (University of Kalyani, Kalyani)	26 th - 28 th February 2020

Name of the faculty member/ Scientist	Title of the talk	Title of the event	Date
Shailendra Asthana	Discovery of novel autophagy inducers by exploring the dynamic protein-protein interaction interfaces through computational approaches	ICAL2020, international conference of Autophagy and Lysosome, EMBO funded (IISc, Bangalore)	16 th - 18 th January 2020
	Basics of computational drug discovery	Drug discovery (GITAM University, Hyderabad)	2019
	Conformational ensemble enriches the accuracy of molecular docking	Drug Discovery training program (GZB, UP)	2019
	Peptidomimetics unveil the cryptic pockets at protein-protein interaction interfaces	International symposium on Integrative Approaches in Life Sciences (JNU, Delhi)	2019
	Molecular dynamics in drug discovery	National Workshop on Biomolecular Recognition and Dynamics (Banasthali Vidyapeeth, Rajasthan)	2019
	The role of advanced computational tools in drug discovery	AICTE sponsored XXIX QIP: Current Trends in Pharmacy (DIPSAR, New Delhi)	2019
	Computational strategy to discover anti-SARS-CoV-2 molecules	Online webinar on Covid-19 (Amity University)	2020
Madhu Dikshit	New Drug Discovery & Development in India	International Conference on Emerging techniques in drug discovery and drug delivery: current challenges and future prospects (Chandigarh Colleges of Pharmacy, Mohali)	10 th February 2020
	Reversal of Insulin resistance in chow-fed iNOS ^{-/-} mice by nitrite supplementation	Society of Free Radical Research -2020 (BARC, Mumbai)	12 th February 2020
	New Drug Discovery in India: Challenges in Industry - Academia Interaction	Innovators cum Entrepreneurs (NEIST Jorhat)	8 th - 10 th May 2019

List of Projects sanctioned during the Financial Year 2019-20

	Project title	Principal Investigator	Collaborators	Funding Agency	Sanctioned budget (in INR)	Project duration
1	Typhoid infection model in India	Dr. Gagandeep Kang	NA	Wellcome Trust	31.18 Crores	5 years
2	Screening of novel Active Pharmaceutical Ingredients (API) using a combinatorial library of small chemical compounds	Dr. Madhu Dikshit	BogaR Laboratories	-	-	5 years
3	Structure-based designing of novel Sirt-1 modulators	Dr. Shailendra Asthana	AIIMS	-	10 Lakhs	5 years
4	Inform development of broadly neutralizing antibody combination suitable for prevention and treatment against HIV in Indian patents with and without comorbidities	Dr. Jayanta Bhattacharya	NA	DBT/ Wellcome Trust India Alliance	3.84 Crore	5 years
5	Translational Research Consortium for Establishing Platform Technologies to Support Prophylactic and Therapeutic Strategies for Dengue – Discovery to Proof-of-Concept	Dr. Sweety Samal, Dr. Guruprasad R. Medigeshi	NA	BIRAC	7.97 Crore	4 years
6	Improvisation of CRISPR/Cas9 and CRISPRi-based approaches and their application in <i>Mycobacterium tuberculosis</i> gene manipulation to assist ongoing TB research and drug discovery programs in India	Dr. Nisheeth Agarwal	Gitam University, Hyderabad	DBT	54.5 Lakhs	3 years

	Project title	Principal Investigator	Collaborators	Funding Agency	Sanctioned budget (in INR)	Project duration
7	Developing HIV broadly neutralizing antibodies as a prevention product for global access through antibody half-life extension engineering	Dr. Jayanta Bhattacharya	University of Oslo, IAVI	Norway Research Council	6.06 Crore	3 years
8	Repositioning Fluoxetine and Salmeterol for treatment of dengue infection-preclinical development and proof-of-concept studies	Dr. Guruprasad R. Medigeshi	NII	DBT	97.52 Lakhs	3 years
9	Understanding the role of GntR family of transcription regulators in Mycobacterium tuberculosis physiology, stress adaptation and pathogenesis	Dr. Ramandeep Singh	NA	DBT	94.54 Lakhs	3 years
10	Identifying regulatory network critical for cholesterol/ utilization in <i>M. tuberculosis</i> and its implications on mycobacterial pathogenesis	Dr. Amit Kumar Pandey	Bennett University, IGIB, NCBS, RGCB and THSTI	NA	-	3 years
11	Understanding the transcriptional landscape of IL-10 producing anti-inflammatory T-cells in IBD	Dr. Amit Awasthi	NA	SERB	57.01 Lakhs	3 years
12	Understanding the metabolic perturbation in cancer using mathematical models	Dr. Samrat Chatterjee	NA	SERB	18.47 Lakhs	3 years

	Project title	Principal Investigator	Collaborators	Funding Agency	Sanctioned budget (in INR)	Project duration
13	Identification of new scaffolds targeting "persisters" in Mycobacteria	Dr. Shailendra Asthana, Dr. Amit K. Pandey	NA	SERB	39.52 Lakhs	3 years
14	Identification, characterization and optimization of monoclonal antibodies that broadly neutralize entry of HIV-1 subtype C predominantly circulating in India	Dr. Jayanta Bhattacharya	NA	SERB	55.67 Lakhs	3 years
15	National Surveillance system for enteric fever in India	Dr. Gagandeep Kang	NA	CMC Vellore	2.24 Crores	3 years
16	Understanding the mechanisms of novel scaffolds active against <i>Mycobacterium tuberculosis</i> : Development of lead molecule that will be effective against tuberculosis	Dr. Padam Singh (SERB-NPDF)	NA	SERB	19.20 Lakhs	2 years
17	To identify unique differential signatures for the diagnosis of stage-specific non-alcoholic fatty liver disease using the multi-omics approach: A pilot study	Dr. Madhu Dikshit	AIIMS	-	10 Lakhs	2 years
18	Development of aptamer-based assay for the diagnosis of breast tuberculosis (Breast TB)	Dr. Tarun Kumar Sharma	AIIMS	-	10 Lakhs	2 years
19	Identification of novel biomarkers for prediction of renal allograft rejection	Dr. Amit Awasthi	AIIMS	-	10 Lakhs	2 years

	Project title	Principal Investigator	Collaborators	Funding Agency	Sanctioned budget (in INR)	Project duration
20	Evaluation of inflammatory and immune modulation mechanisms in bronchoalveolar lavage of patients with Post Tubercular sequelae and chronic airflow obstruction	Dr. Amit Kumar Pandey	AIIMS	-	10 Lakhs	2 years
21	Metabolic profiling as per glycemic status [normoglycemia/ prediabetes/ diabetes] in women with history of gestational diabetes mellitus (GDM) - A cross-sectional study	Dr. Pallavi Kshetrapal	AIIMS	-	10 Lakhs	2 years
22	Screening and identification of small molecules that enhance the tumour suppressor function of PDCD4 by targeting the FAT1/ PDCD4 axis	Dr. Sanjay Banerjee	AIIMS	-	10 Lakhs	2 years
23	Development of novel method for rapid pathogen culture to facilitate their identification and antimicrobial susceptibility testing	Dr. Niraj Kumar	AIIMS	-	10 Lakhs	2 years

	Project title	Principal Investigator	Collaborators	Funding Agency	Sanctioned budget (in INR)	Project duration
24	Developing and implementing a Gall Bladder Cancer Registry to collect clinico-pathological data (demography, treatment patterns, therapeutic outcomes) and collect bile/tissue samples from registered patients for a biorepository for molecular profiling/microbial analysis focussing on identifying an association between GBC and microbial infection	Dr. Pallavi Kshetrapal	AIIMS	-	10 Lakhs	2 years
25	Investigation of the mechanism(s) underlying the antiviral activity of Zinc on Hepatitis E Virus replication and evaluation of its cooperativity with Zinc ionophores in inhibiting viral replication	Dr Milan Surjit	NA	ICMR	39.16 Lakhs	2 years
26	Novel Eco-friendly Nano-composite Coatings for Prevention of Surgical Site Infections	Dr. Susmita Chaudhuri	Mangalore University	DBT	16.18 Lakhs	2 years
27	Nanocurcumin-based combinatorial therapy for Tuberculosis	Dr. Ramandeep Singh	KIIT-Technology Business Incubator	DBT	23.12 Lakhs	2 years

