thsti

ट्रांसलेशनल स्वास्थ्य विज्ञान
एवं प्रौद्योगिकी संस्थान

TRANSLATIONAL HEALTH SCIENCE
AND TECHNOLOGY INSTITUTE

Annual Report 2020-2021

Mission

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

Vision

As a networked organization linking many centers of excellence, THSTI is envisioned as a collective of scientists, engineers, and physicians that will effectively enhance the quality of human life through integrating a culture of shared excellence in research, education and translational knowledge with the entrepreneurial spirit to take technologies into the public sphere. In fulfilment of its vision, the THSTI will work with other constituents of the technology cluster at Faridabad through long term partnerships.

CONTENTS

THSTI Society

S. No.	Member	Designation in Society
1.	Ms. Shailaja Chandra Former Secretary in the Ministry of Health and former Chief Secretary, Delhi	President
2.	Secretary to the Government of India , Department of Biotechnology, New Delhi	Member, Ex-officio
3.	Director General , Indian Council of Medical Research, and Secretary, Department of Health Research, New Delhi	Member, Ex-officio
4.	Additional Secretary and Financial Advisor, Department of Biotechnology, New Delhi	Member, Ex-officio
5.	Adviser- DBT Coordinator-THSTI	Member, Ex-officio
6.	Director, National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi-110067	Member, Ex-officio
7.	Dr. M. Radhakrishna Pillai Director, Rajiv Gandhi Centre for Biotechnology, Thycaud PO, Poojappura, Thiruvanthapuram-695014	Member
8.	Dr. Apurva Sarin Director, Institute for Stem Cell Biology and Regenerative Medicine, GKVK-Post, Bellary Road, Bangalore-560065	Member
9.	Dr. Randeep Guleria Director, AIIMS, Ansari Nagar, New Delhi-110029	Member
10.	Dr. Vineeta Bal Visiting Faculty. Biology Division, IISER, Pune, Dr. Homi Bhabha Road, Pashan, Pune-411008	Member
11.	Dr. UdayKumar R. Yaragatti Director, MNIT, Jaipur	Member
12.	Dr. Ashalatha R Professor, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram-695011	Member

13.	Executive Director, Translational Health Science and Technology Institute, NCR-BSC cluster, Faridabad-121001	Member Secretary, Ex-officio

THSTI Governing Body

S. No.	Member	Position
1.	Secretary to the Government of India, Department of Biotechnology, New Delhi	Chairperson, Ex-officio
2.	Director General, Indian Council of Medical Research, and Secretary, Department of Health Research, New Delhi	Ex-officio member
3.	Additional Secretary and Financial Advisor, Department of Biotechnology, New Delhi	Ex-officio member
4.	Joint Secretary, (Administration), Department of Biotechnology, New Delhi	Ex-officio member
5.	Advisor / Scientist-G, Department of Biotechnology, New Delhi	Ex-officio member
6.	Executive Director, RCB, Faridabad, NCR	Ex-officio member
7.	Director, NII, New Delhi	Ex-officio member
8.	Director, NIPGR, New Delhi	Ex-officio member
9.	Director, NBRC, Manesar, NCR	Ex-officio member
10.	Scientist-E (Nodal Officer), Department of Biotechnology, New Delhi	Ex-officio member
11.	Mr. S. Gopalakrishnan Chairman, Axilor Ventures, Bengaluru	Nominated member
12.	Dr. Prabhakaran Dorairaj Vice President (Research and Policy) and Director, Centre for Control of Chronic Conditions, PHFI, Gurugram	Nominated member
13.	Prof. Sandhya S. Visweswariah, Professor, Dept. of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bengaluru	Nominated member
14.	Dr. Tapas Kumar Kundu, Director, Central Drug Research Institute, Lucknow	Nominated member
15.	Dr. Vaskar Saha Senior Consultant in the Department of Paediatric- Haematology& Director of Tata Translational Cancer Research Center (TTCRC), Kolkata	Nominated member

16.	Dr. Ramesh Byrapaneni Managing Director, Endiya Partners, Hyderabad	Nominated member
17.	Dr. Rakesh Aggarwal, Director, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry	Nominated member
18.	Dr. Sharmila Sengupta, Professor, National Institute of Biomedical Genomics, West Bengal	Nominated member
19.	Prof. Narendra Kumar Arora Executive Director, The INCLEN Trust International, New Delhi	Nominated member
20.	Dr. Neena Valecha, Regional Advisor, World Health Organisation (WHO)	Nominated member
21.	Executive Director, THSTI, Faridabad	Member Secretary, Ex-officio

FROM THE EXECUTIVE DIRECTOR

Humanity would rather forget the past one year marked by tumultuous events which shocked the entire world. But the year would be remembered for the triumph of science over cynicism, optimism over skepticism and service over self. From increasing our capacity vastly in diagnostics to optimizing rationale treatment protocols and to vaccine development, India has done exceedingly well. I am extremely happy that THSTI has contributed immensely in our collective fight against the COVID-19 pandemic. The pandemic tested our capabilities to the limit but also provided an opportunity by forcing us to innovate, indigenize, and rapidly scale up our efforts for *atmanirbhar* Bharat. The research ecosystem at THSTI was just ripe and flourishing to stand up to the challenge and come up swiftly with practical solutions, be it in diagnostics or vaccines. This year's annual report largely focuses on THSTI's contribution to COVID-19 pandemic led by my predecessors Dr. Gagandeep Kang and Dr. Subeer Majumdar under the able leadership of Dr. Renu Swarup, Secretary, Department of Biotechnology who motivated the scientists and provided crucial financial and infrastructure support. Let me briefly highlight the achievements of THSTI here which would bring out the true translational nature of this unique institution.

In the beginning of 2020, when SARS-CoV-2 virus struck affecting countries globally, nobody had imagined that a pandemic of such a scale which has infected more than 120 million people and resulted in >2.5 million deaths worldwide would devastate the mankind. India, as a nation, invested heavily early on to fight the pandemic at all fronts with great success.

THSTI played a key role in basic, clinical and translational science spheres. THSTI was the lead coordinator of DBT's Consortium for COVID-19 research, a multi-institutional platform for developing a clinical cohort of patients with COVID-19, collecting biospecimens, and conducting vaccine effectiveness and re-infection studies. The consortium led by Prof. Shinjini Bhatnagar, provided huge resources which are being used by multiple stakeholders. One of the highlights from the consortium study, at a time when not much was known about the virus, was antibody responses in patients with COVID-19 according to severity of the illness and how antibody levels might decline over time. Another important ongoing project is to assess the effect of COVID-19 on pregnancy in terms of maternal, fetal and neonatal outcomes in the pre-term birth cohort '*GARBH-INi*'. THSTI is also a participating centre in a multi-country study to

examine the severe SARS-CoV2 related disease in children and expected to yield useful data on Multisystem Inflammatory Syndrome in Children (MIS-C).

The Biorepository Facility at THSTI houses more than 3000 samples from corona positive patients. It is a unique resource of well-phenotyped COVID-19 cases followed longitudinally since early 2020. This bioresource has helped tremendously both the academia and industry for the development of diagnostic kits and vaccine development.

One of the core facilities of THSTI, Bioassay Laboratory provides accredited assays, kit validations and in collaboration with Foundation for Innovative New Diagnostics (FIND) trained manpower from various organizations for capacity building. Bioassay Laboratory has tested more than 83,000 clinical samples for SARS-CoV-2 using RT-PCR. It is NABL accredited and has the distinction of being recognized the only lab from India of the 7 labs affiliated with and recognized by the international organization 'Coalition for Epidemic Preparedness Innovations' (CEPI) network. On 5th January 2021, Dr. Harsh Vardhan, Hon'ble Minister for Health & FW, Science & Technology, and Earth Sciences inaugurated the Bioassay Lab.

An important and first of its type, a mobile testing laboratory, the iLAB, with RT-PCR facility was launched by THSTI. It is the first indigenously designed mobile COVID-19 testing unit and has conducted onsite nearly 15, 000 RT-PCR tests in urban and rural areas of Faridabad.

THSTI was the first institution in the country to establish two models of SARS-CoV-2 infections in Hamster and ACE2 Transgenic mice models. These models proved extremely effective for carrying out challenge studies for vaccines and therapeutics and contributed immensely to the regulatory approvals for COVAXIN and SPUTNIK vaccines in India.

Live virus neutralization assays are essential to test efficacy of vaccine and therapeutic molecules. THSTI was one of the few institutions in the country to have this rare facility. Our infectious disease research facility has been carrying out neutralization assays for many vaccine developers and vaccine effectiveness studies.

For newer in-house diagnostics against SARS-CoV-2 amidst global shortage, THSTI developed antibody ELISA technology which has been transferred to industry and used for serosurveillance. THSTI also developed the first APTAMER-based SARS-CoV-2 detection assay targeting Spike and Nucleocapsid antigens that has also been transferred to industry.

With respect to COVID-19, THSTI has published 15 international peer reviewed papers, filed seven patents and transferred four technologies to industry.

Although COVID related work led to diversion of some of our resources, THSTI continued to be engaged in cutting edge research integrating medicine, biology, and technology into translational knowledge for finding solutions for public health problems in India.

GARBH-Ini (interdisciplinary Group for Advanced Research on Birth outcomes-DBT India Initiative) is the largest longitudinal cohort of pregnant women in LMIC. This is being followed up for developing risk stratification algorithm and candidate interventions in order to prevent preterm birth. Under the Immediate and continuous kangaroo mother care (iKMC) intervention in very low-birth-weight infants, a randomized controlled trial led by WHO and carried out in India (THSTI), Ghana, Malawi, Nigeria and Tanzania showed an improvement in the survival of low-birth-weight infants (*NEJM 2021*).

Cutting edge research is ongoing in immunobiology laboratory to elucidate the role of T cells in autoimmune diseases and cancer particularly to understand the molecular pathways that are essential for the generation and function of effector and regulatory T cells. In addition, the immunology core laboratory is involved in identifying the cellular pathways that are modulated by intracellular pathogens for their own survival and has found that host-directed therapy modulates host cellular factors that are essential to support the growth and survival of the intracellular pathogens.

Now we know that humans are not alone and the human microbiome represents a perfect example of collective living and wellbeing. Studying microbiome in health and disease has been a major thrust area at THSTI. Some important findings have emerged at the population level through collaboration with countries such as Japan and Denmark. Gut microbial signatures in various disease states such as diabetes, gallbladder cancer and inflammatory bowel disease have been discovered. Vaginal microbiome was also found to be different and predictive of pre-term birth in a large cohort study.

Tuberculosis (TB) is a major cause of morbidity and mortality in the world. In concerted efforts towards finding a cure for TB, scientists at THSTI have been working towards understanding the regulation and function of complex networks in *Mycobacterium tuberculosis*, identifying

metabolic pathways as drug targets and exploring new vaccine candidates. THSTI is in advanced stages of commercialization for one of its novel anti-tubercular molecules.

Research for other viral diseases that include Hepatitis E virus (HEV), HIV and Dengue is ongoing at THSTI. For Hepatitis E virus, efforts are on to understand virus biology and develop a recombinant vaccine against HEV. With respect to HIV, THSTI in collaboration with IAVI is working towards designing antibody-based intervention strategies to address unmet health needs in India and worldwide. In the field of Dengue, investigators at THSTI have screened a library of pharmacologically active compounds to identify dengue replication inhibitors. They have successfully designed and characterized Dengue structural E protein, stabilized dimers from different serotypes and are now working towards developing a preclinical animal model for Dengue virus infection and assessment of immune responses. THSTI has bagged 3 major grants for influenza research and is working towards developing next-gen low-cost and effective influenza vaccines in collaboration with institutions in the European Union.

Anti-microbial resistance (AMR) is a huge challenge worldwide. Researchers at THSTI are working towards understanding the molecular basis of AMR, developing a rapid test for pathogen identification and antimicrobial susceptibility testing (AST) which will provide affordable diagnostics for detecting AMR.

In addition to developing detection kits for SARS-CoV-2, researchers at THSTI have been working towards developing ultrasensitive point of care tests for detection of acute febrile illnesses and one such aptamer-based technology has been transferred to industry.

Non-alcoholic fatty liver disease (NAFLD), an important public health problem, has no effective treatment. A team of scientists is working on drug discovery for NAFLD, utilizing targeted and non-targeted approaches and have set up relevant *in vitro* & *in vivo* models.

Our major research platforms viz., Bioassay Laboratory, Biorepository, Data management centre and Small animal facility (SAF) provide excellent resources. These facilities contributed immensely to the COVID-19 research work at THSTI. Bioassay Lab provides services for kit validation, trains manpower and provides accredited assays to both academia and industry. Biorepository houses a large number of biospecimens that are provided to investigators on request. Small animal facility has proved to be immensely useful. In the coming months, a new ferret facility will also be set up which will be the first of its kind in the country.

Clinical Development Service Agency (CDSA) has been functioning as an academic Clinical Research Unit (A-CRU) towards supporting and nurturing clinical product development and clinical research in India. This year CDSA has provided clinical study support for investigators, sponsors and SMEs. It has also provided training to personnel both in India and other nations towards capacity building. Despite the pandemic and lockdowns, CDSA continued to conduct online educational courses regularly.

In our outreach program, THSTI has conducted –10 *Science Setu* programs in – schools and colleges to inspire young minds and also organized webinars for women scientists.

THSTI has cemented its place among our nation's top research institutions and is working towards becoming an epitome of translational research by its focussed research on developing indigenous vaccines, affordable diagnostic kits, providing a conducive and enabling environment for research, and encouraging entrepreneurship for its scientific community. We need to keep moving forward and not bask in the glory of our achievements. In my opening remarks when I joined the institute I promised my wholehearted support to our scientists in every possible manner to help navigate their research and translate it into products for the greater good of the mankind. I wish to see the fruits of their labour in the coming years.

Our plans for the next year include setting up a Medical Research Centre which was envisaged in the cabinet note when THSTI was established, developing a GLP facility, upgrading the experimental animal facility, establishing a dedicated influenza virus research program, creating a dedicated AMR research group, and expanding the computational biology, big data and bioinformatics platform. I hope to deliver on my promise.

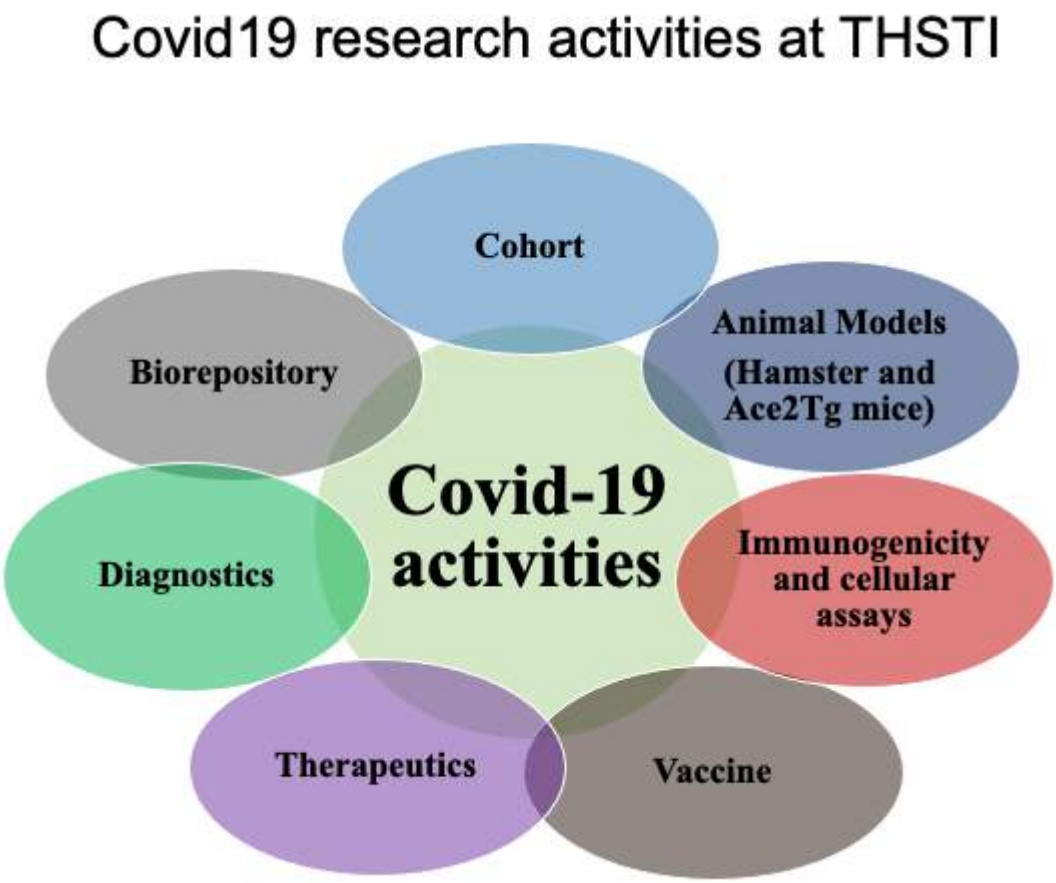
On behalf of the THSTI family, I wish to express our gratitude and sincere thanks to the esteemed members of the THSTI Society, Governing Body, Finance Committee, Scientific Advisory Committee, and the Department of Biotechnology for their constant guidance and kind support.

Jai Hind,

Pramod Garg

SARS-CoV-2 Pandemic: Meeting the Challenge

With the mandate of developing innovative solutions for public health challenges faced by India, THSTI has been one of the first research institutes in the country to meet the challenge posed by SARS- CoV-2. To deal with the biggest threat to humanity in more than 100 years, a comprehensive approach was taken where THSTI has worked closely with both national and international institutions including academia, industry, and government. THSTI has established clinical cohorts, bioresources, and cutting age technology for the assessment of candidate vaccines and drug molecules both in the *in vitro* settings and *in vivo* in different animal models. THSTI not only provided laboratory services to the community but also provided training to the technical staff from different hospitals on diagnostic testing of SARS-CoV-2. THSTI has also been involved in the in-house development of diagnostic tests, candidate vaccines, and monoclonal antibodies for therapeutics. THSTI has contributed significantly for the development of several vaccines in the country.