	Project title	Principal Investigator	Collaborators	Funding Agency	Sanctioned budget (in INR)	Project duration
28	Structure determination and targeting of ubiquitously expressed membrane integrated form of chloride intracellular channels (CLICs) for discovery of small molecular anti-cancer therapeutics	Dr. Dinesh Mahajan	ICGEB, Bennett University, RGCB, NCBS	DBT	21.41 Lakhs	2 years
29	Development and evaluation of a rapid phenotypic antimicrobial susceptibility test for clinical use	Dr. Susmita Chaudhuri	IICT, Hyderabad	BIRAC	44.96 Lakhs	1.5 years
30	Building vaccine confidence among Accredited Social Health Activists to address vaccine hesitancy and accelerate the uptake of childhood immunization in Nuh district of Haryana	Dr. Gagandeep Kang	CORE Group Polio Project, India	Sabine Vaccine Institute	16.71 Lakhs	1 year 5 months
31	Deciphering Interferon regulatory factor 8 mediated gene regulation in protection against <i>M. tuberculosis</i>	Dr. Ramandeep Singh	PGI, AIIMS	NCR Cluster Collaborative Project	10 Lakhs	1 year
32	Novel Inhibitors of DENV RNA-dependent RNA polymerase	Dr. Sankar Bhattacharyya	NII, AIIMS, CMC Vellore, IIT Delhi, Manipal Academy of Higher Education, ICGEB	No funding agency	-	1 year
33	Role of RNA methyl transferase machinery in B-cell Acute Lymphoblastic Leukemia	Dr. Amit Awasthi	NII	-	-	1 year

	Project title	Principal Investigator	Collaborators	Funding Agency	Sanctioned budget (in INR)	Project duration
34	Understanding and targeting inorganic polyphosphate-regulated metabolic pathways in <i>Mycobacterium tuberculosis</i>	Dr. Ramandeep Singh	NA	ICMR	10.30 Lakhs	1 year
35	Smart safety surveillance supporting the collaboration of regulators, PVPI, and immunization programme in India activities	Dr. Gagandeep Kang	NA	WHO	14.29 Lakhs	10 months
36	Smart safety surveillance supporting the collaboration of vaccine vigilance stakeholders in India	Dr. Gagandeep Kang	NA	WHO	33 Lakhs	10 months

PATENTS

	Application No.	Filing Date	Title	Inventors
1	201911013062	1 st April 2019	Mutant HEV pORF2-based antigens and uses thereof	Dr. Milan Surjit, Jyoti Gupta
2	201911013070	2 nd April 2019	Zinc Chelators as inhibitor of Dengue Virus Replication and Uses thereof	Dr. Guruprasad Medigeshi
3	201911015320	16 th April 2019	Method of preparing nutraceutical and pharmaceutical products based on <i>Musa balbisiana</i> and uses thereof	Dr. Sima Kumari, Dr. Sanjay Banerjee, Dr. Rajlakshmi Devi, Dr. Dinesh Mahajan, Dr. Yashwant Kumar
4	201917016835	27 th April 2019	Method of converting carbon dioxide into carbonyl compounds	Dr. Dinesh Mahajan, Dr. Varun Kumar, Dr. Anil Rana
5	201911025444	26 th June 2019	A recombinant mycobacterium strain with constitutively activated toxin VapC12 and uses thereof.	Dr. Amit Kumar Pandey, Mr. Manitosh Pandey, Sakshi Talwar
6	201911027069	5 th July 2019	A H1N1 Influenza virus M2e ectodomain protein obtained from an arrangement of M2e ectodomain polypeptides and uses thereof	Dr. Sweety Samal, Dr. Rajesh Kumar, Dr. Tripti Srivastava, Naresh Kumar, Manish Kumar Bansal
7	201911027455	9 th July 2019	Aptamers against phospholipase A2 in snake venom and uses thereof	Dr. Tarun Kumar Sharma, Dr. Robin Doley, Anjali Anand
8	201911036350	10 th September 2019	Novel DNA aptamers against venom of <i>Bungarus caeruleus</i> (Common Indian Krait), method of its chemical synthesis and uses thereof	Dr. Tarun Kumar Sharma, Anjali Anand

9	201911036660	12 th September 2019	Engineered Hiv-1 Envelope Immunogen	Dr. Jayanta Bhattacharya, Dr. Rajesh Kumar, Dr. Vivek Kumar, Dr. Suprit Deshpande, Dr. Murugavel Kailapuri Gangatharan
10	201911046890	18 th November 2019	Integrative expression vector	Dr. Bhabatosh Das, Dr. Archana Pant, Ms. Jyoti Verma, Dr. Shailendra Vyas
11	201911053300	21 st December 2019	Design, synthesis of novel oxyindole inhibitors of DENV RNA-dependent RNA polymerase	Dr. Rambabu Gundla, Dr. Shailendra Asthana, Dr. Sankar Bhattacharyya, Dr. Venkat Narayana, Lovika Mittal
12	202011004692	3 rd February 2020	High-affinity aptamers against dichlorvos and a process for producing the same	Dr. Tarun Kumar Sharma, Dr. Bandhan Chatterjee
13	202011005513	7 th February 2020	1,2-disubstituted benzimidazolyl amino acids as selective sirtuin-1 lysine deacetylase inhibitors	Dr. Shailendra Asthana, Dr. Boja Poojary, Dr. Sanjay Banerjee, Dr. Nikhil Purushotham, Mr. Mrityunjay Singh, Bugga Paramesha, Mitul Srivastava
14	202011005512	7 th February 2020	Amino acid derived 5-pyrazolylmethylidene rhodanine carboxylic acids as selective sirtuin-1 lysine deacetylase inhibitors	Dr. Shailendra Asthana, Dr. Boja Poojary, Dr. Sanjay Banerjee, Dr. Nikil Purushotham, Mrityunjay Singh, Bugga Paramesha, Mitul Srivastava
15	202011007117	19 th February 2020	Novel autophagy-inducing peptides and combinations thereof, composition and a process for manufacturing the same	Dr. Shailendra Asthana, Dr. Charu Suri, Dr. Manjula Kalia, Dr. Amit Awasthi, Dr. Ajit Chande, Ambadas Rode, Surinder Prajapati, Srikanth Sadhu, Vipin Bhardwaj, Divya Ojha

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Professor Gagandeep Kang
FRS
Fellow

Elected: 2019

Biography

Gagandeep Kang is known for her inter-disciplinary research studying the transmission, development and prevention of enteric infections and their sequelae in children in India. To develop practical approaches to support public health, she has also built national rotavirus and typhoid surveillance networks, established laboratories to support vaccine trials and conducted phase 1-3 clinical trials of vaccines, a comprehensive approach that has supported two WHO pre-qualified vaccines, made by two Indian companies. She is investigating the complex relationships between infection, gut function and physical and cognitive development, and seeking to build a stronger human immunology research in India.

Based at the Christian Medical College, Vellore, she has established strong training programmes for students and young faculty in clinical translational medicine aiming to build a cadre of clinical researchers studying relevant problems in India.

[↑ Hide full biography](#)

Professional positions

Professor of Microbiology, Division of Gastrointestinal Sciences, Christian Medical College

[← Fellows Directory](#)

Prof. Gagandeep Kang was elected as the Fellow of Royal Society of London. With this, she became the first woman working in India to be elected to the Society.



Dr. Tarun Kumar Sharma was awarded the prestigious Indian National Science Academy (INSA) medal for young scientist 2019. He was awarded the medal in recognition of his "expertise in aptamer-based diagnostics and significant contributions in the field of TB diagnostics".

ACADEMICS

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. The thematic areas for research are under the broader areas of Infection & Immunology, Maternal & Child Health, Non-Communicable Disease, and Multidisciplinary Clinical & Translational Research. As on 31st March 2020, 57 students are enrolled for the THSTI doctoral programs.

Table 1 lists students who joined in 2019-20.

List of PhD Students joined in 2019-20

Name of the Student	Supervisor	Funding Agency
Mr. Oinam Ningthemmani Singh	Dr. Milan Surjit	CSIR
Ms. Pragya Tailor	Dr. Pallavi Kshetrapal	THSTI
Mr. Shivam Kumar	Dr. Samrat Chatterjee	THSTI
Ms. Manisha Priya	Dr. Ramandeep Singh	CSIR
Ms. Manisha Yadav	Dr. Susmita Chaudhuri	UGC
Ms. Rita Singh	Dr. Niraj Kumar	CSIR
Mr. Arun Sharma	Dr. Ramandeep Singh	THSTI
Ms. Arpita Singh	Dr. Pallavi Kshetrapal	UGC
Ms. Shashi Kumari	Dr. Bhabatosh Das	UGC
Ms. Sweta Pandey	Dr. Susmita Chaudhuri	DBT

Postdoctoral program

Postdoctoral programs at THSTI attract bright young researchers from across India. Table 2 lists the ones who joined us in 2019-20 and the program they enrolled in:

List of postdoctoral fellows who joined THSTI in 2019-20

Name of the fellow	Funding Agency
Dr. Surabhi Lavania	DBT
Dr. Adarsh Kumar Chiranjivi	BIRAC
Dr. Ankita Gupta	ICMR
Dr. Jigme Wangchuk	DBT/Wellcome Trust INDIA ALLIANCE
Dr. Hilal Ahmad Parray	BIRAC
Dr. Md. Jahangir Alam	ICMR
Dr. Naveen Yadav	BIRAC
Dr. Sumana Ghosh	DBT
Dr. Neeti Kalyani	SERB
Dr. Padam Singh	SERB
Dr. Charu Suri	DBT
Dr. Jyoti Gupta	ICMR
Dr. Pankaj Sharma	DBT
Dr. Kamalesh Verma	BIRAC

Short-term Training Program

Our previous experience with training young students from undergraduate and post-graduate courses from across the country has been an equally enriching experience for our faculty members and PhD students alike. Last year THSTI hosted and trained 43 students (13 in 2018-19) in the fields of immunobiology, prenatal biology, synthetic chemistry, mycobacterial pathogenesis and others.

Students' Achievements:



Bharti Kumari



Nidhi Kaushik



Praapti Jayaswal



Anith Chaudhary



Sakshi Talwar



Meenakshi Kar



Tarang Sharma



Suyasha Roy



Sakshi Agrawal

- Nine of our students were conferred with doctoral degrees in 2019-20 – Dr. Bharti Kumari, Dr. Tarang Sharma, Dr. Praapti Jayaswal, Dr. Anita Chaudhary, Dr. Nidhi Kaushik, Dr. Meenakshi Kar, Dr. Sakshi Talwar, Dr. Suyasha Roy, and Dr. Sakshi Agarwal.
- Three students were awarded for their oral and poster presentations at the "*International Conference of Cardiovascular Sciences - 2020*" held at Delhi Pharmaceutical Sciences and Research University (DPSRU), New Delhi from 20th to 23th February. Soheb Anwar and Ubaid Tariq received the Prof. Suresh C. Tyagi award for their oral presentations and Bugga Paramesha received the Prof. N.S. Dhalla best poster award. Earlier, Soheb also bagged the third best oral presentation award for the research work entitled "Allyl Methyl Sulfide Ameliorates Pressure Overload Induced-Cardiac Hypertrophy Via Modulation of Mitochondrial Dynamics" in *Cardiovascular Research Convergence- 2019* organized by the Department of Cardiology, All India Institute of Medical Sciences (AIIMS), New Delhi held on 4th August 2019.
- Saurabh Chugh won the best poster award in the category cellular microbiology at the *International EMBO India symposium - 2020* themed "Mycobacterial heterogeneity and host tissue tropism" organized by NII and ICGEB from 11th -15th Feb.
- Jyoti Verma won the Young Investigator Award (1st Prize) at the *10th India Probiotic Symposium*. The theme of the symposium was "Cutting Edge Science and Applications: Intestinal Microbiota and Probiotics".

- Suyasha Roy won the G.P. Talwar Young Scientist Award 2019 at the *46th Annual Meeting of Indian Immunology Society (IMMUNOCON 2019)* held at DAE Convention Centre, BARC, Anushaktinagar, Mumbai, Maharashtra from 14-16 November 2019. She was a recipient of the following travel awards in 2019-20:

- ◀ AAI Travel Award from the American Association of Immunologists (AAI) for giving an oral presentation on her PhD work at *17th International Congress of Immunology* held from 19-23 October 2019 at China Convention Center in Beijing, China.
- ◀ Travel Award from *Federation of Clinical Immunology Societies (FOCIS)* at FOCIS Advanced Course in Basic and Clinical

Immunology held from 26th-29th March, 2019 at Shiv Vilas Resort, Jaipur, Rajasthan, India. Suyasha also received the IIS Bursary Award from Indian Immunology Society (IIS) for Poster Presentation and was the only PhD student from India to receive both FOCIS Travel Award and IIS Bursary Award at the event.

- Naseem Ahmed Khan was awarded for the best poster presentation (Certificate and a cash prize of 250USD) for the research work entitled, "Anti-oxidative function of zinc protects from Respiratory Syncytial Virus infection" in *International Chemical Biology Society 8th Annual Conference* on "Navigating Translational Discoveries" held at CSIR-Indian Institute of Chemical Technology, Hyderabad, India on 2-4th November 2019.

EXTERNAL RELATIONS AND INSTITUTIONAL DEVELOPMENT OFFICE

The External Relations and Institutional Development (ERID) office supports researchers at THSTI with 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.



Vidhya Krishnamoorthy has moved to science administration after spending over a decade in research at academic research institutions. She has held the position of

Professional Expert for the past seven years at THSTI. This scientific administrative position at the External Relations and Institutional Development office of THSTI directly places her to support the research community over the lifecycle of a research project for grants-in-aid operations and ethical compliance.

- THSTI has renewed the registration of the Institutional Ethics Committee, Human Research with the Central Drugs Standard Control Organization (CDSCO) for the purpose of conducting clinical trials. The committee is registered as ECR/167/Inst/HR/2013/RR-19 issued under Rule 122D of the Drugs & Cosmetics Rules 1945.
- THSTI has registered the Institutional Ethics Committee for Biomedical and Health Research with the Department of Health Research (DHR). The registration number is EC/NEW/INST/2019/275.



Dr. Siuli Mitra made a transition to science communications after a doctorate in Anthropology and a short Post Doc in conservation genetics. Currently, she handles

science communications and external relations for THSTI. She coordinates the institute's *Science Setu* initiative and begun an initiative, *Manan* to ensure mental health support for young researchers at THSTI. Here's a list of science communication initiatives over the past one year:

- Coordinated the organization of the play *Monsters in the Dark* by Bangalore Little Theatre where THSTI partnered with the India Cancer Society, and NII, Delhi.
- Assisted content development for infographics and comics on THSTI's thematic areas, facilities and individual projects. Both the assignments were in collaboration with teams of designers, illustrators and comix artists from the Lab Hopping, Data Leads, CSIR-IGIB (Dr. Lipsa Panda), and Tejeswini Padma.
- Student outreach – Coordinated the *Shadow-A-Scientist* program at THSTI wherein 17 students were trained at THSTI by faculty members and scientists in various laboratories and facilities.
- Represented THSTI at the Haryana Gyanotsav 2020 at Sonapat.
- Translated the comics titled *Paul has Measles* and *Paul Stays Home* to Hindi. Both the comics are written by Dr. Susana Lopez, a virologist at the Biotechnology Institute (UNAM) in Cuernavaca, Mexico. The comics are available for free access at <https://www.virology.ws/2018/11/20/paul-has-measles/> and <https://www.virology.ws/2020/05/14/paul-stays-home/>.

ADMINISTRATION



The THSTI Administration comprises several functional sections, namely General Administration, Human Resources (HR), Finance & Accounts, Stores & Purchase, Information Technology (IT), Engineering & Estate Management, Intellectual Property Management, and legal. We elaborate on some of the essential activities performed by various sections under the THSTI Administration below.

General Administration

General Administration section broadly deals with meetings, the constitution of internal committees, and wherever required, follow-up with the decisions of these committees, grievances handling, official language implementation functions, logistics arrangement for the staff, security, and house-keeping services and any other functions as required by the Executive Director.

THSTI Governance: THSTI conducted one Society, two Finance Committee, and two Governing Body meetings during the FY 2019-20. Recommendations made by individual committees were documented, circulated among concerned individuals/departments for implementation.

THSTI Internal Committees: Various internal committees are constituted to advise and support the Executive Director in decision-making.

RTI: THSTI has been implementing the RTI Act in letter and spirit. To ensure transparency in its functioning, THSTI has been regularly making suo moto disclosures on its website, so that the number of RTI applications received are minimized. During the period from 1st April 2019 to 31st March 2020, a total of 55 RTI applications and 4 appeals were received by the institute. All applications and appeals have been disposed of as per the provisions of the RTI Act, 2005.