Cohorts

The DBT India Consortium for COVID-19 Research (including DBT AI institutes: THSTI, RCB, NII, ICGEB; Hospitals primarily: Lok Nayak Hospital, New Delhi and ESIC Medical College Hospital, Faridabad in the National Capital Region (NCR), Delhi) has established cohorts of patients with COVID-19 across different "regions and populations"; a large biorepository with well-phenotyped biospecimens to fulfill the objectives to study the epidemiology of SARS-CoV-2 infection, evaluate immune responses to understand natural infection, aid in seroepidemiology, development and validation of *in vitro* diagnostics, vaccines & therapeutics with the primary objective that the results of these research studies inform policy. The three major cohorts are the adult cohort, the pregnancy cohort, and a cohort of children with COVID-19. These cohort studies are led by **Prof. Shinjini Bhatnagar** at THSTI.

The **adult cohort** is one of the largest cohorts of COVID 19 patients established in South East Asia comprising of 4157 patients enrolled since April 2020. The longitudinal immune responses of these patients were documented at different time points (10-28 days, 6-10 weeks, and more than 6 months). It was observed that there is a decline in memory T cells in SARS-CoV-2 seropositive individuals after 8-10 months of recovery from asymptomatic infection. A less robust response to vaccines is expected in individuals recovered from asymptomatic infection, who represent the majority of COVID-19 patients. Important immunological findings from the cohort studies are:

(i) All with severe disease, 89.6% with mild to moderate infection and 77.3% of asymptomatic participants showed seroconversion (IgG antibodies against RBD ag)

(ii) Seropositivity decreased by 22% between 6-10w & 6m from the onset of illness

Evaluation of cellular immunity among COVID-19 recovered patients post-immunization with different vaccines is ongoing.

In the **pregnancy cohort**, associations between antenatal COVID-19 and pregnancy outcomes are being studied in collaboration with European Union Consortium and DBT India (ORCHESTRA).

A cohort of **children (0-19 years)** has been setup across five tertiary hospitals in Delhi NCR and in Bengaluru to understand the clinical course of COVID-19 infection more specifically

the clinical severity, immune responses, and the multi-system inflammatory syndrome in neonates, children, and adolescents.

Diagnostics

Diagnostics for SARS-CoV-2 are critical for detecting infection, understanding the immune response generated after the infection and vaccination, and epidemiological studies. THSTI generated high-quality recombinant proteins, monoclonal antibodies, and aptamers for SARS-CoV-2, which were used for the development of both the antibody as well as antigen detection assays.

THSTI has developed Antibody detection ELISA and Antibody detection Rapid POCT. **Dr. Gaurav Batra**'s team developed a rapid and stable RBD-based IgG ELISA test kit obtained through extensive optimization of the assay components and conditions. The ELISA kit is stable even at room temperature for at least a month. This test, with a shorter runtime, showed higher sensitivity than parallelly tested commercial ELISAs for SARS-CoV-2-IgG, i.e., Euroimmun IgG and Zydus kavach IgG, even when equivocal results in the commercial ELISAs were considered positive. It was also found to be more sensitive, particularly at early time points compared to Abbott Architect IgG CLIA. RBD ELISA also showed higher concordance with neutralization titers than Abbott IgG Assay (nucleocapsid based). The RBD IgG ELISA has been transferred to Xcyton Diagnostics Limited for manufacturing and commercialization.

Apart from this, **Dr. Tarun Kumar Sharma** and his team developed the first APTAMER-based SARS-COV-2 detection assay targeting Spike and Nucleocapsid antigen using SELEX and post-SELEX strategies. Selected aptamer candidates against both the proteins to show high affinity (for spike-trimer ~22nM and nucleocapsid ~4nM) with no cross-species binding. These developed aptamers can detect the low amount of antigen in an ALISA method. The spike binding aptamer was able to discriminate the infected and non-infected nasopharyngeal swab samples with high specificity (~98%) and sensitivity (~91%). These technologies have been transferred to MolBio Diagnostics and Cambrian Bioworks.

In addition, a DNAzyme based visual detection method for SARS-CoV-2, which is compatible with a conventional thermocycler, was also developed by **Dr. Tarun Kumar Sharma** and **Dr. Guruprasad R Medigeshi**. This technology has been transferred to Genei Labs, Bengaluru.

Dr. Milan Surjit in collaboration with Dr. Manish Soneja's laboratory at AIIMS (New Delhi), reported the diagnostic utility of gargle lavage for Real-Time PCR detection of SARS-CoV-2 infection. A study was carried out to compare the efficacy of gargle lavage and nasopharyngeal/oropharyngeal (NP/OP) swab-mediated RNA isolation techniques. They observed that gargle lavage was equally effective in detecting the infection. Easier self-collection mode of gargle lavage sample by the patient (without any assistance from a trained healthcare worker) offers an edge to this technique over the classical NP/OP swab mediated sample collection.

Vaccines

Vaccination is considered to be the most effective strategy for the prevention and control of SARS-CoV-2 infection, as expected from an ideal vaccine candidate to provide protection inducing both humoral and cell-mediated immune responses in the body.

THSTI has helped in the development of several vaccine candidates by offering state of the art facilities for both *in vitro* and *in vivo* experiments. **Dr. Amit Awasthi** and his team at the Immunology Core Lab performed the efficacy testing of Sputnik V vaccine. The study was a part of clinical trials for Phase II and Phase III for the vaccine. The data contributed significantly in the emergency use authorization for Sputnik V vaccine from Drug Controller General of India (DCGI). Dr. Awasthi's lab also carried out pre-clinical studies of ZyCoV-D vaccine manufactured by Zydus Candila and Biological E's Corbevax vaccine. Pre-clinical trials were carried out in hamster model for SARS-CoV2 infection at THSTI. ZyCoV-D is a DNA-plasmid-based vaccine which carries the gene encoding the spike proteins of SARS-CoV-2. At THSTI, challenge studies were carried out with live SARS-CoV2 virus (Wuhan strain) in immunized hamsters. Following the successful pre-clinical trial of the ZyCoV-D at THSTI and its subsequent success in clinical trial studies, ZyCoV-D vaccine has been given emergency approval by DCGI. Corbevax is a subunit vaccine candidate which contains subunit proteins

derived from SARS-CoV2. The pre-clinical studies of Corbevax showed good protection in hamsters. These studies at THSTI contributed in Corbevax getting approval for Phase III trials by the Government of India.

THSTI has also been working on different platform-based technologies (mRNA, protein subunit, conserved peptidomimetic approaches) to develop in-house vaccine candidates with broad potency and improved efficacy.

THSTI is developing a self-amplifying mRNA vaccine candidate against the SARS-CoV-2 wherein S-protein ectodomain and receptor-binding domain (RBD) are being tested as vaccine antigens. A BSL2 compatible replicon of SARS-CoV-2 is also being developed which will be useful for antiviral screening and investigating the mechanism of viral replication.

Researchers at THSTI are working towards developing conserved multi-epitope-based vaccines using peptides (epitopes) on the SARS-CoV-2 spike domain as potential immunogens or as anti-viral blocking agents. **Dr. Sweety Samal** in collaboration with **Dr. Shubbir Ahmed** has stitched the immunogenic epitopes of SARS-CoV-2 and produced it as a soluble immunogen that will generate neutralizing antibodies. As an initial proof-of-concept, conserved epitopes were identified and 20-meters synthetic peptides mainly at the RBD and heptad repeat 2 regions of SARS-CoV-2 spike protein were found to be immunogenic *in vivo* which elicited neutralizing antibodies and blocked the entry of virus *in vitro*. Furthermore, the group has evaluated different routes of immunization and demonstrated the conserved RBD peptides could elicit strong humoral and cell-mediated immune responses both in intra dermal and intra muscular immunization. The designed immunogen candidate is currently under evaluation for its immunogenicity and the potential to induce neutralizing antibodies in the mice model. Since the team has used only the small part of the receptor-binding domain that is mostly targeted by the neutralizing antibodies in the natural course of infection, they believe this designed construct will potentially induce neutralizing antibodies in an immune-focusing manner. Once established, the antigen will be evaluated for its potency for protection through a direct challenge or after passive immunization. Based on the study results, further improvement in the design will be implemented for better immunogenicity.

Scientists at THSTI are also working on developing a protein-based immunogens using different folds and domains displayed on a fragment crystallizable (Fc) domain from IgG. **Dr. Shubbir Ahmed's** team designed protein-based immunogens that have CH3 domains of IgG heavy chains at the either end with the receptor binding motif (RBM) fold of RBD displayed on the CH3 scaffold (CH3-RBM-CH3). Structure modeling and energy minimization of structure suggest a well folded conformation displaying the grafted fold of the RBM from SARS-CoV-2 displayed on the CH3-scaffold. They have successfully purified this construct from bacterial expression system for characterization and immunogenicity studies.

Dr. Tripti Shrivastava and the team at THSTI have designed and characterized SARS-CoV-2 spike protein fragment (330-526) as Receptor Binding Domain ($RBD_{330-526}$) with two native glycosylation sites (N331 and N343) as a potential subunit vaccine candidate. $RBD_{330-526}$ was characterized biochemically and investigated for its thermal stability, humoral and T cell immune response of various RBD protein formulations (with or without adjuvant) to evaluate the inherent immunogenicity and immunomodulatory effect. This purified RBD immunogen is stable up to 72 hours, without any apparent loss in affinity or specificity of interaction with the ACE2 receptor. Upon immunization in mice, RBD generates a high titer humoral response, elevated IFN-γ producing CD4+ cells, cytotoxic T cells, and robust neutralizing antibodies against live SARS-CoV-2 virus. These results indicate the potential of RBD330-526 as a promising vaccine candidate against the SARS-CoV-2. Dr. Shrivastava plans to evaluate this potential protein subunit-based vaccine candidate against SARS-CoV-2 infection in the Syrian Golden Hamster.

Therapeutics

Preclinical evaluations of AYUSH herbal extracts/formulations for mitigating SARS- CoV-2 and associated pathologies are being carried out at THSTI by a team of researchers led by **Dr. Madhu Dikshit** and **Dr. Amit Awasthi**. The following natural products/formulations for testing have been provided by NMPB: Aswagandha (*Withania somnifera*), Guduchi (*Tinospora cordifolia*), Yashtimadhu (*Glycyrrhiza glabra*), AYUSH-64 (its constituent extracts), and four nasal formulations (Anu Taila, Shadbindu Taila, Tila Taila (Sesamum oil) & Go Ghrita. Anti-viral screenings of these compounds are carried out in animal models.

To elucidate the mechanism of SARS-CoV-2 replication and assembly of progeny viruses, researchers at THSTI are developing and characterizing the RNA-protein interactome of SARS-CoV-2 and also trying to characterize genome encapsidation by the viral nucleocapsid protein.

With the broad goal of discovering new targets for the development of antiviral therapeutics against the SARS-CoV-2, a team of researchers at THSTI led by **Dr. Milan Surjit** identified the RNA-protein-protein interaction (RPPI) network assembled at the 5'- and 3'-UTRs of SARS-CoV-2 genomic RNA. 57 host proteins were found to be involved in viral replication, translation, and RNA metabolism machinery. Ongoing studies of the group aim at the molecular characterization of some of the RPPIs, with the ultimate goal of testing their potential as potent targets for antiviral screening. Dr. Surjit's team at THSTI was the first to show the importance of host lysosomal associated membrane protein 2a (Lamp2a, receptor for chaperone-mediated autophagy) during SARS-CoV-2 infection.

THSTI has also been working on antibody-based therapeutic interventions for SARS-CoV-2. Phage display antibody (PDA) libraries allow the rapid isolation and characterization of high specificity monoclonal antibodies for therapeutic and diagnostic applications.

Dr. Rajesh Kumar and his team screened the Tomlinson I and J library against receptor-binding domain (RBD) of SARS-CoV-2 and found that eight clones showed positive binding in phage ELISA and contained one or more amber stop codons in their single-chain antibody fragment (scFv) gene sequences. The presence of amber stop codons within the antibody sequence causes the premature termination of the soluble form of scFv expression in non-suppressor Escherichia coli strain. Dr. Kumar's team used a novel strategy that allows soluble expression of scFvs having amber stop codon in their gene sequences (without phage PIII protein fusion), in the suppressor strain. This strategy of the introduction of the Ochre (TAA) codon at the junction of scFv and PIII gene, speeds up the initial screening process which is critical for selecting the right scFvs for further studies.

A naïve human semisynthetic phage library against RBD was also screened by **Dr. Rajesh Kumar** and **Dr. Chandresh Sharma** and a high-affinity scFv was identified. The scFv was further engineered into antibody formats (scFv-Fc and IgG1). All three antibody formats showed high binding specificity to SARS-CoV-2, RBD, and spike

antigens and the spike antigens in different assay systems. Flow cytometry analysis demonstrated specific binding of the IgG1 format to cells expressing membrane-bound CoV-2 spike protein. They performed identification/screening of epitopes using a 12 mer peptide library in a phage system and evaluated experimentally through *in vitro* assay, for the binding of phage displaying peptides, in three rounds of panning with the generated anti-RBD mAb in ELISA format. Further, the *in vitro* results were corroborated with docking and modeling in silico. Docking studies revealed that the scFv recognizes an epitope that partially overlaps with angiotensin-converting enzyme 2 (ACE2)–interacting sites on the CoV-2 RBD. These anti-CoV-2 antibodies will be useful as valuable reagents for accessing the antigenicity of vaccine candidates, as well as developing antibody-based therapeutics and diagnostics for CoV-2. These potential neutralizing mAbs will be tested for their therapeutic potential in suitable animal models. The team is expanding on their anti-SARS-CoV-2 antibodies generation through mouse hybridoma technology, targeting, other mutants and variants of SARS-CoV-2.

In a study, **Dr. Rajesh Kumar** found that non-neutralizing SARS-CoV-2 directed polyclonal antibodies demonstrate cross-reactivity with the Hemagglutinin (HA) glycans of influenza virus and gp41 of HIV-1. Epitope mapping suggests that the cross-reactive antibodies were targeted towards the glycan epitopes of the SARS-CoV-2 spike and HA, while, in the case of HIV-1, this cross-reactivity was found to be targeted towards the gp41 region of HIV-1 Env (gp160) protein. These findings address the cross-reactive responses elicited against the RNA viruses and warrant further studies to investigate whether such non-neutralizing antibody responses can contribute towards effector functions such as antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent enhancement (ADE) mediated enhancement.

Dr. Jayanta Bhattacharya and his team have recently isolated many SARS-CoV-2 RBD reactive monoclonal antibodies from one convalescent donor by B cell cloning which are being further characterized for their ability to neutralize wild type and different variants of SARS-CoV-2. This work is being done through collaboration between THSTI and IAVI.

Post-infection, the antivirals (small molecules) need to be administered as they can reduce the morbidity and mortality associated with the infection. Therefore, there is a pressing need for the discovery of newly designed antiviral drugs that potentiate the ability to combat the current pandemic efficiently. The structural bioinformatics team of THSTI led by **Dr. Shailendra Asthana** is working on the discovery of antiviral small molecules against SARS-CoV-2. For the discovery of small molecules knowledge of viral proteins, their dynamics, and key residual information especially when it binds with host proteins and/or with other endogenous binders is essential to map the interactions, as these *hot-spot* interactions are essential to perturb the biological function. Therefore, THSTI scientists are applying the CADD and advanced computational biophysics approaches on different viral proteins such as protease (Mpro), RNA-dependent RNA polymerase, Nucleocapsid protein RBD-ACE2 interface, etc. to understand the architecture of binding/interface sites for screening and designing of small molecules.

Bioassay Laboratory

As head of the Bioassay Laboratory (BL), **Dr. Guruprasad R Medigeshi** was assigned the primary responsibility of setting up the first testing lab in the Faridabad-Palwal regions which was the need of the hour in March-April 2020. Bioassay laboratory (BL) supports clinical studies by providing platforms for various clinical and immunological assays. BL is supported by DBT's IndCEPI mission. BL is a part of the Coalition for Epidemic Preparedness Innovations (CEPI) network lab, the first of its kind in India. During the year 2020-21, BL got accreditation for dengue assays and worked towards developing methods and in-house assays for other diseases. BL has developed in-house quantitative ELISAs and plaque reduction neutralization assays (PRNT) for SARS-CoV-2 by using WHO reference standard for calibration and obtained NABL accreditation for these assays. BL performed microneutralization assay against SARS-CoV-2, for phase I/II samples of BioE's vaccine candidate. It is currently in process of carrying out assays for phase III samples for BioE's vaccine candidate. BL is an ICMR-accredited centre for validation of COVID-19 diagnostic kits and accessory products. BL has tested more than 80, 000 clinical samples of SARS-CoV-2 using RT-PCR. BL has a collaboration with Foundation for Innovative new Diagnostics (FIND) for validation of diagnostic kits and training and has helped over 25 companies with validation of diagnostic kits and accessory production. It has provided training to 55 lab personnel of various organizations for COVID-19 testing. Dr. Medigeshi's team also took the

responsibility of running the first-in-the-country mobile diagnostic facility (iLAB) for COVID-19 in rural areas of Haryana. iLAB, the first indigenously designed mobile COVID-19 testing unit made by the Andhra Pradesh Medtech Zone Ltd was assigned to THSTI by DBT for testing samples from remote locations in and around Faridabad. The iLAB has completed testing for more than 15,000 samples to date.

Biorepository

Biorepository facility headed by **Dr. Pallavi Kshetrapal** houses >3800 samples collected from COVID-19 positive individuals. The HVTR lab at THSTI under the guidance and supervision of **Dr. Suprit Deshpande** has contributed to providing hands-on training to the key staff of the THSTI Biorepository team in the separation of plasma and PBMC (peripheral blood mononuclear cells) from blood samples collected from SARS-CoV-2 convalescent donors and their appropriate storage, revival and assessing PBMC viabilities. The biorepository has designed and developed sera panels for R&D as per the requirement for quantitative and qualitative assessment in serological testing that has been stratified by day of collection post symptoms & the level of antibodies against SARS-CoV-2. A set of negative sera pools has been developed utilizing the pre-pandemic sera samples archived at the biorepository. Positive pooled standard controls (sera) calibrated to the National Institute for Biological Standards and Control (NIBSC) standards have been also been developed. The National Bio-Resource for COVID-19 at THSTI NCR Biotech Science Cluster has received 55 requests from academia and industry for COVID-19 bioresource and has responded to all the requests through an external access control committee set up by DBT and coordinated by the THSTI biorepository. To date, biorepository has shared more than 16000 biospecimen/aliquots developed as COVID-19 Bioresources at THSTI with various academia and industries (Industry17; Academia 28).

THSTI's indigenous anti-SARS-CoV-2 IgG ELISA has been developed using the bioresource at the biorepository and has been transferred to the industry partner Xcyton Diagnostics, Bengaluru. This kit has been successfully been used for a serosurvey study for the region of Karnataka.

Animal Models

Dr. Amit Awasthi and his team developed Hamster and ACE2 transgenic animal models for studying SARS-CoV-2 infection. SARS-CoV-2 infection in the golden Syrian hamster animal model causes lung injury and immunopathologies resemble human coronavirus disease (COVID-19).

At THSTI, using the hamster model, Dr. Awasthi and the team observed that the early phase of SARS-CoV-2 infection leads to an acute inflammatory response and lung pathologies while the late phase of infection causes cardiovascular complications (CVC) characterized by ventricular wall thickening associated with increased ventricular mass/ body mass ratio and interstitial coronary fibrosis. They observed that SARS-CoV-2-infected hamsters showed elevated levels of serum cardiac Troponin-I (cTnI), cholesterol, low-density lipoprotein, and long-chain fatty acid triglycerides. Serum metabolomics analysis of SARS-CoV-2 infected hamsters revealed N-acetylneuraminate, a functional metabolite found to be associated with CVC, as a metabolic marker common to both SARS-CoV-2-infected hamsters and COVID-19 patients. These results show that hamsters are a suitable animal model to study post-COVID sequelae associated with CVC and could be extended to therapeutics interventions. THSTI provides these animal models for challenge studies to various pharmaceutical companies for evaluating vaccine candidates' viz., Mynvax, Zydus Cadila, Biological E, etc.

Immunogenicity Assays

Cellular Assays

Immunogenicity is a complex measure to determine the type of immune response generated in convalescent or vaccinated individuals against a pathogen or vaccines respectively. The understanding of anti-SARS-CoV-2 immune response relies on the type of immune cells that are activated over a period of time. To determine the T cell response elicited by SARS-CoV-2 in COVID-19 convalescent and/or vaccinated individuals, antigen-specific and sensitive assays are required. THSTI-Immunology Core laboratory led by **Dr. Amit Awasthi** has standardized various assays like ELISPOT, T cell proliferation assay, Cytokine detection via ELISA, bead-based assays and intracellular cytokine staining, and Activation Induced Marker (AIM) Assay to study virus-specific T cell immune response in a range of human samples, including active COVID-19 infection, COVID-19 convalescent and vaccinated samples. Using the above-mentioned assays, the group is trying to understand the T cell immune response in COVID-19 convalescent or vaccinated individuals. THSTI-Immunology Core lab

supported double-blinded clinical trials and bridging clinical trials by measuring T cell immune response induced by COVID-19 vaccines.

SARS-CoV-2 Pseudo and Live Virus Neutralization Assays

In order to provide support to academic and corporate organization in their endeavor to understand the dynamics of infection and to counter the pandemic through the development of vaccines, **Dr. Sankar Bhattacharya and Dr. Shailendra Mani** at THSTI established SARS-CoV-2 neutralization assays viz., Plaque-reduction neutralization assay (PRNT) and Microneutralization assay (MNT) for evaluation of neutralizing antibodies (NAb) against SARS-CoV-2 in plasma/serum samples from patients/vaccinated individuals or therapeutic antibody formulations. NAb services were performed for various academia and industries such as All India Institute of Medical Sciences (AIIMS), Tata Institute of Fundamental Research (TIFR) Mumbai, National Institute of Immunology (NII), New Delhi, Institute of Genomics and Integrative Biology (IGIB), Delhi, Indian Institute of Science, Education and Research (IISER), Pune, etc. Dr. Bhattacharya and Dr. Mani were also instrumental in collaborating with industry partners for the development of therapeutic antibodies against SARS-CoV-2 which includes INTAS Pharma in Gujarat, Bharat Serum in Mumbai, Vins Biotech in Hyderabad, Syngene International in Bangalore, etc.

To understand the SARS-CoV2 spike protein plasticity in escape mutants, **Dr. Sweety Samal** generated SARS-CoV-2 spike protein pseudotype viruses in env-defective HIV-1 backbone vector carrying luciferase which can be handled inside BSL2+ lab. The mutant pseudoviruses will help to understand the role of the specific mutation in virus infectivity and will aid in the evaluation of the neutralization potency of vaccine candidates.

The work-related to SARS-CoV-2 done at THSTI has resulted in 15 publications, seven filed patents and four technologies have been transferred to industries.

INFECTION AND IMMUNOLOGY

Tuberculosis

Nearly 25% of the world's population is latently infected with M. tuberculosis (Mtb) bacilli and India accounts for almost one-fourth of the global TB burden. Mtb bacilli are one of the few notorious microbes which reactivate during an immunocompromised state to cause active TB. Additionally, in the last decade, there has been a constant increase in the number of drug-resistant TB (DR-TB) cases posing a bigger challenge to clinicians and researchers alike. Keeping in view of the above facts, TB research is one of the main pivots of research at THSTI.

Understanding persistence mechanism of *M. tuberculosis* and validation of new drug target pathways to combat tuberculosis

Eradication of TB requires new strategies aimed at targeting non-replicating bacteria that characterize the latent disease. It has been hypothesized that these bacilli are metabolically inactive due to orchestrated shutdown of microbial metabolism in response to hypoxic, nitrosative and nutrient stress and that these persistent bacilli are drug-tolerant. The focus of **Dr. Ramandeep Sigh's** laboratory is to identify the pathways that enable the bacteria to adapt to these stress conditions.

Understanding the function and regulation of the complex network of Toxin-antitoxin (TA) systems from *M. tuberculosis*

Toxin-antitoxin (TA) systems are auto-regulatory operon encoding for a labile antitoxin and stable toxin. The toxin-mediated growth arrest is mostly bacteriostatic, reversible, and regulated by the expression levels of cognate antitoxins. Type II is the most well-characterized TA family wherein the virulence-associated protein B and C comprising of VapB antitoxin and VapC toxin is the most abundant. Dr. Singh's team had earlier shown that VapBC3, VapBC4, VapBC11, and VapC22 are essential for *M. tuberculosis* to establish infection in guinea pigs. Experiments are in progress to characterize other active VapC toxins and Type IV family of TA systems from *M. tuberculosis*. During last year, Dr. Singh's team performed a detailed functional and biochemical characterization of the VapBC21 (Rv2757c-Rv2758c) TA system from *M. tuberculosis*. Dr. Singh's team demonstrated that the inducible expression of VapC21 inhibited *M. smegmatis* growth in a bacteriostatic manner. They also performed RNA-seq experiments to compare the transcriptional profiles of parental and

VapC21 overexpression strains. It was observed that the overexpression of VapC21 in *M. tuberculosis* altered the expression of 445 genes. His team also determined the effect of VapC21 overexpression on the emergence of drug-tolerant persisters. They observed that the overexpression of VapC21 conferred an increase in the emergence of ethambutol tolerant persisters by 16.0-folds. Co-expression studies were also performed to determine whether VapC21 can interact with non-cognate antitoxins. They observed that co-expression of non-cognate antitoxin VapB32 was able to restore the growth defect associated with VapC21 toxins. To determine the contribution of VapC21 in the survival of *M. tuberculosis* under different stress conditions, a Δ*vapC21* mutant strain of *M. tuberculosis* Erdman was constructed using temperature-sensitive mycobacteriophages. It was observed that the deletion of *vapC21* did not impair the ability of *M. tuberculosis* to survive upon exposure to either oxidative, nitrosative, nutrient starvation, acidic, lysozyme, or detergent stress. Using the mice model, the team led by Dr. Singh showed that parental, mutant, and complemented strains displayed comparable growth during both acute (4 weeks) and chronic (8 weeks) stages of infection. These findings suggest that VapC21 individually does not contribute to the survival of *M. tuberculosis* in lung tissues.

Evaluation of various vaccine candidates in mice and guinea pig model

Dr. Singh's group evaluated the ability of attenuated mutant strains to impart protection against challenge with *M. tuberculosis* in both mice and guinea pigs. Significantly less disease and tissue damage were seen in animals infected with the mutant strain in comparison to the parental strain via the aerosol route. In vaccine efficacy experiments, the animals were immunized with various strains via the intradermal route. At 10 weeks post-immunization, animals were challenged with *M. tuberculosis*. It was observed that these mutant strains were comparable to BCG in their ability to impart protection in mice models of infection. These strains were able to impart protection by ~ 5.0- folds and ~10.0-folds at 4 weeks and 10 weeks post-immunization in mice model of infection, respectively. However, in guinea pigs, the mutant strains were able to impart better protection than BCG. Immunization with attenuated strains resulted in 5.0-10.0-folds better protection in lungs of 4 weeks and 8-weeks immunized animals. However, in spleens immunization with either BCG or attenuated strains resulted in complete clearance of *M. tuberculosis*. Experiments are in progress to understand the correlates of protection.

Identification of small-molecule inhibitors against *Mycobacterium tuberculosis*

To identify small molecule inhibitors, Dr. Singh's team performed whole cell-based assays and identified small molecules that can show synergy with first-line and second-line TB drugs. Among these, thiophene-based compounds showed 2.0-folds better activity than isoniazid. This series of compounds show additive effects with first-line TB drugs and synergistic effects with Bedaquiline. In another study, phenotypic screening of a collection of pharmacologically active compounds (~ 500 compounds) resulted in the identification of two small molecules which are active against drug-resistant strains and also show synergy/additive effect with first line-TB drugs. These compounds were also able to inhibit the growth of intracellular *M. tuberculosis* in the macrophage model of infection.

In addition to phenotypic-based screening, Dr. Singh's group also performed target-based screening to identify small molecule inhibitors with anti-tubercular activity. Dr. Singh's group purified various enzymes involved in the biosynthesis of different amino acids in the laboratory. Among the purified enzymes, MetA (involved in L-methionine biosynthesis) and ArgA (involved in L-arginine biosynthesis) enzymes from *M. tuberculosis* have been extensively characterized. Using standardized assay conditions, Dr. Singh's team identified small molecule inhibitors that target these enzymes. These small molecules are bactericidal in their mode of killing and the group has also shown that supplementation with either L-methionine or L-arginine restores the killing of *M. tuberculosis* in the presence of these drugs. Transcriptomic studies revealed that small molecules targeting either MetA or ArgA enzymes alter the redox status of the bacteria.

Dr. Singh's team carried out target-based screening to identify small molecule inhibitors against ClpB, an enzyme that protects *M. tuberculosis* from various stress encountered in the host environment. The identified small molecules inhibited the enzymatic activity competitively and were able to inhibit the growth of intracellular bacteria. The team showed that the identified small molecules inhibited ATP inducible conformational changes, suggesting that nucleotide-induced shape changes are crucial for ClpB activity.

Dr. Shailendra Asthana identified 2,5-Bis (2-chloro-4-guanidinophenyl) furan as an ArgA inhibitor using high throughput studies. The diaryl furan derivative displayed bactericidal killing of nutrient-starved bacteria. Computational and dynamic analysis revealed that the identified hit and its active derivatives share similar binding site residues with L-arginine, however, with slight variations in their interaction pattern.

Mycobacterium proteasome is considered to be a significant target for drug designing as it is responsible for resisting the effect of NO (nitric oxide) immune system defense mechanism against the bacterial cells. Dr. Asthana's team reanalyzed two compounds viz., Z1020863610, Z106766984 from the Enamine database. They carried out molecular dynamic simulation studies and in vitro validations (in vitro susceptibility assay, enzyme inhibition assay, and MTT assay). In silico outcomes were consistent with *in vitro* results. These two compounds are, therefore, potential leads for future studies.

In a study, Dr. Asthana's team identified small molecule inhibitors against Mycobacterium tuberculosis proteasome using high throughput screening. They identified two potent small molecules MMV019838 and MMV687146 which actively interacted with the catalytic domain/active domain of Mycobacterium tuberculosis proteasome and inhibit the *Mycobacterium tuberculosis* growth *in vitro*.

Way ahead

Dr. Singh plans to investigate the role of remaining active toxins in the physiology and pathogenesis of *M. tuberculosis*. They aim to understand the role of various metabolic pathways such as inorganic polyphosphate metabolism, itaconate dissimilation pathway, and GntR family of transcription regulators in the physiology of *Mycobacterium tuberculosis*. Also, his team would be performing high through put screening against the targets identified from the CRISPRi screen. Finally, computational tools and a medicinal chemistry approach to develop SAR for active molecules will be used. The most active compounds identified from SAR would be evaluated in animal models.

Dr. Asthana plans to carry out further in vitro and in vivo studies of identified small molecule inhibitors to understand the molecular mechanism(s) and discover a novel and potent therapeutic agent against Tuberculosis.

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

Dr. Nisheeth Agarwal and his group have focussed to identify and characterize Mtb genes involved in the regulation of essential metabolic pathways, which can be explored as new druggable targets. In the last year, his group has researched the following aspects: 1) characterization of the proteomic landscape of Mtb

persisters, 2) investigating the therapeutic potential of *clpX* and *clpC1* in Mtb by assessing their role in bacterial virulence using a mouse model of infection, 3) understanding the role of essential membrane proteases, HtrA, MycP3 and LepB in mycobacterial physiology, and 4) improvisation of CRISPRi approach for silencing of multiple genes in mycobacteria.

Characterization of the proteomic landscape of Mtb persisters

In the year 2019, Dr. Agarwal's group reported the emergence of phenotypic drug tolerance in Mtb upon CRISPRi-mediated silencing of essential genes, *gyrA/B*. Induction of various SOS-response genes including LexA-RecA regulons was attributed to this phenotype. Subsequently, they planned a study to characterize the proteomic landscape of Mtb persisters, wherein an *in vitro* model of persistence was set up. The quantitative proteomic analysis by iTRAQ revealed that intermediary metabolism and respiration, cell wall and cell processes, lipid metabolism, information pathways, and virulence, detoxification & adaptation functional categories are primarily modulated in drug-induced persisters. Further, they demonstrated that various surface-localized proteins are crucial for mycobacterial survival during a persistent growth state. His study has identified various proteins differentially regulated in drug-induced persister subpopulation of Mtb that might be critical in mitigating the antimicrobial effect of drugs and can be further explored to develop novel anti-TB agents.

Investigating the therapeutic potential of *clpX* and *clpC1* in Mtb by assessing their role in bacterial virulence using a mouse model of infection

To understand how the protein homeostasis, also known as proteostasis, is maintained in Mtb, Dr. Agarwal's group has characterized the mechanism of protein homeostasis by Clp proteolytic machinery assisted by unfoldases, ClpX, and ClpC1 in Mtb. They reported that the degradation of putative Clp substrates is sequence-nonspecific and relies on the conformation of target proteins. It was shown that the disordered ends are critical for interaction and subsequent degradation of the substrate proteins by Clp machinery. To further assess the requirement of ClpX and ClpC1 in mycobacterial virulence, they set up the mouse infection experiments with *clpX(-)* and *clpC1(-)* knockdown strains of Mtb H37Rv. A significant decline by ~1000-folds in the intracellular bacterial load of both the mutant strains compared to control was observed during the course of infection, which suggests that these proteins play a critical role in determining mycobacterial virulence and could be explored as drug targets.

Understanding the role of essential membrane proteases, HtrA, MycP3, and LepB in mycobacterial physiology

Under this multi-institutional project, Dr. Agarwal and the group aimed at characterizing the role of putative membrane proteases, namely HtrA, MycP3, and LepB that are predicted essential for bacterial growth. With the help of the CRISPRi approach, the conditional knockdown strain of Mtb H37Ra depleted with each of the three genes was created. Further studies are ongoing to determine the proteome profile of these strains and their role in biofilm formation.

Improvisation of CRISPRi approach for silencing of multiple genes in mycobacteria

With the realization that disruption of multiple ORFs remains challenging in mycobacteria, Dr. Agarwal conceptualized a study wherein he performed construction of the guide sequence expressing plasmid, pGrna to facilitate cloning and expression of multiple guide sequence cassettes targeting a versatile set of Mtb genes from a single plasmid. Using the modified plasmid, pGrna2, it was shown that the expression of as many as 12 sigma factor genes can be efficiently repressed in Mtb expressing dCas9. Interestingly, it was observed that cumulative knockdown of these non-essential transcriptional regulators is lethal for Mtb growth. Importantly, the Δ12sig strain exhibits sensitivity to transcriptional inhibitor rifampicin and oxidative stress diamide, further implying the involvement of these genes in controlling bacterial stress response. To the best of his knowledge, this is the first study wherein 12 genes have been silenced together in a single recombinant strain of Mtb.

Building up a repository of CRISPRi mutant strains of mycobacteria

Apart from the above activities, Dr. Nisheeth has also initiated a program to build a repository of CRISPRi constructs and select mutants for users within and outside THSTI. In the last year, they have added 50 more CRISPRi plasmid constructs resulting in ~200 constructs distinctly targeting genes in Mtb, *M. smegmatis*, and *M. marinum*. His is the only laboratory in India having such a vast collection of CRISPRi constructs against a variety of genes from different species of mycobacteria. Several of these plasmid constructs, as well as bacterial strains, have also been shared with TB researchers at various institutes such as IISC, NII, NIPER Mohali, CSIR IMTECH, NCBS, South Asian University, Bose Institute, etc.