Public Grievances: An essential pre-requisite to make the public service delivery system more citizen-centric is to have a robust public grievance redressal and monitoring mechanism. All of the public grievances received through various portals instituted by the government were disposed of within the stipulated period.

Implementation of the official language policy:

The Institute made efforts to promote the use of Hindi in official work with a view to ensuring proper implementation of the Official Language Policy of the Government. Hindi *Pakhwada* was organized from 9th to 14th September 2019 in the institute. Various Hindi competitions were organized, and successful participants were given cash awards.

Human Resources

Human Resource Section of the Institute deals with all employees' matters, some of which are recruitment, deputation, promotion, training, probation, transfer, travel, employee benefits, discipline, employee welfare, and exit.

Recruitment: THSTI posted 50 recruitment notifications for filling 209 positions. The rolling recruitment for faculty positions was uploaded on the institute's website and published in leading newspapers and journals. The rolling advertisement in case of JRF/SRF/RA positions continued successfully every month to cater to the requirements, which would arise frequently.

Promotion: Under the Modified Flexible Complementing Scheme (MFCS), Dr. Bhabatosh Das and Dr. Samrat Chatterjee were promoted

from the position of Assistant Professor to Associate Professor; Dr. Guruprasad R. Medigeshi, Associate Professor was promoted to the position of Professor.

Foreign travel: The Institute provided an opportunity for the young students and scientists to attend the International scientific events and network with their counterparts in various places across the world. A total of 87 international visits were done by students and scientists during the year 2019-20

Employee benefits: THSTI continues to strive for providing its personnel with benefits like LTC, Medical reimbursement, telephone and newspaper reimbursement, and children's education allowance by following the Government of India directions. All these cases of reimbursement and other allowances were processed as per rules and in a time-bound manner.

Employee welfare: To motivate the employees and students, various recreational facilities are available within the campus. During winter, various sporting competitions were held.

To recognize exceptional research work and contribution to the overall development of the institute, THSTI has instituted the following awards by creating an endowment deposit supported through the extramural non-governmental funds. These awards will be distributed every year during the Foundation Day celebrations:

	Name of the award	Cash award (in Rs)
1	Dr. M.K. Bhan Group Award for the most impactful collaboration	Rs. 50000/-
2	Award for a faculty for the best-published paper	Rs. 15000/-
3	Award for a Ph.D. student for the best-published paper	Rs. 15000/-
4	Award for a Ph.D. student for the 5 years all-round performance	Rs. 15000/-
5	Award for a faculty for overall contribution to the institutional development during the previous financial year	Rs.10000/-
6	Award for an administrative staff for overall contribution to the institutional development during the previous financial year	Rs.10000/-
7	Award for technical staff for overall contribution to the institutional development during the previous financial year	Rs.10000/-

Human resource development: As part of human resource development (HRD) activities, the institute plans and implements programs for providing opportunities to technical and administrative employees to update and upgrade their knowledge and skills so that they may perform their duties effectively. The programs are also aimed at enhancing the pride and satisfaction they feel in their work.

Finance and Accounts

The Finance and Accounts section of the institute attends to the day-to-day financial matters, payments to contractors/ suppliers, payment of salaries to staff, etc. The section is also responsible for preparing the annual statement of accounts, which is provided at the end of this chapter.

The financial highlights of the institute (as on 31.03.2020) are mentioned here:

S. No	Head	Op. Balance	Re-appropriation	Receipt	Expenditure	Balance
1	GIA Manpower	160.80	0.00	750.00	783.10	127.70
2	GIA General	-7.64	0.00	1950.00	1942.36	0.00
3	GIA Capital	-55.65	0.00	2750.00	2689.85	4.50
	Total	97.51	0.00	5450.00	5415.31	132.20

The section has adopted various digital methods for payment disbursements/collections in order to avoid the cash transactions. During the FY 2019-20, the institute has generated internal revenue to the tune of Rs 183.86 lakhs and also received the non-governmental funds from abroad of Rs 1295.16 lakhs in addition to the Govt. of India funding, as shown above.

Stores and Purchase

The day-to-day purchase work is managed by

the Standing Purchase Committee, a team of senior officials assisted by Section Officer who supervises the stores & purchase activities. The section deals with all the purchase-related works as per guidelines / SOP framed for this purpose. The procurement processes are within the ambit of comprehensive Rules and Regulations available in the General Financial Rules (GFR), 2017; Government orders guidelines issued time to time by the Ministry of Finance and Central Vigilance Commission to increase transparency and objectivity in public procurement 2020.

Some important figures of this section during the financial year 2019-20 is provided below:

Procurement for Financial Year 2019-2020				
Particular	Total Orders		Total Order Value (INR)	
	Non-GeM	GeM	Non-GeM	GeM
Consumable	2410	134	149,803,729	2,095,460
Equipment	183	93	69,075,359	9,684,737
Total	2593	227	218,879,088	11,780,197

Information Technology

Some of the major achievements of the IT team during the financial year 2019-20 are listed below:

- The ERP's purchase module was made live, and the Finance module started execution in parallel mode.
- Deployment of the THSTI cloud server for providing storage/backup facility.
- Complete digitalization of the Recruitment module starting from the candidate registration till the result.
- Deployment of new 125 Mbps fibre-based internet leased line circuit.
- LAN cabling and arranging other infrastructure for Bioassay lab and Aryabhata Data science and AI Program center.
- Provided support to conduct around 300 online meetings, 26 seminars, and 8 events.
- In-house deployment of websites/web services like CDSA Toolkit, eTHSTI, CDSA Training, and migration of THSTI's website to NIC Cloud.

Engineering and Estate Management

The FY 2019-20 saw a substantial transformation of infrastructure with new labs and office spaces coming up, and the department was instrumental in the 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 the implementation of the ecological work plan, fencing of 85 acres of cluster land, and functionalization of faculty housing, among other assignments. Besides the developmental and construction activities, the estate management division of the institute has been leading the ecological works in the bio-cluster to ensure sustainable development with minimum impact on the environment. The creation of ponds in the catchment areas is yet another initiative of the division to retain rainwater to enhance the water table in the cluster land. The division has ensured 100% recycling and utilization of used water and has taken initiatives to produce compost from the waste generated from the cluster.

The department successfully executed an energy audit and took action to save electricity worth INR 20 lakh per annum. Currently underway are a 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 is being planned to reduce power consumption in the cluster and improve power supply, respectively.

Intellectual Property Protection, Legal, and Collaborations

During the year 2019-20, THSTI has made significant advances in terms of patents, technologies developed and commercialized and national/international collaborations. Our sphere of influence is visible from the productive

collaborations we have had with scientific organizations, academic institutions, and industries. In the last year, THSTI filed a total of 15 patent applications and developed 6 technologies. The institute was a primary part of the 22 research collaborations/MoUs which were executed with different agencies during the FY 2019-20.

TRANSLATIONAL HEALTH SCIENCE AND TECHNOLOGY INSTITUTE, FARIDABAD

BALANCE SHEET AS AT 31ST MARCH, 2020

Amount (In Rs.)

CORPUS/CAPITAL FUND AND LIABILITIES	Schedule	31.03.2020	31.03.2019
Corpus / Capital Fund	1	1,92,33,67,334	1,70,58,71,477
Reserves and Surplus	2	10,07,82,346	9,98,36,749
Earmarked/Endowment Funds	3	-	-
Secured Loans and Borrowings	4	-	-
Unsecured Loans and Borrowings	5	-	-
Deferred Credit Liabilities	6	-	-
Current Liabilities and Provisions	7	59,34,99,641	44,34,26,823
TOTAL		2,61,76,49,321	2,24,91,35,049
ASSETS			
Fixed Assets	8	1,72,17,91,391	1,76,43,18,802
Investment From Earmarked/Endowment Funds	9	-	-
Investment-Others	10	2,700	2,700
Current Assets, Loans, Advances etc.	11	89,58,55,230	48,48,13,547
Miscellaneous Expenditure (to the extent not written off or adjusted)		-	-
TOTAL		2,61,76,49,321	2,24,91,35,049
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


(MANOJ KUMAR)
SECTION OFFICER (F & A)


(M.V.SANTO)
HEAD ADMINISTRATION


(Dr. GAGANDEEP KANG)
EXECUTIVE DIRECTOR


(SANJIV RAI MEHRA)
PARTNER
M. No. 80402



Place: Faridabad
Date: 21/07/2020



TRANSLATIONAL HEALTH SCIENCE AND TECHNOLOGY INSTITUTE

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31ST MARCH, 2020

Amount (in Rs.)