Way ahead

The focus of Dr. Agarwal's laboratory has been to identify new drug targets- both in the host as well as in the pathogen. His group is working towards identifying novel host pathways that are differentially regulated upon infection, and control mycobacterial growth in the host cells. Simultaneously, they are also identifying potential target genes in Mtb itself to understand the

cause(s) of the emergence of drug resistance in bacteria and to come up with potential drug targets and new scaffolds for future evaluation against drug-resistant as well as susceptible populations. In the coming years, they plan to screen small molecule inhibitors against some of these genes that play a critical role in Mtb pathogenesis. In addition, they are working towards building up a repository of CRISPRi plasmids and knockdown mutants that are freely available to TB researchers across the globe.

Mechanistic understanding of antibiotic and disease persistence in tuberculosis

The *Mycobacterium tuberculosis*'s (Mtb) ability to survive inside the host for decades (disease persistence) and evolve new strategies that induce drug tolerance (antibiotic persistence) that eventually leads to the evolution of multi-drug (MDR) and extremely-drug (XDR) resistance strains are the two main hurdles in finding a cure to TB. **Dr. Amit Kumar Pandey** hypothesizes that the compounds administered as an adjunct along with the existing standard therapeutic regimen would enhance the pathogen clearance rate thereby reducing the duration of treatment. This, they believe would eventually lead to a decrease in the frequency of generation of MDR and XDR cases in tuberculosis. Dr. Pandey's lab has been working on the hypothesis that the differentially regulated critical metabolic pathways, triggered by the stress associated with limited intracellular nutrient availability and host-mediated insult, contribute significantly towards the generation of both disease and antibiotic persistence observed during Mtb infection. Their group had earlier demonstrated that Mtb could metabolize and survive on media containing cholesterol as a sole carbon source and that cholesterol metabolism is very critical for Mtb persistence. Utilizing genetic and high-dimensional informatics approaches, they have successfully identified specific pathways critical for the generation of persisters in mycobacteria.

Nutrient utilization pathways

Using cholesterol-rich media, Dr. Pandey's lab successfully identified one of the Mtb pathways critical for the generation and enrichment of persisters during mycobacterial infections. Dr. Pandey's lab is also exploring the role of nutrient storage mechanisms and pathways in long-term disease and antibiotic persistence.

Pathways regulating metal ion homeostasis

Metal ions are one of the essential micronutrients that Mtb scavenges from the host for its long-term survival inside a very hostile intracellular niche. Dr. Pandey's lab for the first time demonstrated that the Mtb transcription repressor protein $SufR_{TB}$ regulates the ISC operon and has a role in controlling the intracellular iron homeostasis in Mtb. Disruption of the iron homeostasis in $\Delta sufR_{TB}$ decreased the fitness of the mutant strain to grow inside mouse bone marrow-derived macrophages. Further, they demonstrated that the $SufR_{TB}$ protein-mediated regulation of iron homeostasis in Mtb is required for Mtb to persist inside the host. Currently, the focus of the lab is to identify additional Mtb pathways that are critical for maintaining the iron and manganese homeostasis inside Mtb. Preliminary *ex-vivo* data from his lab has demonstrated that these proteins have a role in both antibiotic and disease persistence during Mtb infection.

Designing novel intervention strategies against "persisters"

Based on the information derived out of the above studies, Dr. Pandey's lab has developed recombinant Mtb and *M. bovis* BCG strains that could potentially be used as an *in vitro* model of persistence in mycobacteria (Patent under process). Currently, the lab is developing protocols for high-throughput screening of compounds that could potentially be inhibiting the generation of persisters. Both target and phenotype-based screening approaches will be used to identify potential hits. Their goal is to validate these molecules in both *in vitro* and *in vivo* models of tuberculosis infection. Using X-ray crystallography and homologous modeling Dr. Pandey's lab has successfully solved the crystal structure of the vapBC12 ribonuclease complex. Dr. Pandey's lab has preliminary data that implicates the role of a transcription factor in antibiotic and disease persistence in tuberculosis. Further, his lab is trying to understand the mechanism and pathways that are regulated by this transcription factor.

Identifying host correlates of persistence during Mtb infection

Dr. Pandey's lab has demonstrated that activation of RNase toxin (VapC12) results in cholesterol-specific growth modulation that increases the frequency of generation of the persisters in a heterogeneous *M. tuberculosis* population. Based on the above findings, the lab is working on the hypothesis that differential protein expression profiling between the mutant and the wild-type strains would enable in identifying critical proteins/pathways involved in the downregulation of host immune response. Dr. Pandey's lab also demonstrated that the vapC12 mutant fails to persist inside the mice in a high-dose infection model. This model was exploited to identify the differential expressed host genes that were critical for the long-term survival of the Mtb inside the host. Currently, his lab is trying to identify protein critical for

the host-pathogen interaction essential for disease and antibiotic persistence in tuberculosis. The long-term goal is to design intervention strategies directed at the host pathways (HDT) that would prevent long-term disease persistence during Mtb infection.

TB vaccines and exploring Mtb-encoded virulent proteins as novel therapeutic targets

Despite the BCG vaccination of most neonates, Tuberculosis (TB) continues to be a challenge. BCG fails to protect adolescents & adults and act as a booster. Despite DOTS, >40% of TB patients don't adhere to treatment regimens. Despite TB-NAAT and GeneXpert MTB/RIF in use, limited access to timely diagnosis continues. With raising extra-pulmonary TB, MDR, and XDR-TB burden, existing diagnostic tools, anti-TB therapy, and BCG's utility are insufficient and inefficient. Consequently, **Dr. Krishnamohan Atmakuri**'s group is involved in identifying novel vaccine candidates, potential therapeutic targets, and new diagnostic markers.

Mycobacterial extracellular vesicles as novel candidate vaccine

Previously, Dr. Atmakuri's group and others had demonstrated the ability of mycobacteria in releasing extracellular vesicles (EVs) from their outer surfaces. This group had earlier characterized EVs from (i) an avirulent Mycobacterium smegmatis and (ii) a pathogenic and (iii) an attenuated M. tuberculosis strains and then compared their contents. They reported that (i) mycobacterial EVs (mEVs) carry a subset of proteins, metabolites, DNA, and RNA either within them or on their surface; (ii) EVs proteomes from pathogenic, attenuated, and avirulent mycobacteria significantly overlap; and (iii) mEVs trigger pro-inflammatory cytokines in vitro in THP-1 macrophages. Consequently, Dr. Atmakuri's group compared *in vivo* whether mEVs derived from either pathogenic, attenuated, or avirulent when used as subunit vaccine booster to BCG in Guinea pigs (GPs) model for TB, enhance protection against a virulent mycobacterium challenge. They reasoned that if EVs especially from attenuated or avirulent mycobacteria extend BCG-mediated protection, culturing such mycobacteria for EVs enrichment would be safer, cheaper, and convenient. They first subcutaneously administered the GPs under study with the BCG vaccine. Then, they administered them with three boosters' doses of EVs derived from one of the three mycobacteria. Four- and eight weeks post-challenge with pathogenic mycobacterium, they euthanized the GPs and enumerated the pathogen load in their lungs. Surprisingly, in contrast to their expectations, none of the mEVs enhanced protection imparted by BCG. Most importantly, all three mEVs abrogated BCG-

mediated protection. Histopathology analyses also indicated pathogen burden similar to those in unvaccinated GPs. Thus, their work indicates that mEVs carry high pathogenic potency and poor protective efficacy. They speculate that their data indirectly shows that mEVs of environmental mycobacteria perhaps are the major contributing factor to BCG's failure as a vaccine.

A nucleoid-associating factor as a potential therapeutic target

Typically, essential proteins for bacterial growth are primary therapeutic targets. However, this strategy invariably leads to the emergence of MDR and XDR-TB. Therefore, here, Dr. Atmakuri's group explores targeting an important in vivo essential, nucleoid-associating protein HupB, whose protein levels showed significantly altering in response to different host-mediated stresses. They also demonstrated earlier that the KO for hupB grows very slow in rich media and is highly sensitive to detergent exposure indicating a potential alteration in the composition of its cell surface proteins and lipids. Their detailed TLC analyses of polar and non-polar lipids of KO indicated several lipids either enhanced or reduced in quantity when compared to WT. Upon complementation of the KO, most of these levels were restored to WT levels. They next tested if the KO would also be sensitive to various stresses that alveolar macrophages impose upon Mtb. When tested in vitro, interestingly, the KO was found to be highly sensitive to very low pH, oxidative, and nitrosative stresses. While the complemented and the WT strains continue to grow for 14 days at all pH tested, all KO bacteria perished within seven days. Similarly, by one day, all KO exposed to oxidative and nitrosative stresses died, while the WT and complemented continued to exhibit growth. In THP-1 macrophages, by seven days, the KO CFUs reduced by 75% while the WT and complemented strains continued to grow. Since HupB levels increase in the presence of Isoniazid (INH), Dr. Atmakuri's group (in collaboration with Dr. Ramandeep Singh) also evaluated if SD1, a small molecule that efficiently targets HupB works synergistically together with INH to reduce Mtb growth in vitro. Their checker-board analyses showed that as a combination, their MICs reduced by 50-75%. This synergy also extended to their in vitro macrophage infection model where the combination exhibited superior killing of the infecting Mtb over INH or SD1 alone. In summary, the data shows for the first time that an in vivo essential virulent protein of Mtb works in synergy with the existing first-line drug INH to efficiently kill Mtb in vitro. In vivo testing is currently underway.

Development of a small molecule-based new drug leads for TB infection

Dr. Dinesh Mahajan's group is working towards the identification and development of small molecule-based drug discovery in the field of Tuberculosis. They have used two approaches for new drug discovery. One is based on repositioning of the approved drugs or drug leads evaluated in phase 2/3 trials. The other approach involves the identification and development of New Chemical Entities (NCEs) focused either on a specific molecular pathway or a drug target. These efforts resulted in the identification of many new chemical hits which showed promise in inhibiting the growth and replication of Mtb in vitro. Few hits were further optimized leading to the identification of initial drug-like leads (DR514 and DR516; MIC= 0.7 µM) as well as novel series of small molecules by exploiting phenotypic screening and optimizing SAR. These molecules are under evaluation for further pharmacological characterization and animal efficacy studies. Dr. Mahajan's team is designing the second generation of analogs and optimizing PK-ADME properties along with in vitro potency. The team is also planning to pursue optimization (SAR and DMPK) of other new chemical hits identified in a tuberculosis research group for further development leading to the identification of new leads. This will ensure a pool of drug leads to evaluate for in vivo pharmacological studies including efficacy studies in animals i.e., standalone treatment as well as a combination with existing drugs.

RNA VIRUSES INFECTING HUMANS - HOST PATHOGEN INTERACTIONS, IMMUNE RESPONSE, AND VACCINE DEVELOPMENT

Dengue

Zinc homeostasis and oxidative stress in RNA virus infections

Zinc is one of the most relevant elements associated with the regulation of the redox environment and zinc deficiency leads to excessive formation of reactive oxygen species (ROS). Different RNA viruses with positive, negative, or double-stranded RNA genomes may show varied susceptibilities to ROS levels depending

on the host factors and pathways hijacked by these viruses for replication. **Dr. Guruprasad R Medigeshi**'s group focuses on the role of zinc-dependent pathways in the life-cycle of RNA viruses with a specific focus on the antioxidant functions of zinc.

Dr. Medigeshi and his team had earlier shown that zinc chelation by N, N, N, N'-tetrakis (2-pyridinylmethyl)-1,2-ethanediamine (TPEN) led to specific inhibition of dengue virus (DENV) without affecting other RNA viruses such as respiratory syncytial virus or rotavirus. This year, they tested whether the antioxidant function of zinc is perturbed due to zinc chelation and if oxidative stress has a role in the inhibition of DENV replication in cells treated with TPEN. They found that zinc-chelation leads to an increase in ROS levels in Caco-2 cells. This effect was reversed after the addition of zinc, but not other divalent cations, thus indicating the antioxidant properties of zinc. Salubrinal, an ER stress inhibitor also blocked ROS induction and reversed virus inhibition suggesting that the ER stress may contribute to ROS production under zinc chelation conditions.

Oxidative stress leads to a reduction in DENV infection

The team determined the direct effect of inducing oxidative stress by the addition of ROS on DENV infection in Caco-2 cells. H_2O_2 treatment was used to create an oxidative environment *in vitro*. This treatment led to a significant reduction in DENV titers and DENV genome levels in a dose-dependent manner. The effect of H_2O_2 was reversed by co-incubating with ebselen, an antioxidant, further suggesting that the effect is due to the addition of ROS. The effect of peroxynitrite, an oxidizing agent on DENV infection was also tested similarly and nitrosative stress was also shown to reduce DENV infection in a dose-dependent manner.

Oxidative stress specifically inhibits positive-strand RNA virus infections

ROS triggers a number of signaling cascades and affects metabolic functions that have either a positive or a negative impact on RNA viruses. Therefore, Dr. Medigeshi and his team investigated the specificity of the effect of ROS treatment on RNA viruses from different families. They included Japanese encephalitis virus (JEV), Chikungunya virus (CHIKV), SARS-CoV-2, and DENV as representatives of positive-strand viruses. Respiratory syncytial virus (RSV), a negative-strand virus, and rotavirus (RV), a double-stranded RNA virus was also used. Cao-2 cells were infected with DENV and treated with H_2O_2. They found that positive-strand RNA levels started declining from 4 h to 16 h in a time-dependent manner. There was a moderate increase in negative-strand RNA levels initially but no further production of fresh negative-strand intermediates was observed at 16 h post-infection. Next,

A549 cells were infected with DENV, JEV, CHIKV, and RSV, Calu-3 cells were infected with SARS-CoV-2 and Caco-2 cells were infected with RV. After viral adsorption, all cells were treated with H_2O_2. A ten-fold reduction in titers of positive-strand RNA viruses in H_2O_2 treatment was observed whereas RSV and RV titers were unaffected. These results suggest that H_2O_2 treatment has an inhibitory effect only on positive-strand RNA viruses suggesting that this class of viruses are more susceptible to oxidative stress.

DENV infection leads to a reduction in ROS formation in blood cell subsets

Oxidative stress may influence viral infections in multiple ways. The observations *in vitro* suggest that DENV replication is sensitive to ROS induction. Dr. Medigeshi's team measured intracellular ROS levels in blood cell subsets from whole blood samples collected from patients with dengue or other febrile illnesses (OFI). A significant reduction in ROS levels was observed in dengue samples relative to samples from OFI or healthy controls. As the sample numbers were low in each of the three disease conditions, any association of ROS levels with disease severity could not be detected, however, a negative trend between disease severity and ROS levels was observed with most severe cases showing the lowest levels of ROS. They measured DENV RNA levels in total RNA isolated from whole blood by quantitative RT-PCR. ROS levels were lower in samples with high viremia and viral RNA levels negatively correlated with ROS levels. The team also investigated which of the peripheral blood mononuclear cell (PBMC) subsets from dengue patients show altered ROS induction in DENV patients. Relative to healthy controls, almost all the leucocyte subsets showed increased ROS production in OFI cases but samples from DENV patients did not show any elevated levels of ROS and, contrary to OFI samples, showed downregulation of ROS in most of the cellular subsets. The results suggest that redox balance during DENV infection may be altered and modulation of pathways dealing with oxidative stress may have an important role in the clinical outcome of dengue disease.

Way ahead

Dr. Medigeshi and his team have identified the critical role of zinc homeostasis in RNA virus infections and showed that zinc levels may specifically impact positive-strand RNA viruses either by directly modulating virus replication or by regulating host pathways that are necessary for productive virus infection. Zinc homeostasis is regulated by zinc transporters; therefore, his team is investigating to further understand the role of specific zinc transporters involved in the life-cycle of these viruses.

Translational Research Consortium (TRC) on Dengue virus research

Under National Biopharma Mission-Translational Research Consortium (TRC) on Dengue virus research, Dr. Medigeshi's team is generating a biorepository of low passage clinical isolates of dengue virus as a national resource for academic research and vaccine development. They have generated 36 dengue virus low passage isolates and are now processing the samples for whole genome sequencing and molecular characterization. They have also established and validated methods for dengue virus serotyping, dengue viremia estimation, and dengue ELISA in the bioassay lab for dengue vaccine development, and a high-throughput virus neutralization assay is being validated. They have identified the role of zinc homeostasis in dengue virus replication in cell culture models and clinical samples.

Dr. Sweety Samal and the team are developing an ADE AG129 mouse disease model for Dengue virus infection. This model will be used for screening potent and broad monoclonal antibodies against the Dengue virus and also in understanding the disease biology.

Way ahead

With the translational research program on Dengue, Dr. Medigeshi's team is a part of a consortium involved in sero-epidemiology of dengue across India to generate knowledge for vaccine trials. They are in the process of completing the establishment of dengue virus neutralization assays for NABL accreditation. In addition, they would generate information on the circulating dengue virus strains at the molecular level which will also augment the efforts to develop effective dengue vaccines.

Effect of megakaryopoiesis on DENV replication and vice versa

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** lab 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 differentiates 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 the pattern of gene expression. In their study, Dr. Bhattacharya's team found that pharmacologically induced differentiation of K562 into megakaryocytes promoted the replication of DENV in these cells. On the other hand, 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. The results indicated 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 has been performed and is currently under analysis.

Way ahead

Dr. Bhattacharya plans to carry out a detailed analysis of comparative transcriptome data to score for reversal of gene expression associated with megakaryopoiesis. In the coming years, his team will analyze the 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

In collaboration with Dr. Shailendra Asthana, Dr. Bhattacharya's team has identified novel oxindole compounds as 'hit' molecules active against DENV replication through inhibition of viral RNA-dependent RNA polymerase. These molecules showed differential efficacy against all serotypes of DENV.

Way ahead

Using docking studies, these molecules are predicted to interact with the RNA-template entrance site on the RdRp protein. Therefore, Dr. Bhattacharya plans 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 Isothermal Calorimetry (SPR). We will study biophysical interactions using both Isothermal calorimetry and Surface-plasmon resonance.

Identification of novel inhibitors of CXCR3, a surface protein involved in cytokine signaling

In collaboration with Dr. P Guchhait, RCB, and Dr. Shailendra Asthana, THSTI, Dr. Bhattacharya's team is exploring the rational design of CXCR3 inhibitors that can reverse CXCL4 mediated upregulation of DENV replication in infected monocytes.

Design and Development of immunogens for Dengue

The surface-exposed E protein on infectious mature dengue virus (DENV) plays an important role in virus entry and is the major target of neutralizing antibodies. The E (envelope) protein dimer is, therefore, suitable for immunogen design. **Dr. Supratik Das** used a newly designed construct DENV4 TVP/360 prM-E4v.2 to produce soluble, cleaved, well-ordered, native-like DENV4 E (envelope) protein dimers in mammalian expression system wherein the furin cleavage site has been optimized by replacing RRREKR sequence proximal to prM junction and containing the furin cleavage site (P1 to P4) and P5-P6 with the R6 (RRRRRR) sequence to maximize prM cleavage. A CD5 leader sequence was put at the N-terminus to allow for efficient transport through the secretory pathway and an Avi–tag followed by His-tag was put at the end of the coding sequence, attached to the C-terminus of E protein [truncated to amino acid 395 to remove the TM (transmembrane) and CT (cytoplasmic tail) domains] by a Gly-Ser (G4S) linker. An A259C mutation was introduced in the E polypeptide sequence to promote dimerization. DENV RVPs prepared from DENV4 strain TVP/360 C-prM-E are potently neutralized by homotypic WHO serum and therefore prM-E amino acid sequence from the DENV4 strain TVP/360 was chosen as the base sequence for the study. The coding region for the entire design was codon-optimized for mammalian cell expression and cloned into pcDNA3.1 vector. Using this construct, Dr. Das and his team showed that E is efficiently cleaved and separated from prM polypeptide. He has used a novel two-step purification strategy to purify the DENV4 E protein dimers wherein in Ni-NTA chromatography and immuno-affinity chromatography has been carried out using A11 EDE2 (E-dimer epitope 2 directed) dengue antibody coupled to cyanogen bromide activated sepharose. Using this purification strategy, he purified the protein to >99% homogeneity. This protein is entirely present as a dimer. Digestion with both Endo H and PNGase F led to a single faster-migrating band that migrates faster than the undigested doublet band of DENV4 E(A259C) dimer suggesting that the protein is deglycosylated and therefore the purified protein contains glycosylation as is on the viral membrane. In ELISA, the purified protein bound to dengue antibodies directed against the major epitopes of E namely domain III (antibody 513), EDE1

(antibody C8), and EDE2 (antibody A11). Negative stain EM (electron microscopy) and 2D class averages showed that the dimers were well-ordered. The crystal structure of the mature dengue virus E dimer fits in well with the low-resolution 3D model generated from the 2D classes. Taken together his results demonstrated that using the construct DENV4 TVP/360 prM-E4v.2 he was able to produce soluble, cleaved, well-ordered, native-like DENV4 E protein dimers from mammalian expression system with high purity, and antigenic and structural integrity. He has used a similar strategy to purify DENV3 E protein dimers using the construct DENV3 CH53489 prM-E4v.2 and currently characterization is ongoing.

Dr. Tripti Shrivastava has purified disulfide-bond stabilized dengue envelope protein dimers to apparent homogeneity. Stabilized Dengue 1 E disulfide-bond, previously not known, has also been produced successfully in stabilized conformation by Dr. Shrivastava. These dimers have been characterized for their binding specificity towards dengue neutralizing antibodies targeting the three major epitopes on E protein by ELISA and BLI studies. BLI studies showed that E dimer binds to the neutralizing antibodies at nanomolar affinity. Further characterizations and standardization to increase the purification yield of these dimers are ongoing.

Way ahead

Dr. Das plans to further stabilize the DENV3 and DENV4 E protein dimers using additional disulfide bonds and use these proteins to immunize BALB/c mice to investigate the types of antibodies that are elicited. In addition, his group plans to investigate and identify protein-protein interaction partners of dengue non-capsid structural proteins using biochemical tools e.g. GST pull-down of GST-tagged prM followed by silver staining of interacting protein and their identification by LC/MS-MS.

Dr. Shrivastava is working towards the establishment of Dengue reagent resource at THSTI as 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

Hepatitis E

Understanding the biology and development of intervention strategies against the Hepatitis E virus

Dr. Milan Surjit and his team have been investigating the role of host-pathogen interactions in HEV pathogenesis and exploring the mechanism of viral translation and replication. Apart from that, his group has been evaluating the antiviral activity of Zinc against HEV, intending to determine the possible therapeutic benefit of Zinc in HEV patients. His laboratory is also actively engaged in the development of a recombinant vaccine against HEV. In the coming year, his group aims at initiating preclinical studies on the HEV vaccine candidate, which should provide crucial information regarding the safety and protective efficacy of the vaccine formulation in a non-human primate model of HEV infection.

While working on the host-pathogen interactions in HEV, Dr. Milan's team has identified the RNA-protein-protein interactions (RPPI) assembled at the 5'- and 3'- untranslated regions (UTR) of HEV genomic RNA and constructed the RPPI network. Further studies are ongoing to elucidate the functional significance of the HEV 5'- and 3'- UTR RPPI network. In another important study, they have identified the proviral properties of HEV protease. Their work demonstrates that HEV protease inhibits the activity of host eIF2AK4 (eukaryotic initiation factor 2 alpha kinase 4) to allow the viral proteins to be translated into infected cells through a cap-dependent translation process. Note that like host cell mRNA, HEV genomic RNA is capped at the 5'-end and poly-A tailed at the 3'-end. eIF2AK4 is a key stress-induced kinase of the host that blocks host cellular translation under conditions of nutritional stress. It acts by phosphorylating and inhibiting the activity of eIF2α, which is essential for the initiation of translation in eukaryotes. Their study demonstrates that HEV protease binds to and inhibits the dimerization of eIF2AK4, leading to inhibition of its activity. Therefore, HEV protease plays a significant role in promoting viral replication and progeny virus production in infected cells.

In an earlier study, Dr. Milan's group reported the antiviral activity of Zinc salts against HEV. However, exogenously supplemented Zinc salts are known to be of limited use owing to tight control of Zinc homeostasis inside the cells as well as toxicity associated with Zinc salts. In the past year, they have evaluated the possible therapeutic benefit of Zinc Oxide

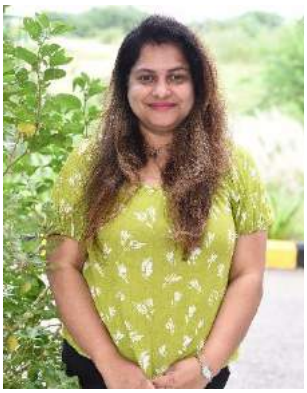
nanoparticles (ZnO-NP) and tetrapods (ZnO-TP). Their study demonstrates that ZnO-NP and ZnO-TP are significantly less toxic to the cells while retaining the antiviral activity against HEV. In the future, they aim at exploring the possible therapeutic usage of ZnO-NP and ZnO-TP against HEV.

Another major area of their research focuses on developing a recombinant vaccine against HEV. In the past year, his laboratory has established a multi-institutional translational research consortium (TRC) and secured funding from the BIRAC (Department of Biotechnology, Govt of India) to evaluate the efficacy of a recombinant virus-like particle (VLP) based vaccine against HEV. In collaboration with partner laboratories at AIIMS, New Delhi, and PGIMER, Chandigarh (clinical partners), his group plans to complete the preclinical studies of the HEV vaccine candidate.

Influenza

The emergence and re-emergence of Influenza have underlined the necessity for the development of therapeutic and preventive strategies to combat viral infection. In recent times infectious disease has appeared and reappeared in a more virulent form or a new epidemiological setting. Influenza virus, which caused the pandemics of 1918, 1957, 1968, and 2009 exhibits high antigenic diversity with antigenic drift, shift, and resortment.

Development of next-generation Influenza H1N1 matrix ectodomain (M2e) based soluble immunogen in a mammalian expression system



Dr. Sweety Samal's team has successfully developed next-generation Influenza H1N1 matrix ectodomain (M2e) based soluble immunogen in a mammalian expression system. The group has developed BALB/c mouse disease and challenge model for studying Influenza virus pathogenesis and for testing Influenza vaccine candidate's immunogenicity and protecting efficacy. To evaluate the protective efficacy as a vaccine candidate, they immunized the BALB/c mice with M2e-5X immunogens consisting of five peptides of conserved M2e to produce the protein (30 µg/mouse) intra muscularly with a prime-boost approach. After the second boost, the mice were challenged intranasally with the Influenza PR8 mouse-adapted virus at a dose of LD_{100}. The preliminary results suggested elicitation of potent humoral and cellular responses and protection against the Influenza PR8 challenge. Dr. Samal's team is repeating the study to include more challenging viruses; Influenza A H3N2- X-31virus and

Influenza H1N1 A/CA/04/2009 pandemic H1N1 to check the efficacy of the M2e-5x immunogen against heterosubtypic Influenza A/ H1N1 challenge viruses.

Understanding the viral factors that influence the severity of influenza disease

Dr. Samal and her team evaluated the virulence of clinically similar strains of pandemic 2009 influenza viruses (A/California; A/South Carolina; A/Mexico) in mice by intra nasal infection. They found that the A/Mexico/4108/2009 virus showed efficient replication and high lethality in mice, which was the parent pandemic strain of the 2009 Influenza pandemic. Sequence analysis displayed prominent differences between polymerase subunits (PB2 and PA) and neuraminidase (NA) of viral genomes that might correlate with their different phenotypic behavior. The group is further analyzing the underlying mechanism between virus and host factors.

Production of inactivated Influenza viruses to study the effect on antigenicity and assay development

Under Horizon 2020 project INDIGO, Dr. Samal and the team are collaborating with Christian Medical College, Vellore, and St. John's Research Institute (SJRI), to study immune responses of Influenza vaccines. As the inactivated viruses are helpful for B and T cell activation and ELISA, Dr. Samal's team inactivated the influenza A viruses (seasonal and pandemic A (H1N1) virus isolates). The group is further analyzing the *in vivo* effect of these inactivated viruses.

Validation of structurally occluded conserved epitopes as novel universal Influenza vaccine

Dr. Tripti Shrivastava and her team designed an Influenza HA monomer-based vaccine candidate displaying conserved structurally occluded epitopes which have been characterized biochemically and biophysically. Immunogenicity assessment studies showed a very high antigenic titer upon a single boost. The sera from protein boost in all the immunized groups were further studied for 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. Next, the immunized animals were evaluated for their potency for protection through direct virus challenge post-immunization. The direct challenge with the PR8 virus showed that the magnitude of potential shown by the

monomeric vaccine candidate was equivalent to HA trimer, with no loss in body weight post-challenge with semi-lethal dose. The histopathological studies done on lungs tissues post-challenge showed no pathological markers, in terms of lymphocytes, neutrophils, and inflammation, hence leading to the validation of the concept of monomeric vaccine candidate displaying structurally occluded conserved epitopes are the novel addition towards universal Influenza vaccine

Way Ahead

Dr. Samal's group aims to incorporate M2e-5X with an adjuvant (developed by the Indian Institute of Chemical Technology, Hyderabad as a collaborator) and measure the efficacy to develop indigenous Influenza A next-generation vaccine candidate.

Dr. Shrivastava plans to carry out further studies based on Structurally Occluded conserved epitopes for designing better Influenza vaccine candidates with implementation and application towards other respiratory diseases.

HIV Vaccine and Antibody Translational Research

The HIV Vaccine Translational Research laboratory (HVTR), under the THSTI-IAVI partnership program led by **Dr. Jayanta Bhattacharya** at THSTI primarily, carries out basic, and translational research in the following areas:

(a) Characterization of the association between genetic, antigenic, and neutralization properties of primary HIV-1 with particular reference to subtype C that is predominantly circulating in India

(b) Discovery and characterization of neutralizing antibodies elicited in natural HIV and COVID-19 infection.

Towards achieving these scientific goals, Dr. Bhattacharya's team has built successful collaborations and partnerships with national and international organizations. The overall goal of the laboratory is to leverage strengths in developing strategies to identify and optimize neutralizing monoclonal antibodies suitable for broadly dissecting the circulating HIV clade C variants in India and also to utilize the existing expertise and platform for tackling emerging infectious diseases like COVID-19.

HIV-1 intra-subtype C envelope diversity and its association with altered sensitivity to broadly neutralizing antibodies (bnAbs)

As subtype C accounts for half of the global HIV-1 burden, limited information on the intra-clade C sequence and neutralization diversity is a major hindrance to the development of efficacious vaccine strategies. In this year, genetic attributes of globally circulating HIV-1C gp120 sequences were compared by Dr. Bhattacharya's team. They observed evidence of region-specific clade C *gp120* variabilities with variable susceptibility to different gp120-targeting bnAbs. As a proof of concept, they next examined neutralization susceptibility of 70 env-pseudoviruses isolated from Indian donors to four clinically relevant bnAbs targeting distinct gp120 specificities wherein significant variations were observed. Trend analysis indicated increasing region-specific neutralization resistance over time. These findings warrant the need for a more comprehensive extended analysis which will be necessary for selecting optimal bnAb combinations suitable for region-specific HIV-1 circulating subtypes.

Antigenic and immunogenic property of a novel HIV-1 India clade C Env trimer with near-native pre-fusion conformation

The interplay between circulating HIV and broadly cross-neutralizing polyclonal antibodies developed in a subset of elite neutralizers is widely believed to provide strategies for rational immunogen design. In the present study, Dr. Bhattacharya's team studied the structural, antigenic, and immunogenic properties of a novel soluble HIV-1 clade C trimeric protein (1PGE-THIVC) with near-native pre-fusion conformation prepared using the primary sequence of an HIV-1 clade C *env* isolated from the broadly cross-neutralizing plasma of an elite neutralizer. The novel 1PGE-THIVC Env trimer displayed a native-like pre-fusion closed conformation in solution as determined by small-angle X-ray scattering (SAXS) and negative stain electron microscopy (EM). This closed spike conformation of 1PGE-THIVC Env trimers was correlated with weak or undetectable binding of non-neutralizing monoclonal antibodies (mAbs) compared to neutralizing mAbs. Furthermore, 1PGE-THIVC SOSIP induced potent neutralizing antibodies in rabbits to autologous virus variants. Our study demonstrated neutralization of sequence matched and unmatched autologous viruses by serum antibodies induced in rabbits by 1PGE-THIVC and also highlighted a comparable specificity for the 1PGE-THIVC SOSIP trimer with that seen with polyclonal antibodies elicited in the elite neutralizer

Isolation & characterization of broadly neutralizing monoclonal antibodies (bnAbs)

Recently, many HIV-reactive monoclonal antibodies were isolated from one HIV+ elite neutralizer by antigen-specific B cell sorting and cloning by Dr. Bhattacharya's team. Two of these mAbs have been found to demonstrate cross-neutralization when tested against

pseudoviruses expressing heterologous *envs*. Further characterization of these mAbs is ongoing.

Way ahead

Dr. Bhattacharya's team plans to work on further characterization of the novel HIV neutralizing mAbs. In addition, they plan to examine the PK profiles of a few engineered bnAbs in the NHP model in collaboration with the National Institute of Immunology, New Delhi as part of antibody development. They have initiated recruitment of HIV+ donors across different geographical sites in India a part of the Team Science project supported by India Alliance, in studying and comparing HIV-1 clade C genetic and neutralization diversities with that of Africa towards developing prophylactic and therapeutic antibodies to tackle currently circulating HIV-1 in India.

Sepsis

The timely and accurate diagnosis and treatment of sepsis in neonates continue to be a huge challenge. Since a wide array of bacterial pathogens trigger sepsis, until appropriate antibiotics are administered within 24 h of sepsis suspicion, one in every two, blood culture positive neonates die in ~3-7 d. With MDR- and XDR-ESKAPE pathogens infecting most neonates, case fatality rates become much higher. To establish their niche, pathogens deliver an array of effector molecules dominated by proteins. Since antibiotics resistance has no direct correlation with virulence, and most often clinicians simply used a broad-spectrum antibiotic regimen, **Dr. Krishnamohan Atmakuri's** group hypothesize that identifying and stopping pathogens' secreted (into the host) virulent functions will lead to a positive outcome from sepsis. At any given time, one pathogen cell may deliver into a host, a wide array of virulent molecules in several hundred to thousand numbers. These are the true "derailers" of immune system cellular processes. Therefore, post-infection, beyond a certain duration, eliminating the pathogen per se may not be sufficient to contain sepsis. Thus, the assumed "dysregulated" immune response may not be so dysregulated as the infecting pathogen's virulent molecules continue to prevail and perform their designed host-specific functions, even post pathogen elimination. Thus, identifying them especially after their delivery into the host environment is imperative to a superior understanding of pathogen-mediated sepsis. They hypothesize that therapeutic targeting of key pathogen players will alleviate sepsis. However, direct mass spectrometry to identify the secreted pathogen proteins in the backdrop of thousands of different host molecules yields poor dividends. Thus, no pathogen proteins get detected until specifically enriched. Hence, Dr. Atmakuri's group employed the click

chemistry strategy to specifically label all pathogen proteins and later among them enrich the secreted proteins from infected host cells environment. This technique involved first metabolic labeling with an azide molecule such as a derivative of amino acid and later use an alkyne key to trap and enrich the azide-labeled pathogen proteome for MS-based identification and downstream analyses. To achieve that, Dr. Atmakuri's group first evaluated if the metabolic labeling of the pathogen proteome with an azide molecule would indeed work. Employing *Acinetobacter baumannii* (Acb) as a model pathogen, and using an azide methionine-derivative viz. Aha (L-Azidohomoalanine) in in vitro axenic cultures, they first demonstrated that indeed the nascent synthesized proteins of Acb are Aha-labelled. They confirmed this by using a fluorescent tag with an alkyne that specifically recognizes the azides labeled to surface proteins of Acb. All Aha labeled bacteria turned fluorescent confirming metabolic labeling of Acb proteome. They also enhanced the labeling by growing the bacterium in a minimal media depleted of L-Methionine amino acid. Then, to specifically-label nascent pathogen proteome (and not host proteome) that responds to host environment during infection in vitro in cell culture settings, they employed site-directed mutagenesis and mutated three specific amino acids in Acb's Methionyl-tRNA ligase such that the recombinant protein can specifically take up a methionine-derivative azide viz. Anl (Azidonorleucine). Again, by fluorescent microscopy, they confirmed Anl incorporation both in axenic broth and in cell cultures where lung epithelial cells were infected with unlabelled Acb. They further enhanced the intensity of Anl-specific labeling of the pathogen proteome by supplementing a cocktail of specific amino acids lacking L-Methionine. Then, to identify the pathogen proteome labeled by Aha (in broth cultures) and Anl (during host cell invasion in vitro), they have standardized several intermediary steps of (i) enrichment of the labeled proteins for downstream mass spectrometry and (ii) conditions for superior cell infections. The team is currently performing infection experiments in different cell lines to identify the pathogen secretome that may be useful for therapeutic and diagnostic strategies.