INCOME	Schedule	31.03.2020	31.03.2019
Income from Sales/ Services	12	1,13,77,978	1,15,71,668
Grants/Subsides	13	27,00,00,000	20,69,80,000
Fees/Subscriptions	14	3,88,775	1,40,000
Income from Investments (Income on Invest.from earmarked/endow.Funds transferred to Funds)	15	-	-
Income from Royalty, Publication etc.	16	-	-
Interest Earned	17	3,25,15,138	2,76,31,588
Other Income	18	57,49,078	30,95,842
Increase/decrease in stock of Finished goods and works- in- progress	19	-	-
Deferred Income-Fixed Assets		13,30,22,481	9,41,24,776
TOTAL (A)		45,30,53,450	34,35,43,874
EXPENDITURE			
Establishment Expenses	20	7,39,79,530	8,12,60,554
Other Administrative Expenses etc.	21	19,26,59,810	16,26,90,407
Expenditure on Grants , Subsidies etc.	22	-	-
Interest	23	3,25,15,138	77,59,879
Depreciation (Net Total at the year-end-corresponding to Schedule-8)		13,30,22,481	9,41,24,776
Prior period Adjustment A/c (ANN-A)		-	-
TOTAL(B)		43,21,76,959	34,58,35,617
Balance being excess of Income Over Expenditure (A-B)		2,08,76,491	(22,91,743)
Transfer to special Reserve(Specify each)		-	-
Transfer to /from General Reserve		2,08,76,491	(22,91,743)
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

(MANOJ KUMAR)
SECTION OFFICER (F & A)(M.V.SANTO)
HEAD ADMINISTRATION(Dr.GAGANDEEP KANG)
EXECUTIVE DIRECTORAs per our separate Report
of even date attached
For Mehra & Sistani
Chartered Accountants(SANJIV RAI MEHRA)
PARTNERPlace: Faridabad
Date: 21/07/2020

TRANSLATIONAL HEALTH SCIENCE & TECHNOLOGY INSTITUTE (THSTI)
Faridabad

CONSOLIDATED RECEIPTS AND PAYMENTS ACCOUNT FOR THSTI, PROJECTS & FELLOWSHIP FOR THE YEAR ENDED 31ST MARCH, 2020

AMOUNT-IN-RUPEES

RECEIPTS	31.03.2020		31.03.2019	
OPENING 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	
Grant-in Aid Received:-				
Fellowship	2,34,24,252		2,75,54,336	
Projects	53,17,08,569		41,63,45,445	
THSTI	54,50,00,000		41,94,32,000	
Other Receipts -THSTI				
Application Fees	51,775		25,750	
Admission Fee	-		31,500	
Guest House Receipt	6,85,437		2,54,708	
HRA Recovery	24,48,652		20,99,836	
Income from Sales and Services	1,53,41,391		1,15,54,168	
Interest Received from Banks	1,61,15,378		1,98,71,709	
Miscellaneous Receipts	4,61,254		13,673	
Penalty Receipt	1,11,712		1,71,675	
Receipt from STTP	3,37,000		1,40,000	
Recruitment Fee	12,34,507		1,95,700	
RTI Receipt	478		2,000	
Sales of Scrap	51,486		17,500	
Tender Fee	66,700		1,93,000	
Vendor Registration Fee	1,10,000		1,08,000	
Accrued Interest Received	9,77,921		5,89,620	
Advance Receipt From Debtors	4,248		-	
Building Contribution From Constituents	7,56,00,000		-	
Decrease in advances	85,75,331		78,03,843	
Earnest Money Deposit	41,00,379		47,11,938	
Govt. Dues Payable	5,65,759		9,39,737	
Other Liabilities/Payable	2,66,12,050		2,28,68,438	
Security / Hostel Deposit Received	5,72,240		12,88,235	
TOTAL		1,70,76,23,420		1,45,67,27,377

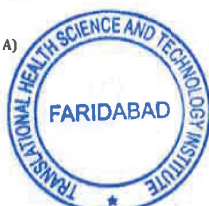
AMOUNT-IN-RUPEES

PAYMENTS	31.03.2020		31.03.2019	
Particulars				
Fellowship Paid	2,29,49,928		2,67,61,340	
Projects Expenditure	43,16,42,494		42,61,04,264	
THSTI Expenditure:-				
Fixed Assets	6,61,66,252		6,37,83,962	
Work -in- Process- Building	28,18,850		17,44,75,059	
Consumables	4,46,92,973		4,88,47,763	
Manpower	7,83,09,701		7,08,90,662	
Administrative Expenses	14,95,43,299		11,25,09,578	
Advances , Receivables & Liabilities	31,19,19,364		7,98,88,848	
Closing Cash & Bank Balance				
Fellowship	(65,95,190)		(70,69,514)	
Projects	49,79,32,562		39,78,66,486	
THSTI	10,82,43,187		6,26,69,930	
TOTAL		1,70,76,23,420		1,45,67,27,377

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

(MANOJ KUMAR)
SECTION OFFICER (F & A)

PLACE: Faridabad
DATE: 21/07/2020



(M.V SANTO)
HEAD ADMINISTRATION

(DR. GAGANDEEP KANG)
EXECUTIVE DIRECTOR

(SANJIV RAI MEHRA)
PARTNER



SCIENTIFIC EVENTS AND OUTREACH

Important Visitors:

Coalition of Epidemic Preparedness Innovations (CEPI) team visits THSTI



L-R: Gunnstein Norheim, Dawn O Connell, Gagan-deep Kang, Richard Wilder, Melanie Saville, Amrita Sekhar, Arun Kumar, Debra Yeskey

A team from the **Coalition of Epidemic Preparedness Innovations (CEPI)**, a global organization aimed at strengthening global epidemic preparedness visited THSTI on the 2nd of August 2019. The purpose of their visit was to get an overview of the infrastructure and bioassay and diagnostic facilities available at THSTI and to interact with the scientific community engaged in vaccine and assay development to understand the research capabilities at THSTI. The team also interacted with CDSA to get an overview of the training and research support services offered.

THSTI hosts Dr. Trevor Mundel, President for Global Health, Bill and Melinda Gates Foundation



"No other country among the world's emerging economies has the same combination of world-class biomedical research, effective translational research expertise, capacity to design and implement highly powered clinical trials, and capacity to produce health products that meet the stringent regulatory standards of the World Health Organization (WHO)", said Dr. Trevor Mundel, President of Global Health, Bill and Melinda Gates Foundation a day before his visit to THSTI during a lecture on the role India can play in the global effort for reaching the Sustainable Development Goals. His visit to THSTI was significant given the Foundation's long-standing support for different projects at THSTI, primarily research focused on vaccine development and maternal & child health. After a quick roundabout around THSTI and RCB facilities, the Executive Director, Dean (Clinical Research), faculty and scientists presented an overview of work at THSTI and specific thematic areas.

Special NCR Cluster event organized by THSTI - Prof. VijayRaghavan discusses career opportunities for postgraduates in biological sciences

Prof. K. VijayRaghavan, Principal Scientific Adviser to the Government of India, spoke to the students from across NCR Biotech Science Cluster institutes and college students of NCR about career opportunities for postgraduates in biological sciences in his talk "Chance and Necessity in making a bright future" at THSTI, Faridabad on 20th December 2019. He went on to discuss the global situation of postgraduate careers (in biological sciences). He elaborated on what institutions can do for us and what we can do to further our career opportunities.



2nd edition of THSTI's Immunology course from 1st to 3rd April 2019



THSTI organized the second edition of the course on Immunology titled "**Overview of Cellular**

and Molecular Immunology" from 1st to 3rd April 2019. This course is designed to cover basic and recent advances in the field of Immunology. Distinguished immunology researcher and teacher, Dr. Shiv Pillai who is currently a Professor at Harvard Medical School, Boston, USA delivered most of the lectures. Dr. Vineet Ahuja who is Professor at the Dept of Gastroenterology of AIIMS, Delhi along with Dr. Amit Awasthi, Associate Professor at THSTI also delivered lectures. Dr. Awasthi was also the faculty-in-charge for the course. This year saw more than 250 participants comprising faculty members, research fellows and PhD students from institutes in NCR and across the country. One of the highlights of the course this year was that researchers at Banaras Hindu University, AIIMS Delhi, PGIMER Chandigarh, SRM Sonipat, Tezpur University and others could attend the course by joining a live streaming session.