HUMAN MICROBIOME and ANTIMICROBIAL RESISTANCE

Currently, the major focus of **Dr. Bhabatosh Das**'s lab is oriented to understand (i) composition, diversity, and dynamics of the healthy human microbiome (ii) the role of the microbiome in health and diseases, and (iii) genetics of antimicrobial resistance traits in bacterial pathogens associated with (a) Gastroenteritis (b) Sepsis. In addition, his lab is working to

understand the evolution of the SARS-CoV-2 virus.

Gut microbiome diversity in acute severe colitis

The gut microbiome is the key player in gut health and the altered microbiome or dysbiosis is a signature of several gastric disorders including inflammatory bowel disease (IBD). The group compared the gut microbiome of patients with ulcerative colitis (UC), acute severe colitis (ASC), and healthy controls (HC). Ulcerative colitis was diagnosed using ECCO guidelines and ASC was diagnosed using Truelove and Witts' criteria. The alpha diversity was significantly lower in ASC than mild-moderately active UC ($p<0.05$), or HC ($p<0.001$). The gut microbiome in ASC was very heterogeneous, as reflected by high intra-cohort variation, which was significantly greater than in UC or HC. On principal coordinate analysis, the microbiome of HC and UC were similar, with the ASC cohort being distinct from both. Comparison of ranked abundances identified four distinct clusters of genera (G1, G2, G3, G4), with specific trends in their abundance across 3 groups: G1/G2A clusters had the least, whereas G3 had the highest abundance in the ASC cohort. Dr. Das and the team identified that the gut microbial diversity is lower in ASC than mild-moderate UC or healthy controls. In addition, gut microbiome composition is very heterogeneous in ASC patients, with a distinct abundance profile of specific bacterial taxa varying between healthy controls and ASC.

Trans-ethnic gut microbial signatures of prediabetic subjects from India and Denmark

Type 2 diabetes mellitus (T2D) is a prevalent disease, which is characterized by imbalances in the level of blood glucose. Several studies have reported an association of gut microbiota and microbial metabolites with T2D. Identifying robust gut microbiota or microbial genomic signatures of prediabetes and characterizing early prediabetic stages is important for the understanding of disease development and could be crucial in early diagnosis and prevention. Dr. Das and his team profiled and compared the gut microbiota composition and diversity of prediabetic individuals (N=262) with normoglycaemic individuals (N=275) from two cohorts located in India and Denmark. In addition, fasting serum inflammatory biomarkers were also profiled from the enrolled study participants.

After correcting for strong country-specific cohort-effect, 16 operational taxonomic units (OTUs) including members from the genera Prevotella9, Phascolarctobacterium, Barnesiella, Flavonifractor, Tyzzerella_4, Bacteroides, Faecalibacterium, and Agathobacter were identified as enriched in normoglycaemic subjects with respect to the subjects with

prediabetes using a negative binomial Wald test. Dr. Das's team also identified 144 OTUs enriched in the prediabetic subjects. Comparative analyses of relative abundance bacterial taxa revealed that the Streptococcus, Escherichia/Shigella, Prevotella2, Vibrio, and Alloprevotella OTUs exhibited more than four-fold enrichment in the gut microbiota of prediabetic subjects. When considering subjects from the two geographies separately, they were able to identify additional gut microbiome signatures of prediabetes. The study reports a probable association of Megasphaera OTU(s) with impaired glucose tolerance, which is significantly pronounced in Indian subjects. While the overall results confirm a state of proinflammation as early as in prediabetes, the Indian cohort exhibited a characteristic pattern of abundance of inflammatory markers indicating low-grade intestinal inflammation at an overall population level, irrespective of glycaemic status. The results present trans-ethnic gut microbiome and inflammation signatures associated with prediabetes, in Indian and Danish populations.

Vaginal microbial signatures in Indian women

Dr. Das and team are doing a comparative analysis of vaginal microbiome composition and microbial genomic repertoires of women who enrolled in the Interdisciplinary Group for Advanced Research on Birth Outcomes – A DBT India Initiative (GARBH-Ini) pregnancy cohort to identify bacterial taxa and specific genomic signatures associated with term birth (TB) and PTB in Indian women. They collected high vaginal swab (HVS) samples during all three trimesters from pregnant Indian women who delivered spontaneous term and preterm neonates. Vaginal microbiome of both term and preterm samples revealed similar alpha diversity indices. However, a significantly higher abundance of Lactobacillus iners, Megasphaera sp, Gardnerella vaginalis, and Sneathia sanguinegens were identified in preterm samples whereas a higher abundance of L. gasseri was observed in term samples. The relative abundance of L. iners, and Megasphaera sp. were found to be significantly different over time between term and preterm mothers. Analyses of the representative genomes of L. crispatus and L. gasseri indicate that their genomes are enriched with horizontally acquired mobile genetic elements (MGEs). The functions linked with MGEs can be correlated with anti-inflammatory conditions in the vagina. The results reflect that a significant number of genes may have sporadic distribution or accumulated mutations. Such functions may help to understand the molecular basis of geo-specificity different Lactobacillus species living in the reproductive tract of women across the globe.

Developing bacterial reporter strains for the discovery of antibiotic potentiators

The emergence of multidrug-resistant bacterial pathogens and decreasing effectiveness of antibiotics pose a global threat to public health. Resistance to antibiotics in bacteria can occur through four major pathways (i) Acquisition of functions that modify or degrade antibiotics (ii) Alterations in the antibiotic's target (iii) Modulations of cell permeability and (iv) Expulsion of antibiotics from the cytoplasm. Current findings indicate that horizontally acquired antibiotic resistance genes (ARGs) contribute majorly to antibiotic resistance (AMR) in Gram-negative ESKAPE pathogens. We have engineered the genome of Vibrio cholerae and developed reporter strains for rapid and robust screening of small molecule inhibitors against different classes of b-lactamases (blaNDM, kpc, oxa-24, shv) and aminoglycosidases (aph, aadA, aac) which are prevalent in the genome of Gram-negative ESKAPE pathogens.

List of Collaborators

Dr. Ramandeep Singh

Dr. Krishan Gopal, IMTECH, Chandigarh

Dr. Raghavan Vardarajan, IISc, Bangalore

Dr. Mahavir Singh, IISc, Bangalore

Dr. Nagasuma Chandra, IISc, Bangalore

Dr. Deepak Saini, IISc Bangalore

Dr. Diwan Rawat, Delhi University

Dr. Dinesh Mahajan, THSTI

Dr. Raj Kapoormath, University of KwaZulu-Natal, South Africa

Dr. Rohan Dhiman, NIT, Rourkela

Dr. Indu Pal, Punjab University

Dr. Nisheeth Agarwal, THSTI

Dr. Shailendra Asthana, THSTI

Dr. Nisheeth Agarwal

Dr. Ramandeep Singh, THSTI

Dr. Umesh Varshney, IISc, Bengaluru

Dr. Bichitra Biswal, NII, New Delhi

Dr. Vinay Nandicoori, NII, New Delhi

Dr. Preeti Saxena, South Asian University, New Delhi

Dr. Alka Rao, IMTECH, Chandigarh

Dr. Chaaya Iyengar, NIPER, Mohali

Dr. William R. Bishai, Johns Hopkins University, USA

Dr. Amit Kumar Pandey

Dr. Amit Awasthi, THSTI, Faridabad

Dr. Ranjan Nanda, ICGEB, New Delhi

Dr. Amit Singhal, ASTAR, Singapore

Dr. Debasis Dash, IGIB, New Delhi

Dr Vengadesan Krishnan, RCB, Faridabad

Dr. Shailendra Asthana, THSTI, Faridabad

Dr. Renu Goyal, THSTI, Faridabad

Dr. Amit Kumar Yadav, THSTI, Faridabad

Dr. Bichitra K. Biswal, NII, New Delhi

Dr. Niti Kumar, CDRI, Lucknow

Prof. Parmod Mehta, Center of Biotechnology, MDU, Rohtak

Dr. Tarun Kumar Sharma, THSTI, Faridabad

Dr. Yashwant Kumar, THSTI, Faridabad

Dr. Krishnamohan Atmakuri

Dr. Vengadesan, RCB

Dr. Ramandeep Singh, THSTI

Dr. Tarun Kumar Sharma, THSTI

Dr. Tripti Shrivastava, THSTI

Dr. Guruprasad R Medigeshi

Prof. Rakesh Lodha, AIIMS - New Delhi

Prof. Anil Kumar Pandey, ESIC-Faridabad

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

Dr. Sampath Kumar, IICT-Hyderabad,

Dr. Nagendra Hegde, NIAB, Hyderabad

Dr. Madhuri Subbiah, NIAB, Hyderabad

Dr. Rajesh Gokhale, NII, New Delhi

Dr. Soumen Basak, NII, New Delhi,

Dr. Sudhir Krishna, NCBS-Bengaluru

Dr. Milan Surjit

Dr. Baibaswata Nayak, AIIMS, New Delhi

Dr. Shalimar, AIIMS, New Delhi

Dr. Manish Soneja, AIIMS, New Delhi

Dr RK Ratho, PGIMER, Chandigarh

Dr. T K Maiti, RCB, Faridabad

Dr. Prasenjit Guchhait, RCB, Faridabad

Dr. Avinash Bajaj, RCB, Faridabad

Dr. Ranjith-Kumar, IPU, New Delhi

Dr. S K Lal, Monash University, Malaysia

Dr. Q Pan, Erasmus MC, Rotterdam

Dr. Tripti Shrivastava, THSTI

Dr. Shubbir Ahmad, THSTI

Dr. Sankar Bhattacharya, THSTI

Dr. Shailendra Mani, THSTI

Dr. Samrat Chatterjee, THSTI

Dr Shailendra Asthana, THSTI

Dr. Supratik Das

Dr. Manidipa Banerjee, IIT-Delhi, India

Dr. Anmol Chandele. ICGEB-Delhi, India

Dr. Dilip Kumar, Baylor College of Medicine, USA

Dr. Sweety Samal

Dr. Anmol Chandale, ICGEB, New Delhi

Dr. Arockiasamy Arulandu, ICGEB, New Delhi

Dr. Raghavan Varadarajan, IISc, Bengaluru

Dr. H.M Sampath Kumar, IICT, Hyderabad

Dr. Annapurna Vyakarnam, St. John's Research Institute (SJRI), Bengaluru

Dr. Savit.B. Prabhu, CMC, Vellore

Dr. R. Subasri, ARCI, Hyderabad

Dr. Amit Awasthi, THSTI

Dr. Shubbir Ahmed, THSTI

Dr. Supratik Das, THSTI

Dr. Tripti Shrivastava, THSTI

Dr. Rakesh Kumar, THSTI

Dr. Tripti Shrivastava

Dr. Manidipa Banerjee, IIT-Delhi, India

Dr. Amit Awasthi, THSTI

Dr. Milan Surjit, THSTI

Dr. Rajesh Kumar, THSTI

Dr. Sankar Bhattacharya, THSTI

Dr. Shailendra Asthana, THSTI

Dr. Shailender Mani, THSTI

Dr. Sweety Samal, THSTI

Dr Supratik Das, THSTI

Dr. Jayanta Bhattacharya

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

Dr. Devin Sok, IAVI- Neutralizing Antibody Center, The Scripps Research Institute, USA

Dr. Jan Terje Andersen, University of Oslo, Norway

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

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

Dr. Saravanan, YRG Center for AIDS Research & Care, Chennai

Prof Thumbi Ndungu, University of KwaZulu-Natal, Durban, South Africa

Prof Pontiano Kaleebu, Uganda Virus Research Institute, Entebbe, Uganda, Africa

Dr. Eunice Enduati, KEMRI-Wellcome Trust Research Programme, Nairobi, Kenya, Africa

Prof Kalpana Luthra, AIIMS, New Delhi

Prof Bhaswati Bandyopadhyay, Calcutta School o Tropical Medicine, Kolkata

Prof Jayanthi Shastri, TNMC & BYL Nair Hospital, Mumbai

Dr. Bhabatosh Das

Prof. Kiyoshi Takeda, Osaka University, Japan

Prof. Francois-Xavier Barre, Institute for Integrative Biology of the Cell, France

Prof. Vineet Ahuja, AIIMS, New Delhi

Prof. Sourabh Dutta, PGIMER, Chandigarh

Dr. Shalimar, AIIMS, New Delhi

Dr. Animesh Ray, AIIMS, New Delhi

Dr. Ankur Mutreja, University of Cambridge, United Kingdom

Dr. Sanjay K Banerjee, NIPER, Guwahati

Dr. Tushar K Maiti, RCB, Faridabad

Dr. Chittur V Srikanth, RCB, Faridabad

Dr. Hemanta Koley, National Institute of Cholera and Enteric Diseases, Kolkata

Dr. Asish K. Mukhopadhyay, National Institute of Cholera and Enteric Diseases, Kolkata

Dr. Thandavarayan Ramamurthy, National Institute of Cholera and Enteric Diseases, Kolkata

Dr. Santanu Chattopadhyay, Rajiv Gandhi Centre for Biotechnology, Kerala

Prof. Shinjini Bhatnagar, THSTI

Dr. Nitya Wadhwa, THST


thsti


ट्रांसलेशनल स्वास्थ्य विज्ञान एवं प्रौद्योगिकी संस्थान


ट्रांसलेशनल स्वास्थ्य विज्ञान एवं प्रौद्योगिकी संस्थान

Maternal and Child Health

“Bridging clinical epidemiology, multi-omics, and data science to improve maternal and child health”

Maternal and Child Health (MCH) is critical because it is a key determinant of the health of a society and the focus of MCH program is on finding effective solutions that have major public health implications. The strategy is to encourage discovery research that is inspired by societal need and innovation which is driven by new or existing knowledge in order to create applications that can go back to society. Led by **Dr. Shinjini Bhatnagar**, the MCH team fulfills its mandate by the following approaches:

- Analytical observational studies with multidisciplinary contributions in discovery research to develop novel biomarkers, tools, and interventions that facilitate the translational pipeline
- Randomized controlled trials to evaluate interventions for reducing early life morbidity that will have a quick impact on improving childhood outcomes
- Augmenting infrastructure for clinical research to create national resources by establishing clinical research units in partner hospitals, data management centers, and a biorepository.
- Training programs to develop capacity among young investigators.

Analytical observational studies with multidisciplinary contributions in discovery research to develop novel biomarkers, tools, and interventions that facilitate the translational pipeline

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

The abysmally high infant mortality rate in our country is largely due to babies dying at birth, or within the first month with major killers being pre-term birth (PTB), neonatal infections, and encephalopathy due to birth asphyxia. Nearly 3.6 million of the 27 million babies born annually in India are preterm (defined by WHO as babies born less than 37 weeks of gestation), 300,000 of them die soon after birth contributing to a third of the neonatal deaths and a quarter of the global neonatal deaths. Globally, we rank first for deaths due to PTB complications. Those who survive are at a very high risk of cognitive and metabolic disorders.

Despite decades of research, there are no effective solutions for PTB and this is because of large knowledge gaps. PTB is a complex syndrome with multiple etiology that includes interacting biological, psychosocial, and environmental factors. Deep fundamental knowledge is required for innovative applications and it is critical that efficient solutions have to be prioritized both at the national and global levels. Driven by this societal need an inter-institutional and interdisciplinary program was established in 2014 by DBT, GOI, coordinated by THSTI on preterm birth. The year 2020-21 marked the successful completion of the first phase of our institute's flagship program and the DBT Atal Jai Anusandhan Mission program: GARBH-INi. It is unique because the research institutes (THSTI, RCB, NIBMG) have collaborated with district hospitals in Gurugram and large tertiary hospitals SJH and MAMC as partners. It is truly multidisciplinary with strengths across clinical, epidemiological, statistical, biological, and imaging sciences. The team posits that attributable risks to PTB of various clinical and epidemiological factors vary during pregnancy. Secondly, PTB may be a result of static (genomic) predispositions and dynamic (epigenomic, proteomic, metabolomic) modulations at different stages of pregnancy. Therefore, multidimensional data collected in a time series manner across pregnancy will help in stratifying women into defined risk groups for PTB.

Their primary deliverables are (i) to have a dynamic multi-dimensional predictive model that provides a decision algorithm for early prediction and timing of intervention of PTB and (ii) candidate leads emerging out of multi-omics analyses that can be further evaluated as interventions.

The objectives to achieve this critical health system deliverable are:

(a) Epidemiological, clinical and ultrasound-based risk factor analysis that will feed into the risk stratification algorithm

(b) Biochemical/Omics biomarkers to provide more customized decision-making tools for clinicians

(c) Tools for more personalized & futuristic prediction with ultrasound (using machine learning approaches) & omics technology

(d) Inferences from multi-omics and clinical and epi risk factor analysis on mechanistic pathways to identify candidate leads

Led by **Prof. Shinjini Bhatnagar**, a cohort of pregnant women known as the GARBH-INI Cohort was established in May 2015 at the Civil hospital in Gurugram (GCH), Haryana, India

after establishing a challenging but highly standardized infrastructure at the clinical sites (GCH as the primary clinical site and Safdarjung Hospital as the referral hospital) including a research laboratory at the district hospital. Women are enrolled within 20 weeks of gestation and are followed until delivery and once at postpartum. The enrolled women are followed up at 4-5 time-points across 3 trimesters of pregnancy to document extensive clinical & epidemiological information, for maternal and neonatal biospecimens and serial ultrasonographic examination. Till June 2021, a total of 27652 pregnant women were screened, 8757 participants were enrolled and 7567 outcomes were determined in the study. The decision to enroll 8000 women in the first phase of this study was taken apriori based on the hypothesis.