Third Annual Meeting of South-East Asia Regulatory Network (SEARN) on 24th and 25th April 2019



L-R: Madhur Gupta, WHO India Country Office, Manisha Sridhar, WHO SEARO, Eswara Reddy, DCGI, Raj Long, BMGF and Gagandeep Kang, THSTI

The WHO South-East Asia Regional Office (SEARO) had the 3rd Annual Meeting of South-East Asia Regulatory Network (SEARN) on 24th and 25th April 2019 in Delhi. Regulators from across the

region met to develop approaches to working together to increase reliance and referral to promote rapid access to high quality, efficacious and safe products for patients.

Network National Regulatory Authorities is responsible for regulation of medical products, including medicines, vaccines, biological and medical devices and diagnostics for human use in the eleven countries of the South East Asia Region. Here's the link to the press release of the meeting.

Fifth collaborators meeting of the Rotavirus Vaccine Impact Assessment and Intussusception Surveillance Study at THSTI



The fifth collaborators meeting of the Rotavirus Vaccine Impact Assessment and Intussusception Surveillance Study was held at THSTI on 17th December 2019. The meeting is the fifth one in the series of meetings that are held to discuss key issues, challenges, and progress made in the introduction of the rotavirus vaccine in India. This one was attended by Vaccine Impact investigators from 30 hospitals across the country. The study is led by Prof. Gagandeep Kang, Executive Director, THSTI.

THSTI and AIIMS, Delhi co-organize One-Day symposium on NAFLD Therapeutics and Diagnostics



THSTI and AIIMS, New Delhi co-organized a one-day symposium titled "Current Trends in Therapeutics and Diagnostics of Non-alcoholic Fatty Liver Disease" on 10th December 2019 at THSTI. The prime objective of the symposium was to deliberate on the current scenario of drug discovery and development as well as the status of biomarker discovery for Non-alcoholic fatty liver (NAFL) disease & non-alcoholic steatohepatitis (NASH). Deliberations by the experts from academia, industry and medical institutions helped in improving the molecular understanding of disease progression and strategize for effective therapy. The panel discussion addressed the current status and pitfalls of the current drugs under clinical trial. Future strategies for therapy and biomarkers identification were also extensively deliberated. The symposium was well received by academicians, clinicians and industry personals. One hundred twenty clinicians and researchers from 16 organizations from across the country attended the symposium. Researchers from Dabur, Jiva Ayurveda, BogaR laboratories and Zydus also participated in the symposium.

THSTI at DBT and BIRAC's Global Bio India 2019



THSTI was represented by a team of scientists, students and fellows at the first edition of Global Bio India 2019, the largest Bio event hosted in India to date to showcase opportunities in the Indian Biotechnology sector to the world. The event was important for THSTI as well, as the GARBH-Ini website and the Clinical Trial Toolkit developed by CDSA were both launched by Dr. Renu Swarup, Secretary, DBT and other distinguished experts from the respective areas. Besides, being a part of sessions on Global Examples of Big Data in Health, Next 10 years for Vaccinology and the Director's Forum: Academic Assets Showcase, a team of students and scientists crewed the institute's stall showcasing THSTI's work on maternal & child health and diagnostics.

Team of scientists and students represent THSTI at IISF 2019 Mega Expo



A team of scientists, students, and fellows comprised the THSTI contingent at the Mega Expo of the fifth India International Science Festival from 5th to 8th November 2019 at Kolkata's Science City. In addition to posters showcasing THSTI's research programs, students hosted science games for school children who formed the major chunk of the visitors to the expo.

THSTI Open Day to mark the India International Science Festival (IISF) 2019



THSTI celebrated its second Open Day for 2019, this time as a pre-event of the India International Science Festival (IISF) 2019 to be held at Kolkata from 5th to 8th November 2019. One hundred fifty students and teachers from Government Senior Secondary School, Bhankri village and Manav Rachna International School in Faridabad and DAV, Sector 14 in Gurgaon attended. Prof. Shinjini Bhatnagar, Dean (Clinical Research) welcomed the students to THSTI, briefing them on our research, THSTI's Science Setu (outreach) program and IISF 2019. The students visited the Biorepository and Small Animal Facility, Central Instrumentation Facility for a demonstration by first-year PhD students. The visits were followed by a chalk-talk session by Dr. Tarun Kumar Sharma (in Hindi) and Ms. Hina Lateef Nizami, a PhD student (in English). A science poetry session and painting competitions were organized for our young visitors.

Sampoorti-Poorti System on display at the Ayushman Bharat Conclave



Honorable Union Minister of Health, Dr. Harsh Vardhan being briefed about the Sampoorti-Poorti System by Dr. Pawan Mehrotra of THSTI at Ayushman Bharat Conclave.

The Sampoorti-Poorti System was on display at the Ayushman Bharat Conclave at Vigyan Bhawan, New Delhi. The Sampoorti-Poorti System for post-surgical prosthetic rehabilitation of breast cancer patients who undergo mastectomy was developed at THSTI with support from BIRAC-DBT, Tata Trusts, numerous hospitals, and NGOs spread across India. This non-invasive explant comprises silicone gel-based prosthesis and accessories, available in different sizes and shapes, as per patient's needs.

THSTI completes a decade - Celebrations marked by Prof. CNR Rao's talk, Presidential Address by DBT Secretary and outreach event



ED addressing THSTI and students from schools and colleges on the 10th Foundation Day.

THSTI completed its 10 years on 15th July 2019 with day-long celebrations that included Foundation Day address by Bharat Ratna Prof. C. N. R. Rao, Presidential address by DBT Secretary Dr. Renu Swarup and a report and address by Prof. Gagandeep Kang, the current Executive Director. As the fraternity celebrated, they were also joined by senior officials from DBT, faculty from RCB and other partner institutes. On exhibition was THSTI's work spanning the first ten years. The celebrations also included a colloquium for senior PhD students who eloquently presented their work and a poster competition for senior PhD students and fellows. An outreach program was organized which was attended by 200 students and faculty members from two schools (Govt. Senior Secondary School, Bhankri village, Manav Rachna International School, Charmwood Village) and six colleges (Acharya Narendra Dev College, Shaheed Rajguru College of Applied Sciences for Women, Kirorimal College, Maitreyi College, Ram Lal Anand College, Hansraj college) from across NCR who are part of the THSTI *Science Setu* initiative. The outreach event included visits to the facilities and labs in THSTI, poster competition for colleges and essay competition for schools. The day ended with a cultural program put together by staff and students from THSTI.

THSTI organizes July '19 edition of the NCR Cluster Seminar Series

THSTI organized the July 2019 edition of the NCR Cluster Seminar Series themed '**Importance of Modern Biology in Clinical Research**' on 5th July 2019 at the NCR BSC Auditorium. A series of lectures were organized with a line-up of distinguished speakers from RCB, NBRC and



Top (L-R): Dr. Pallavi Kshetrapal, Prof. Shinjini Bhatnagar, Dr. Pravat K. Mandal (NBRC). Bottom (L-R): Dr. Tushar Maiti (RCB), Dr. Ramachandran T., Dr. Deepika Shukla (NBRC)

AIIMS apart from the host institute. Prof. Shinjini Bhatnagar (Dean, Clinical Research, THSTI) introduced the concept of clinical research to the audience largely comprising students and young fellows from the cluster institutes. This was followed by two sessions each with three lectures.

Course on Vaccinology for Clinical and Public Health Practice



THSTI hosted a short course on Vaccinology for Clinical and Public Health Practice from 18th-21st November 2019 in collaboration with (JIPMER, LSHTM, NUS, HITAP). The 4-day event included a policy symposium on 18 November followed by a 3-day vaccinology workshop. The course participants could explore topics such as

resource mobilisation for national immunisation programmes and use of evidence to inform vaccine policy development at the policy symposium with the regional country representatives. Also included were lectures, hands-on exercises as well as discussions led by leading faculty in the field from LSHTM, NUS Saw Swee Hock School of Public Health and HITAP.

Workshop on Innovative Solutions for Maternal and Child Health Using Medical Image Analysis and Artificial Intelligence on International Women's Day



This year's International Women's Day celebrations at THSTI on 5-6 March was marked with a workshop on 'Innovative Solutions for Maternal and Child Health Using Medical Image Analysis and Artificial Intelligence' was conducted in collaboration with the University of Oxford. This workshop brought together 70 participants and faculty that included clinicians, physician-scientists, biologists, computer vision scientists and young researchers together on a common platform to initiate thought-provoking discussions and identify some important research questions that can form the basis of innovative solutions. The primary objectives were to identify research questions emerging from clinical and public health need for low- middle income countries which can be addressed by image processing and AI, provide deeper understanding of image

processing tools that would facilitate research and do a hands-on-training /attempt to address one of the research questions identified. Two workshops were conducted by DMC and ADAPT on **Clinical research methodology** in Sharda University, Greater Noida.

THSTI attends Haryana Gyanotsav - An outreach event for school children



THSTI attended the Haryana Gyanotsav organized by Bhagat Phool Singh Mahila Vishwavidyalay. Thousands of students from schools and colleges in and around Sonapat and other parts of Haryana visited in the event that was held for over two days (14th and 15th February 2020).

Students of ITS Ghaziabad visit THSTI



Thirty students of B.Sc. Bioinformatics III year of the Ghaziabad-based ITS Institute of Health and Allied Sciences visited THSTI's laboratories and facilities on 17th February 2020.

Other events

National Unity Day (राष्ट्रीय एकता दिवस) observed at THSTI



National Unity Day (राष्ट्रीय एकता दिवस) was observed at THSTI's premises on 31st October 2019. This day was observed to reinforce our commitment as a fraternity, to strengthen the security, unity, and integrity of the country. To mark the occasion, Dr. T. Ramamurthy, National Chair, THSTI, and Dr. Guruprasad Medigeshi, Associate Professor, THSTI, administered the National Unity pledge (राष्ट्रीय एकता दिवस) in English and Hindi respectively in the presence of the entire fraternity.

हिन्दी सप्ताह 2019 का आयोजन



राष्ट्रीय हिन्दी सप्ताह को मनाते हुए संस्थान में 11 एवं 12 सितंबर को विभिन्न कार्यक्रम आयोजित किए गए। वैज्ञानिकों, अधिकारियों, कर्मचारियों एवं विद्यार्थियों ने उत्साह सहित भाग लिया। कविता पाठन, निबंध लेखन आदि प्रतियोगिताओं का आयोजन किया गया। हिन्दी सप्ताह के समापन समारोह में मुख्य अतिथि डॉक्टर कुमुद शर्मा एवं कार्यकारी निदेशक डॉक्टर गगनदीप कंग ने सफल प्रतियोगियों को पुरस्कृत किया।

हिन्दी सप्ताह के समान समारोह में मुख्य अतिथि डॉ. कुमुद शर्मा एवं कार्यकारी निदेशक डॉ. गगनदीप कंग ने सफल प्रतियोगियों को पुरस्कृत किया।

THSTI observes Sadbhavna Divas (सद्भावना दिवस)



THSTI fraternity celebrated Sadbhavna Divas (सद्भावना दिवस) in its premises on 20th August 2019. The day marks promotion of national integration and communal harmony among people irrespective of religion, languages and regions. The idea behind observance of Sadbhavna Divas is to eschew violence and to promote goodwill among people. THSTI National Chairs Dr. T. Ramamurthy and Dr. Madhu Dikshit

administered the pledge in English and Hindi respectively.

Fifth International Yoga Day



THSTI and RCB celebrated the 5th International Yoga Day at the NCR Biotech Science Cluster. Hour-long yoga sessions were held for and attended by employees working in the cluster for one week culminating in a Yoga event on the 21st of June. Ms. Niti Singh who is a PhD student at THSTI was the instructor during these sessions. The event received enthusiastic participation of people from the two institutes who practiced various *asanas* and meditation during the hour-long session.

HOW MEDIA COVERED THSTI IN 2019-20



THSTI COMMITTEES

Serial No.	Committee	Members
1	Scientific Advisory Committee	Dr. Partha Majumder Dr. Jaya S. Tyagi Dr. B. Ravindran Dr. Raghavan Varadarajan Dr. Rajesh Gokhale Dr. Ashok Venkataraman Prof. Judi Allen Dr. B. V. Ravi Kumar Chairperson - Dr. Partha Majumder
2	THSTI Management Committee	Executive Director and Heads of all the centers Chairperson - Executive Director
3	Finance Committee	Financial Advisor, DBT (Chairperson) 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, Bhubaneswar Dean, THSTI Administrative Officer (Finance and Accounts), THSTI Head – Administration, THSTI
4	Maintenance Committee	Dr. Niraj Kumar Dr. Shailendra Asthana Dr. Guruprasad R. Medigeshi Dr. Bhabatosh Das Mr. G. R. Agarwal Mr. Vishal Gupta Mr. Bhawani Singh Chairperson – Dr. Niraj Kumar/Dr. Shailendra Asthana

Serial No.	Committee	Members
5	Purchase Committee	Dr. Amit Awasthi Dr. Shailaja Sopory Dr. Nisheeth Agarwal Dr. Dinesh Mahajan Dr. Gaurav Batra Dr. Shubbir Ahmad Mr. Manoj Kumar Mr. Satish Kumar Chairperson – Dr. Amit Awasthi/Dr. Shailaja Sopory
6	IT and Communications Committee	Dr. Samrat Chatterjee Mr. M. V. Santo Dr. Amit Kumar Yadav Mr. G. R. Agarwal Mr. Tushar Sharma Chairperson – Dr. Samrat Chatterjee/Mr. M. V. Santo
7	Institutional Ethics Committee- (Biomedical and Health Research) (Reg No. EC/NEW/ INST/2019/275)	Prof. Satinder Aneja Ms. Vidhya Krishnamoorthy Prof. Subir Kumar Maulik Dr. Suvasini Sharma Mr. Munawwar Naseem Dr. Ujjayini Ray Ms. Jasmine Singh Dr. Shailaja Sopory Dr. Arti Kapil Dr. Bhabatosh Das Ms. Amandeep Kaur Ahuja Chairperson – Prof. Satinder Aneja Member Secretary – Ms. Vidhya Krishnamoorthy
8	Institutional Animal Ethics Committee	Dr. Sudhanshu Vрати Dr. Niraj Kumar Dr. Krishnamohan Atmakuri Dr. Amit Awasthi Dr. Amit Pandey Mr. M. T. Sambandam Mr. Ranvir Parashar Prof. Harbans Lal Dr. J. P. Mittal Chairperson - Dr. Sudhanshu Vрати

Serial No.	Committee	Members
9	Institutional Committee for Stem Cell Research (IC-SCR)	Prof. Narinder Mehra Dr. Sujata Mohanty Dr. Sam Mathew Dr. Prasad Abnave Dr. Prasenjit Guchhait Dr. Sivaram Mylavarapu Prof. Nalin Mehta Dr. Ujjayini Ray Ms. Jasmine Singh Mr. Munawwar Naseem Chairperson - Prof. Narinder Mehra Coordinator/Member Secretary - Ms. Vidhya Krishnamoorthy
10	Biosafety Committee	Dr. Krishnamohan Atmakuri Dr. Milan Surjit Dr. Susmita Chaudhuri Dr. Shailaja Sopory Dr. Bhabatosh Das Dr. Vinay Kumar Nandicoori Dr. Ramachandran T. Dr. Prasenjit Guchhait Chairperson - Dr. Krishnamohan Atmakuri
11	Academic Committee	Dr. Samrat Chatterjee Dr. T. Ramamurthy Dr. Ramandeep Singh Dr. Pallavi Kshetrapal Dr. Gaurav Batra Dr. Amit Awasthi Mr. Joby Cyriac Chairperson - Dr. Samrat Chatterjee
12	RTI Act Committee	Dr. Krishnamohan Atmakuri – PIO Dr. Nisheeth Agarwal. Appellate Authority Mr. M. V. Santo – Nodal Officer Executive Director – Public Authority