The interdisciplinary MCH team has sub-teams with lead PIs to complete the committed objectives and the deliverables. These are described within each section.

Epidemiological, clinical, and ultrasound-based risk factor analysis that will feed into the risk stratification algorithm

The team has reported a preterm birth rate of 13.2% in the cohort. This is the first time that a systematic serial evaluation has been done in the country for preterm birth. Phenotypically, nearly 60% of all preterm births in the GARBH-INI cohort as spontaneous preterm labor while another 21% had prelabour rupture of membranes. As expected, moderate and late preterm births were the dominant subtypes of clinical severity emphasizing the need for attention. The cohort has provided a platform to assess other adverse pregnancy outcomes: Stillbirth rate - 2.5%, low birth weight - 26.7%, and SGA - 36.2%.

On the evaluation of possible risk factors by conducting uni- and multivariable analysis, particularly BMI at enrolment showed a U-shaped association with both underweight (OR 1.2 (1.03, 1.5)) and overweight/obese (OR 1.4 (1.1, 1.8)) women having a higher risk of PTB. The team has established in an adjusted multivariable model that women with prior preterm birth had 3 times higher risk of PTB in the current pregnancy, short cervix in the 3rd trimester increased the risk 2.3-folds and overweight increased the risk of PTB by 2.1 folds.

Gestational Weight Gain

Dr. Ramachandran Thiruvengadam and his team have described the longitudinal pattern of gestational weight (GWG) for the first time in India. When compared with global standards, 27% of our women had inadequate GWG at 18-20 weeks; this proportion increased

progressively across pregnancy to 43·3% at the time of delivery. In brief, they 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. First trimester BMI, parity, and social factors like the 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.

Air Pollution

An interim analysis of GARBH-INi data, by Dr. Thiruvengadam and his team (in collaboration with Dr. Rengasamy's team at IIT Madras), showed nearly 9.5% of all PTB could be attributed to exposure to indoor air pollution (assessed at enrolment by a history of use of biomass fuel for cooking and exposure to second-hand tobacco smoke). The quantitative estimates of the risk of adverse pregnancy outcomes across different ranges of exposure are still unclear. They have established a low-cost monitoring network created with a coverage of 350 sq.km.; standardized household surveys and initiated satellite data modeling. Ambient air pollution monitoring was done for 350/550 sq. km for six months. Geo-coding of participant addresses was completed. Around 200 participants were enrolled and 85 followed up for exposure assessment. The study is currently facing difficulties in fieldwork and participant household visits because of COVID. Efforts are being made to move towards increasing the satellite component of the ambient air pollution monitoring.

Gestational Diabetes Mellitus

Thirteen (13%) women had gestational diabetes (GDM) in the Garbh-INi cohort; this rate is higher for a secondary level care hospital. The association of GDM on adverse pregnancy outcomes is being evaluated by **Dr. Deepika Murugesan** in collaboration with Prof Nikhil Tandon and Dr. Yashdeep Gupta from the Department of Endocrinology at AIIMS, New Delhi. Further, alternative diagnostic strategies combining fasting blood glucose, random blood glucose, glycated hemoglobin, and clinical risk profiling at various time points are being explored as alternatives. A patient-friendly diagnostic test will improve compliance and aid in capturing more GDM cases. Additionally, the effect of glycemic status at various time points in pregnancy on fetal growth

and uteroplacental blood flow is also being analyzed to establish the importance of glycemic control for fetal wellbeing.

Multiple MicroNutirent (MMN) Deficiencies

Dr. Lovejeet Kaur and her team (in collaboration with **Dr. Shailaja Sopory** and Dr. Uma Chandra Mouli Natchu at SJRI) focus on the nutritional biomarkers of adverse pregnancy outcomes, primarily preterm birth, and neonatal health. Their overall aim is to understand the population and gestation-specific nutritional profiles, and how the coexisting maternal multiple nutritional deficiencies, their absorption, and transport across maternal-fetal dyads, affect maternal, fetal, and neonatal health.

Multiple MicroNutrient (MMN) deficiencies in mothers have been associated with preterm birth across the world. However, due to ethnic and dietary diversity, the deficiency types and their extent vary. Therefore, maternal supplementation interventions need to be population-specific. Further, gestational changes in the maternal nutritional profiles are also observed either due to nutritional transfer from mother to the fetus or superimposed with hemodilution during pregnancy. Hence, to have a population and gestation-specific micronutrient profiles of mothers, the team has initialized investigations on micronutrients (vitamins and minerals) in maternal sera of early pregnancy.

The team has successfully standardized the protocols for the micronutrients specifically Vitamin D, Ferritin, Selenium, Copper, Zinc, Magnesium. The methodology employs a multiplexing approach using Inductively Coupled Plasma Mass Spectrometry and bioanalyzers and has successfully generated quality data on ~400 odd sera samples of pregnant women in early gestation (<20 weeks) from the Garbh-INi Cohort. The remaining vitamin estimations (B12, B9, A & E) are ongoing. The plan is to profile ~2000 mothers at different gestational periods (with ~13% preterm birth rate) for all the above-mentioned micronutrients in the coming year. Robust data on a large homogenous cohort shall help determine if and how these deficiencies affecting maternal and child health could be overcome by prioritizing through strategic maternal supplementation as interventions to prevent preterm birth in the current population. The team will analyze nutritional trajectories across gestation in the present cohort and determine if there are associations of co-existing deficiencies with maternal, fetal, and neonatal outcomes.

Generation of a dynamic prediction model for preterm birth

Conventional risk factors (as described above) in a regression model, explained about 6% of PTB. The team developed a dynamic prediction model for PTB. Two approaches were used to improve the model, (i) identify non-linear and longitudinally varying predictors and (ii) add novel predictors from USG images and -omics markers. At the baseline (< 14 weeks POG), previous PTB, passive smoking, interpregnancy interval, education in years, type of family and cervical length, uterine artery blood flow, resulted in a receiver-operator characteristics curve (AUROC) of 0.59 (Sensitivity: 0.8; Specificity: 0.26). The probabilities from the first-trimester prediction model were then fed forward into the second level of prediction at 18-20 weeks using specific markers (fetal biometry, blood pressure, cervical length, and gestational weight gain, to arrive at revised probability scores (AUROC: 0.75; Sensitivity: 0.8; 0.9; Specificity: 0.55; 0.42). These revised scores were then fed forward into the 3rd level of prediction at 26-28 weeks that included pallor, pedal edema, blood pressure, and gestational weight gain. This improved the predictive efficacy of the model (AUROC: 0.75; Sensitivity: 0.8 & Specificity: 0.53) indicating an improvement in the prediction metrics AUROC as the pregnancy progressed. Interestingly, they were able to identify the most important predictors at each level during this dynamic prediction. The ongoing analysis is being done with the inclusion of candidate predictors from the multi-omics data.

Biochemical/Omics biomarkers to provide more customized decision-making tools for clinicians

The discovery of biochemical and omics biomarkers was planned to balance between analytical robustness while maintaining the efficiency in sample and resource utilization. The GARBH-INI team designed a nested case-control study that minimized (i) selection bias as both cases and controls were derived from the same population, (ii) information bias by ensuring accurate phenotyping of outcomes, and (iii) recall bias for exposure in the cohort. They also ensured all omics analyses were done in the same set of participants in order to permit an integrated analysis. Before they embarked on the comparative analysis between women delivering term and preterm, they evaluated the longitudinal changes among the women who delivered normal term babies.

The team has successfully developed the multi-omics pipelines for the discovery phase and is now focusing on key omics biomarkers.

A summary of the omics analyses:

Proteomics

Proteomic evaluations to identify predictive biomarkers for preterm birth are carried out in 3 different tissues, by Dr. Tushar Maiti's team (from RCB) in collaboration with Dr. Thiruvengadam and Dr. Desiraju from MCH, THSTI.

Salivary Proteome across pregnancy: This analysis revealed significant alterations in the expression of proteins involved in immune modulation, metabolism, and host defense across pregnancy. These proteins could serve as biomarkers if found altered in women delivering preterm (Dey et al. Sci. Rep.2020,10, 8022).

Vaginal Secretory proteome: Sixty-one proteins were differentially expressed across pregnancy (at enrolment, 18-20 & 26-28 weeks) in high vaginal fluid of pregnant women. These proteins indicate antioxidant, antimicrobial, cervical remodeling, and inflammatory functions, providing mechanistic hypotheses that these pathways are altered during pregnancy (Kumar et al. 2021 J. Proteome Research).

Plasma Proteome: The discovery phase proteomic studies of plasma (N=20) have been completed and the results are being analyzed to identify plasma proteins that may discriminate between term and preterm birth.

Genomics and epigenomics

Genomics and Epigenomic analysis are being conducted by Drs. Partha P Majumdar and Arindam Maitra and their teams at National Institute of Biomedical Genomics (NIBMG), Kalyani, in collaboration with the clinical team at THSTI.

They have identified 254 differentially methylated CpG sites from the promoter regions of 251 genes between women delivering term and preterm. ABCA6 gene (cg06815731) exhibited the highest temporal variance in mothers delivering preterm. Biological pathway analysis identified nitric oxide response, androgen metabolic process, steroid dehydrogenase activity, negative regulation of sequestering of calcium ion, and release of sequestered calcium ion into the cytosol (under review). Epigenomic analysis identified significant CpG changes associated with biological pathways such as nitric oxide response and androgen metabolic process and the pathways associated with steroid dehydrogenase activity, negative regulation of sequestering of calcium ion and release of sequestered calcium ion into the cytosol. Genome-Wide Association Study has been conducted in collaboration with the clinical and the data science team of Dr. Thiruvengadam and Dr. Desiraju from MCH, THSTI

using the Global Screening Array (GSA) with ~650,000 markers (N=5000). The genetic variants are being tested for association with quantitative outcomes such as gestational age at delivery, birth weight, placental weight, and blood flow. The analysis is ongoing.

Microbiome Analyses

Microbiome analyses, by Dr. Bhabatosh Das (THSTI) and Dr. Souvik Mukherjee's teams (NIBMG), in collaboration with the clinical and the biorepository teams of MCH have shown that (i) microbiota of reproductive age Indian women is mostly dominated by *L. iners* and *L. crispatus*, (ii) the genome of the Lactobacilli is enriched with mobile genetic elements like GIs (CRISPR-Cas), phages and insertion sequences, and (iii) among the several novel Lactobacillus species/subspecies observed in the vaginal ecosystem of Indian women more than 60 different bacterial species (n=363) have been identified. (Mehta et al. Microb Ecol. 2020 Aug;80(2):487-499). When they evaluated the differences in the microbiome between women delivering term and preterm, they found that *Lactobacillus iners* was significantly higher in all the trimesters in PTB samples. *Lactobacillus gasseri* was significantly higher in term samples only in 3rd trimester of pregnancy. *Sneathia sanguinegens* and *Gardnerella vaginalis* were also found to be significant in the 2nd trimester in PTB (Kumar S et al. Front Cell Infect Microbiol. 2021 May 13;11:402.)

Metabolomics

Metabolomic analysis for clinical biomarker discovery and prediction models for preterm birth is being carried out by **Dr. Pallavi Kshetrapal** and her team. They followed an untargeted approach for profiling of maternal serum collected in the first trimester of pregnancy (<20 weeks) from women delivering term (n= 61) and preterm (n= 61) using UHPLC-MS/MS. Raw data were pre-processed using progenesis QI. Curated data was applied for a data dimension reduction approach using QC-RFSC, modified 80% rule and RSD<20% that decreased the features to 1494 (acquired on positive mode) and 1009 (acquired on negative mode). Identification of the features for a biomarker discovery was carried out using the Progenesis QI software based on m/z, retention time, and fragment information. Data normalization and statistical analysis were carried out on Metaboanalyst 4.0 (online, R-based GUI platform) for biomarker discovery and pathway enrichment analysis. Biologically relevant alterations in the sera metabolome were identified in maternal sera collected at ≤20 weeks of POG in 61 PTB vs 61 TB delivering mothers. Comprehensive analysis of identified

metabolites revealed a significant elevation in the levels of steroids and steroid derivatives, glycerophospholipids, and fatty acids, and their conjugates are associated with spontaneous PTB.

Verification and Validation plan for omics analyses

Verification of the discovery phase results will be performed on different platforms of biomarker analysis to assure analytical robustness. The validation studies will emphasize the applicability of the results of the discovery phase among specific subgroups of the population, for various phenotypes of preterm birth, and different geographic settings in the country.

The sample size needed to validate the predictive biomarkers identified in the discovery phase will depend on the number of candidate markers advanced into the validation phase. The longitudinal validation will require samples collected at all time points of follow-up (V1, V2, V3, and V5). They currently have 200 such preterm birth samples which have been fully utilized for the discovery and verification stages of biomarker development. The team would require additional preterm birth samples for validation. These samples will be acquired in phase-2. The results from GARBH-INI's teamwork have marked important milestones in the development of translatable outcomes that have the potential to improve pregnancy outcomes (Figure 1).

Figure 1. Important translatable outcomes of GARBH-INI Phase 1

GARBH-INI Phase 2

Way Ahead - Establishing Infant Birth Cohort

Children born in the pregnancy cohort (GARBH-INI) shall be included in a birth cohort and this effort will be led by **Dr. Nitya Wadhwa** supported by **Dr. Vidushi Gupta** and **Dr. Thiruvengadam**. The birth cohort is being initiated based on the hypotheses, that (i) babies born with different phenotypes of PTB and FGR have distinct childhood growth trajectories and development, (ii) exposures during the antenatal period have an effect modifier role on childhood growth and development, (iii). exposures at birth and post-natal life are associated with childhood growth & development and (iv) babies born with different birth phenotypes can be stratified early in infancy for their risk of stunting and developmental delay. The technical and financial approvals of the Phase 2-Birth cohort from DBT and the Institutional Ethical Committee approvals from Gurugram Civil Hospital have been obtained. The next phase of the study will allow us to relate both independent association and antenatal effect modifiers that may result in different birth phenotypes and also look at the growth and developmental outcomes of different birth phenotypes born in the cohort.

Tools for more personalized & futuristic prediction with ultrasound (using machine learning approaches) & omics technology

CALOPUS: Computer Assisted LOw Point of care UltraSound

In collaboration with the University of Oxford, Drs Bhatnagar, Thiruvengadam, and Desiraju from MCH, THSTI are developing processes for a portable ultrasound (Computer Assisted LOw Point of care UltraSound (CALOPUS) with an integrated decision support system built by using Artificial Intelligence (AI) algorithms. In summary, the videos are being collected using a previously validated six-step protocol and algorithms are being built to extract useful clinical information about heart rate, the number of fetuses, position of the placenta, and fetal presentation.

Till now 3232 videos have been collected from 1295 participants. Among these, 302 videos are annotated (a bounding box is drawn to identify the fetal and maternal organs) by expert

radiologists. Our preliminary models to identify maternal and fetal organs in these videos have an accuracy of 86% on unseen test videos.

The next step is to extend these models to extract information on the fetal presentation, viability, placenta, and amniotic fluid automatically and build a decision system. The end deliverable will be a portable ultrasound with just three light decision control systems, that are simple enough to be used by a health worker and can act as a triaging tool in resource-limited settings for appropriate referral of high-risk pregnancies. This tool will ensure that the fetal sex is not determined, and will follow all the rules of the PNDT act.

Predicting Preterm Birth using Convolutional Neural Networks and Ultrasonographic Images

The primary objective of **Dr. Bapu Koundinya Desiraju** and his team is to develop a universal screening tool (with high sensitivity and specificity) using Convolutional Neural Networks (CNN) and Ultrasonographic (USG) images, taken during the mid-trimester of pregnancy, to predict preterm birth.

Ultrasonographic (USG) images have been shown and if analyzed systematically, may lead to more accurate predictive models. Machine learning (ML) techniques are capable of extracting features automatically and can learn from the subtle patterns in the image. Convolutional Neural Networks (CNN), a type of ML algorithm, is a state-of-the-art technique that has revolutionized the field of computer vision. Recent studies demonstrated that CNN can achieve accuracies comparable to human experts in medical image recognition tasks like diagnosing skin cancer, pneumonia, diabetic retinopathy, etc. These methods have never been applied to USG images to predict PTB. Sonographic evaluation of cervical length (CL) has been studied extensively to predict PTB, but the data is prone to both inter-and intra-observer variation. Small studies have reported mid-trimester sonographic cervical texture (AUC = 0.77; 95% CI: 0.66-0.87) and consistency index (AUC = 0.84; 95% CI: 0.75-0.93) as better predictors of PTB than CL alone. In this study, the team proposes to leverage the potential of these algorithms to predict PTB using the images of the cervix taken by transvaginal USG.

Preparation of the dataset

The dataset preparation phase was done till September 2020 cervix images were identified and extracted from a large database of images using the labels written on the images. They

used different optical character recognition (OCR) techniques and standardized a pipeline to identify, extract and preprocess the images. A total of 11,231 USG images including 9629 terms and 1602 preterm from 3,266 participants have been identified from the database using different OCR techniques and extracted for the analysis. These images were preprocessed using image inpainting (to remove labels and other text on images) and normalization.

Artificial intelligence modeling

The dataset was divided into three disjoint training, validation, and test sets. All models were trained on the training set, based on the accuracy of the validation set, the hyperparameters such as network architecture, loss functions, etc. were fine-tuned. The final model was then tested on the unseen test set. All the results presented are on the final test set. This is a binary classification task where given an image it can be predicted if the delivery will be term or preterm. This classification had a high-class imbalance problem where the number of women delivering preterm was only 13% as compared to the term. The initial models could not accommodate such imbalance and demonstrated poor accuracy and generalization to the test set. To overcome the problem of class-imbalance minority class-oversampling, majority class under-sampling and applied different loss functions such as Weighted Cross-Entropy & Focal loss have been used. Techniques such as minority class-oversampling and majority class under sampling were used only during training while the original distribution of term and preterm was maintained during the validation and testing phase to estimate the real-world performance. The loss weighing term used in weighted cross-entropy and focal loss was inversely proportional to the number of samples in that particular class. The metrics used for evaluating the model were accuracy, precision, recall, balanced accuracy, and AUROC. The network architectures used are based on ResNet 18, ResNet 34, and Efficient Net.