Serial No.	Committee	Members
13	Complaints Committee (to enquire into complaints of sexual harassment)	Dr. Shinjini Bhatnagar Dr. Nita Bhandari Dr. Nisheeth Agarwal Dr. Pallavi Kshetrapal Dr. Nitya Wadhwa Ms. Amandeep Kaur Ahuja (external member) Dr. Shobha Broor (external member) Mr. M. V. Santo Chairperson - Dr. Shinjini Bhatnagar
14	Student & Employee Welfare, Sports and Hostel Committee	Dr. Sankar Bhattacharya Dr. Susmita Chaudhuri Dr. Tripti Srivastava Dr. Nagender Rao Rameshwaram Dr. Santosh Sadashiv Mathapati Mr. M. V. Santo Chairperson - Dr. Sankar Bhattacharya
15	Tender Opening Committee	Mr. Manoj Kumar Mr. Satish Kumar Mr. Gopal Kishan Chauhan Ms. Rajni Verma Chairperson - Mr. Manoj Kumar/Mr. Satish Kumar
16	Building Committee for Campus - II, Faridabad	Dr. V. S. Chauhan, Ex-Director, ICGEB Executive Director, THSTI Executive Director, RCB Director, NII Director, NIPGR Director, NBRC Dr. Alka Sharma, Advisor, DBT (Scientific Coordinator, NCR-BSC) Mr. Shrikumar Suryanarayan Dr. Partha Majumder, Director, NIBMG Dean (Clinical Research), THSTI Chairperson - Dr. V. S. Chauhan

Serial No.	Committee	Members
17	SC/ST Grievance Redressal Committee	Dr. Niraj Kumar Dr. Milan Surjit Mr. M. V. Santo Chairperson - Dr. Niraj Kumar
18	Vigilance Officer	Dr. Ramandeep Singh
19	Environmental Health & Safety Committee	Dr. Dinesh Mahajan Dr. Nisheeth Agarwal Dr. Sushmita Chaudhuri Mr. Vishal Gupta Dr. T. Ramachandran Dr. Sanjay K. Banerjee Dr. Milan Surjit Chairperson - Dr. Dinesh Mahajan
20	ERP committee	Dr. Amit Kumar Yadav Dr. Nisheeth Agarwal Dr. Ramandeep Singh Dr. Guruprasad R. Medigeshe Mr. M. V. Santo Chairperson - Dr. Amit Kumar Yadav
21	IDRF Committee	Dr. Ramandeep Singh Dr. Prasenjit Guchhait Dr. Avinash Bajaj Dr. Guruprasad R. Medigeshe Dr. Nisheeth Agarwal Dr. Padmakar Tambare Mr. Ramesh Kumar Rathore Mr. G. R. Aggarwal Chairperson - Dr. Ramandeep Singh / Dr. Prasenjit Guchhait
22	Science Setu Committee	Dr. Krishnamohan Atmakuri Dr. Pallavi Kshetrapal Dr. Tarun Kumar Sharma Dr. Siuli Mitra Chairperson - Dr. Krishnamohan Atmakuri
23	Ecological Committee	Dr. Shinjini Bhatnagar Dr. Ramandeep Singh Dr. Sankar Bhattacharyya Dr. Tushar K. Maiti Dr. Feroz Khan Suri Mr. M. V. Santo Mr. Narender Kumar Mr. Ramesh Kumar Rathore Chairperson - Dr. Shinjini Bhatnagar

Serial No.	Committee	Members
24	Sports Committee	Dr. Amit Awasthi Dr. Samrat Chatterjee Dr. Pallavi Kshetrapal Dr. Niraj Kumar Dr. Amit Kumar Yadav Chairperson - Dr. Amit Awasthi
25	Radiation Safety Committee	Dr. Milan Surjit Dr. Guruprasad R. Medigeshe Dr. Dinesh Mahajan Dr. Bhabatosh Das Dr. Krisnamohan Atmakuri Dr. Susmita Chaudhuri Mr. Vishal Gupta Chairperson - Dr. Milan Surjit
26	Specification Sub Committee	Dr. Jayanta Bhattacharya Dr. Milan Surjit Dr. Gaurav Batra Mr. Satish Kumar Chairperson - Dr. Jayanta Bhattacharya

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, NICED, Kolkata

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

Prof. Sudhanshu Vrat, Executive Director,
Regional Centre for Biotechnology, Faridabad

Dr. Jonathan D. Pillai, Project Lead, Jiva Sciences
Pvt. Ltd., Center for Cellular and Molecular
Platforms, NCBS Campus, Bellary Road,
Bengaluru, India

Dr. Sanjay Kumar Banerjee, Associate
Professor, National Institute of Pharmaceutical
Education and Research (NIPER), Guwahati

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

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

Dr. Sagarika Haldar, 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. Jayanta Bhattacharya, Director, International AIDS Vaccine Initiative

Dr. Sameena Khan, Technical Research Expert, Strategy Group, Premas Biotech Pvt Ltd, Gurugram

Dr. Ranajoy Mullick, Manager, Vaccine R & D, International AIDS Vaccine Initiative, 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. Ullas Kolthur Seetharam, Professor, Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai

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

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

LECTURES AT THSTI

Date	Title	Speaker
5 th April 2019	<i>Streptococcus pyogenes</i> Ser/Thr/Tyr kinase and phosphatase-mediated post-translational modifications: Implications in bacterial virulence and therapeutic interventions	Prof. Vijay Pancholi, Associate Professor, Dept. of Pathology, Ohio State University College of Medicine
9 th April 2019	Students' interaction with Dr. Roger Glass	Dr. Roger Glass, Director, Fogarty International Centre & Associate Director for Global Health, NIH
26 th April 2019	Norm, Normal and Normative: Misunderstood Characters of Low Lung Function in India	Dr. Anurag Agarwal, Senior Scientist and Director CSIR-IGIB
26 th April 2019	An Overview of Advanced Proteomic technologies: Case studies on brain tumors	Dr. Sanjeeva Srivastava, Professor, Department of Biosciences and Bioengineering, IIT Bombay
10 th May 2019	R for biomedical text mining	Dr. S. Ramachandran (Senior Principal Scientist, Informatics and Big Data, IGIB)
24 th June 2019	Development of Anti-flavivirus Therapeutics	Dr. Rajeshwar Singh Sankhala, Research Scientist with US Military HIV research program of Walter-Reed Army Institute of Research/HJF
28 th June 2019	Proteomics to Multiomics to Multimodal Approach towards Translation: Glioblastoma model	Dr. Ravi Sirdeshmukh, Institute of Bioinformatics, Bangalore & Mazumdar Shaw Center for Translational Research, Bangalore
19 th July 2019	Drug development, landscape and challenges	Dr. Guru Aithal, Professor of Hepatology and Head of Digestive Diseases Centre, Nottingham Biomedical Research Centre.
23 rd August 2019	Applications of MicroCT in Preclinical INV Imaging for Small Animal	Dr. Alexandre Belenkov, Applications Scientist, Perkin Elmer, Life Sciences & Technology
25 th September 2019	Mechanistic Insights into Processing of Clients by Molecular Chaperones	Dr. Vinay Dahiya, Postdoctoral Researcher in Prof. Johannes Buchner's lab, Technical University of Munich (TUM), Germany
3 rd October 2019	Fc receptor expression on CD4+ T cells: An implication for HIV-1 latency	Dr. Anil K. Chauhan, American immunologist
4 th October 2019	Accelerating biology: 10x Genomics products and vision	Dr. Mike Lucero, 10X Genomics, USA
11 th October 2019	The role of autophagy against radiation stress and in the early stages of human embryonic development	Dr. Kulbhushan Sharma, Scientist D, Institute of Nuclear Medicine and Allied Sciences (INMAS), DRDO

6 th November 2019	Novel techniques in Cell Culturing	Bill Whitford, Strategic Solutions Leader, GE Healthcare Life Sciences Logan, USA Dr. Sanjoy Mukherjee, PhD, GM-Applications, India and South Asia, GE Healthcare Life Sciences, India
8 th November 2019	Method for detection of protein sequence remnants within non-coding genes and their regulations	Dr. Sweta Talyan, Post-Doctoral Researcher at Heidelberg University Hospital, Heidelberg, Germany and a member of Prof. Christoph Dieterich's group
25 th November 2019	Thrombotic susceptibility in aging	Dr. Sanjana Dayal, Assistant Professor, University of Iowa
29 th November 2019	A novel quantitative approach for understanding strain level microbiota dynamics after fecal transplantation for several diseases	Dr. Varun Agarwal, Postdoc at Mount Sinai School of Medicine,
28 th January 2020	Sustained remission in the London patient and tissue sampling for HIV	Prof. Ravindra 'Ravi' Gupta, a Professor of Clinical Microbiology and Wellcome Trust Senior Fellow in Clinical Science at The University of Cambridge and faculty at the Africa Health Research Institute in Durban, South Africa
11 th February 2020	Commensals of two kinds: Promoter or Eliminator of <i>Vibrio cholerae</i> infection	Dr. Sang Sun Yoon from the Department of Microbiology and Immunology of Yonsei University College of Medicine, Seoul.



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