The model with the ResNet 34 architecture with focal loss is showing the best results till now that has an AUROC of 0.58 and an accuracy of 64% in the preterm group.

The next steps would be to identify problems and challenges to improve upon the existing models using the following steps:

(i) Trouble-shooting the existing models by comparing clinical characteristics of correctly identified participants vs incorrectly identified and extract clinically meaningful inferences

(ii) Build models using the data of only high-risk women (with a history of any risk factor for preterm birth)

(iii) Reclassify preterm birth using our own more accurate second trimester gestational age estimation model and use it for the modeling

(iv) Define gestational age at delivery as a continuous outcome and build regression models to estimate gestational age at delivery.

(v) CNN is known to be a 'black box model' and is very difficult to interpret. This hinders the practical application of these models especially in healthcare where the cost of an error is very high. Producing interpretable models is essential to gain the trust of clinicians and patients. Algorithms such as class activation maps and their variants will be implemented to make the models more interpretable

In summary, this model should take away the need to make manual calculations from the images and save on time and effort. Although building these algorithms requires a lot of computation, once developed they can be distributed as a simple software that can be used in hospitals with a standard USG facility.

Observational studies to understand specific biological determinants of pregnancy outcomes

Infection, inflammation, and immune system development

Dr. Shailaja Sopory's group focuses on infection and inflammation. It started with cross-sectional analysis to evaluate differences in immune parameters between small for gestational age (SGA) infants and average for gestational age (AGA) infants (PloS ONE 2015 Apr 21:10(4)). This led to multiple cross-sectional studies where comparisons were made between cord blood and adult peripheral blood immunophenotype (PLoS One. 2016 Sep 9;11(9):e0162242) and between Indian and American infants (PLoS One 2018 Nov 16; 13 (11): e0207297)**.** These studies were followed up by addressing these questions using a longitudinal study design in the GARBH-Ini cohort which would help understand the inflammatory influences in the antenatal period that lead to SGA and FGR. In the initial analysis bacterial vaginosis (OR = 1.26, p= 0.02), gastroenteritis (OR= 1.49, p= 0.007) and cough (OR =1.32, p= 0.0005) at any time during pregnancy are shown as risk factors for SGA and cough (OR = 1.37, p= 0.002) at any time during pregnancy as risk factor for FGR.

The longitudinal analysis for expression of inflammatory markers across pregnancy and in cord blood shows that cord blood has a distinct cytokine profile as compared with maternal

blood. Lower concentrations of chemokines and inflammatory markers were observed in Indian mothers from those reported in the western world. It was also observed increased levels of adhesion molecules and IL-4 and IL-Ra, known anti-inflammatory markers in our cohort. Moreover, anti-inflammatory markers and growth factors (IL-4, M-CSF, Flt-3L, PlGF) increase significantly along gestation. PCA analysis indicated the association of certain cytokines with fetal growth parameters. Subsequently, changes that take place in maternal inflammatory patterns in fetal growth-restricted pregnancies will be evaluated.

Human Placenta Research Program

The Human Placenta Research Program is embedded in the GARBH-INI cohort and is led by **Dr. Pallavi Kshetrapal** and her team. It investigates the role of molecular factors in pregnancy complications and adverse outcomes, primarily in PTB. The other two outcomes that are being addressed are preeclampsia and SGA. The aims are to i) identify molecular signatures of PTB using a multi-omics approach to identify unique placental biomarkers ii) understand the molecular and cellular basis of adverse pregnancy outcomes (PET, PTB, SGA) within the placenta, and iii) identify mechanistic links between these biomarkers and altered placental function/s. Molecular signatures are being identified at the level of transcripts, proteins, and metabolites in maternal blood and placental samples collected in the Garbh-INi cohort. The ultimate deliverable is to dissect the role of the identified molecular signatures in disease pathophysiology using *in-vitro* cell/tissue culture and *in vivo* mice models.

Protein profiling of placental enriched exosomes

The Team conducted a nested case-control study within the longitudinal study design of the pregnancy cohort to determine the protein cargo present in circulating placental EVs in maternal plasma of term and preterm birth across the first, second and third trimesters.

Bioinformatics analysis of differentially expressed proteins revealed consistent upregulation of inflammatory pathways and epithelial-mesenchymal transition pathways at term and downregulation of coagulation/complement activation in preterm (*Menon R, Debnath C, et al*, Endocrinology 2020).

Circulating levels of sHLA-G in pregnant mothers and SGA births.

A study was conducted, by **Dr. Pallavi Kshetrapal** and her team, to determine the association between circulatory levels of sHLA-G in pregnant mothers and SGA births. The expression of HLA-G in the placenta is crucial for the establishment and maintenance of

pregnancy. Its aberrant expression could lead to perturbed immunological interactions in the placenta which could be associated with SGA births. HLA-G protein produced in the human placenta has been reported to enter maternal circulation. The objective of this study was to assess the difference in the trajectories of soluble HLA-G in maternal sera during pregnancy between women delivering SGA and appropriate for gestational age (AGA) neonates. A nested case-control study was performed by selecting the study participants enrolled in the GARBH-INI cohort. SGA was defined as birth weight less than 10th centile for a specific gestational age and sex. The neonates were categorized as SGA or AGA based on their weight at birth, sex, and completed gestational age in weeks using Fenton Growth Chart. Longitudinal profile of sHLA-G during pregnancy was estimated in the maternal sera collected at regular time points in pregnancy of North-Indian pregnant females delivering SGA (N=23) or AGA (N=17) neonates using sandwich ELISA. Linear mixed models were built and compared to study the association between sHLA-G levels during pregnancy and SGA births.

No difference was observed in the trajectory of sHLA-G during the period of pregnancy in mothers delivering SGA as compared to those delivering AGA neonates. However, a trend towards higher levels of sHLA-G at the first trimester and a sharp persistent drop throughout the second trimester was observed in mothers delivering SGA neonates. This can be explored further in studies with larger sample sizes. *(Submitted revised version to AJRI, 2021).*

Identification of biological pathway/s or nodes, using the multi-omics approach, where intervention could be tested will be followed up as leads to improve the function/s of the placenta for a healthy outcome.

Maternal micronutrients and mechanistic impact on fetal body composition

Dr. Suchitra Devi 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. This study is focused on identifying molecular mechanisms mediating lean muscle mass proportions *in utero* and maternal factors, in particular maternal vitamin D levels and maternal body composition, that influence fetal and infant body composition, using human umbilical cord-derived mesenchymal stem cells (uMSCs) and mouse models. The team established a repository of 17 uMSC lines from a

cohort of North Indian women. To understand the role of vitamin D in deciding stem cell fate during fetal development, the team used RNA-seq to identify 347 differentially regulated genes (DEGs), of which 130 skeletal muscle-specific genes were upregulated in the vitamin D treated uMSCs. They have built a comprehensive profile of metabolites in primary human skeletal muscle cells during myogenic progression in an untargeted metabolomics approach, identifying 71 unique metabolites including Pantothenate metabolism and Coenzyme A biosynthesis and Arginine Proline metabolism displaying dominant roles in proliferating myoblasts, while metabolites involved in vitamin B6, Glyoxylate and Dicarboxylate, Nitrogen, Glutathione, and Tryptophan metabolism being upregulated during myogenic differentiation (Kumar et al, 2020). To examine the consequences of vitamin D deprivation in lean muscle mass maintenance, mice lacking vitamin D receptor signaling (vdr-/-) were used. Increased skeletal muscle glycogen storage, leading to energy deprivation as indicated by reduced muscle ATP levels, increased AMPK activity, and hypoglycemia was observed. However, these metabolic defects could be bypassed by switching vdr-/- mice to a milk fat-enriched diet, which led to the alleviation of metabolic and proteostatic defects as well as skeletal muscle atrophy. The major outcomes and deliverables of the study are:

- The creation of a uMSC repository with maternal micronutrient information can be utilized to address relationships between multiple micronutrients (vitamin D, B12, folate, and iron) and any organ development *in vitro*. Using uMSCs, the team showed that vitamin D signaling is a key determinant in fetal muscle development. Additionally, rather than large volumes of cord blood routinely used to obtain MSCs with limited success, cord tissue -MSCs are a more reliable, robust, and convenient source for MSCs
- Mouse models demonstrate that the primary role for vitamin D signaling might be required for the optimal utilization of glucose from carbohydrate-rich diets in mammals.

Understanding fetal brain development

Dr. Yogita K Adlakha, SERB Research Scientist, focuses on understanding fetal brain development, dysfunction, and disorder.

Till now most of the information about the human brain has been obtained from animal models or autopsy human brain samples. These models have failed to mimic the human brain. Therefore, the team

wants to fill this gap by utilizing the human stem cell-based models which will recapitulate human brain cells to a larger extent.

Having expertise in deriving and culturing stem cells, her team has standardized and optimized protocols to derive induced pluripotent stem cells (iPSCs) from umbilical cord blood. They are in the process of deriving fetal-specific brain stem cells from iPSCs to understand fetal brain development using molecular, genetics, and cellular tools.

Randomized controlled trials to evaluate interventions for reducing early life morbidity that will have a quick impact on improving childhood outcomes

Evaluating interventions to reduce the burden of early life morbidity and mortality

Dr. Wadhwa's group with Dr. Bhatnagar as a Co-PI envisages evaluating cost-effective solutions to reduce early life morbidity and mortality. This is the key to achieving universal health coverage and reaching the Sustainable Development Goals (SDG) for health. Late phase clinical trials provide the evidence base and are the cornerstone of advances in clinical practice and/or health policy. The team has been leading large multicentre randomized controlled trials evaluating low-cost, affordable priority interventions for improving outcomes in early life.

Adjunct zinc for young infant sepsis

This large multicountry (India and Nepal) multisite trial with a sample size of 4200 being implemented under a DBT, Govt of India Program of Cooperation with the Kingdom of Norway, is being led and coordinated by **Dr. Wadhwa's group**. The individually randomized double-blind placebo-controlled parallel-group superiority trial is evaluating the efficacy and safety of 10 mg of elemental zinc given orally as an adjunct to standard therapy to infants aged 3-59 days, hospitalized with a clinical severe infection in reducing mortality both during initial hospitalization and in the 12-week follow-up period. Besides the primary outcomes of mortality, the trial investigates important secondary clinical outcomes like treatment failure, time to recovery, re-hospitalizations.

It was initiated in 2017 in 6 sites across India and Nepal and is currently being conducted at 4 hospitals in India Safdarjung hospital (SJH), Maulana Azad Medical College (MAMC), Chacha Nehru Bal Chikitsalaya (CNBC), and Kalawati Saran Children's Hospital (KSCH).

The conduct of this multisite trail has had many challenges with the premature closure of Nepal sites and the impact of COVID-19.

Major outcomes and milestones achieved

i. the **First study** in MCH to develop and use an **electronic data capture platform**.

ii. **Study progress**: Till 31st March 2021, the sites had screened 26,033 infants 3-59 days of age and enrolled 2,798 eligible infants with clinical severe infection. A little less than one-third of enrolled infants with clinical severe infection had associated diarrhea.

There were 235 deaths (8.4%) in the 12-week study period. The overall treatment failure rate is 14% and severe illness requiring rehospitalization of the enrolled infants in the 12-week follow-up period is 9.70% (262/ 2703).

Follow-up: The trial has maintained an excellent 12-week follow-up **rate of 98.6%.**

iii. Protocol paper was published in 2017 (BMC Pharmacol Toxicol. 2017; 18(1):56)

iv. **Safety monitoring and interim analysis:** The Data and Safety Monitoring Board (DSMB) for the trial has regular oversight and performed an interim analysis in December 2019 followed by an independent data review by a statistician. A consensus decision was taken to continue enrolling till November 2021 to improve the precision of the results and collect data for sub-studies and important secondary outcomes.

Three sub-studies have been embedded within this young infant sepsis program

Sub-study 1: Biological mechanisms that explain clinical effects of zinc supplementation

The first is to understand at the molecular level how zinc works under disease conditions, the cellular pathways responsive to zinc supplementation that determines clinical outcomes, and the host gene expression signatures that change on zinc supplementation and can predict clinical outcomes.

Neonates have underdeveloped innate immune responses that include decreased cytokine production, and reduced neutrophil and DC functions. It is now being recognized that the compensatory anti-inflammatory responses in children may be one of the key contributors to the pathobiology of pediatric sepsis. Therefore, understanding the molecular details behind the altered balance between the inflammatory and anti-inflammatory response in neonatal sepsis is essential to improve clinical outcomes. The team is studying the changes in

frequencies and numbers of different immune cells and their intracellular zinc levels at enrolment, 48-72 hours post enrolment, and discharge between the zinc/placebo supplemented groups. Immunophenotyping has been done in real-time for neutrophil and eosinophil subsets with their intracellular zinc levels on 315 enrolled infants. The other subsets are being evaluated in the PBMCs in batches.

Higher frequencies of total neutrophils (largely driven by the mature neutrophils) at enrolment were observed. This is corroborated by the values of absolute neutrophil counts and the percentage of polymorphonuclear cells obtained from the TLC data. Similarly, higher frequencies of monocytes and lower frequencies of T cells were observed at enrolment. This is consistent with what has been reported for sepsis where monocyte and neutrophil counts increase in response to infections and specifically CD4 T cell loss has been seen in septic patients of all ages. A significant difference in the intracellular zinc levels is seen between the recovered and not recovered infants. Infants in the "not recovered" group show higher zinc levels in neutrophils and eosinophils.

Sub-study 2: Initiated in 2021, the study investigates whether infants who have sepsis with associated diarrhea more often have enteropathogens in stool than those with no associated diarrhea. Further, the intestinal microbiome/ metagenome of young infants with sepsis will be characterized.

Substudy 3: The zinc equity study evaluates the health & economic consequences of adjunct zinc in young infant sepsis with a focus on health gains, financial risk protection, cost-effectiveness analysis, and equity impact.

Immediate and continuous kangaroo mother care (CMC) in very low birth weight infants

This multicountry open-labeled randomized controlled parallel-group superiority trial conducted in five hospitals in India, Ghana, Malawi, Nigeria, and Tanzania, evaluated the safety and efficacy of immediate and continuous KMC in newborns with a birth weight between 1 kg-1.799 kg compared to conventional care in an incubator or radiant warmer until their condition stabilized and were given KMC thereafter (control). The primary outcomes were death in the neonatal period and the first 72 hours of life. Initiated in 2017, the study conduct was concluded in February 2020.

Major outcomes and milestones achieved:

- A data analysis workshop was held in April 2020 to jointly analyze the combined data from all partnering sites and prepare the manuscript.
- The study shows a significant 25% reduction in neonatal deaths (relative risk of death, 0.75; 95% confidence interval [CI], 0.64 to 0.89; P = 0.001). The number needed to treat is 27. The study also shows a 35% reduction in risk of hypothermia and an 18% reduction in risk of clinical sepsis during hospital stay which is statistically significant. In the group that received iKMC, there was an interesting dose-dependent effect where the risk of death was lower in infants who received more hours of skin-to-skin contact per day.
- Dissemination: The results of the study have been communicated to the public through publication in a peer-reviewed scientific journal (WHO Immediate KMC Study Group, NEJM 2021 May 27; 384(21):2028-38), and a written press release has been circulated for mainstream media. The team is planning a national results dissemination meeting for relevant Ministry of Health officials. This high-quality impactful trial will have a crucial role in translating the results into recommendations and guidelines by the WHO.

iKMC neurodevelopment follow-up study

The cohort of very low birth weight (VLBW) babies enrolled in the iKMC trial are being followed up to evaluate the long-term impact of iKMC, our hypothesis being that continuous Kangaroo Mother Care initiated immediately after birth in VLBW babies will reduce the risk of ND impairment by 25%. The primary outcome of neurodevelopment is a composite outcome and includes rates of cerebral palsy, hearing impairment, vision impairment, mental and motor development, and epilepsy at two years of life. Secondary outcomes include growth, and feeding practices, mortality, maternal depression, and home environment (parent-child interactions). The follow-up study for the iKMC trial infants was initiated in November 2019.

Major outcomes and milestones achieved:

i. **Training workshop on neurodevelopment outcome assessment:** Training for cognitive, language, and fine motor development including social-emotional scale using Bayley Scales of Infant and Toddler Development (Bayley-III) was conducted in Tanzania.

   Training workshop for Hammersmith Infant Neurological Examination (HINE) assessment, epilepsy diagnosis, hearing, visual acuity, home environment, growth and morbidity, maternal depression was done by experts in India for all participating countries

ii. **Study progress:** Target enrolment is 1201, and 1000 infants have been enrolled till March 2021. Of these 96 infants had died and their verbal autopsies have been performed after all required permissions. 2795 toddlers have completed the 2-year assessments. The study is ongoing

Antenatal corticosteroids for late preterm birth

ACTION 3 trial: Consolidating evidence on available interventions to end preventable deaths and for improving survival of LBW infants, the team has collaborated with WHO on a large 13500 sample multicountry multisite three-arm, parallel-group, double-blind, placebo-controlled, randomized trial of two doses of antenatal corticosteroids for women with a high probability of birth in the late preterm period to test the efficacy & safety of antenatal corticosteroids for improving outcomes in late preterm newborns. The primary outcome is a composite outcome of stillbirth (post-randomization) OR neonatal death within 72 hours of birth OR use of respiratory support within 72 hours of birth or until discharge from hospital, whichever is earlier. The trial will also investigate several important secondary outcomes of efficacy and safety in both the newborn and their mothers. This study will be initiated in the last quarter of 2021

The team is currently setting up the hospital sites in India, Pakistan, Bangladesh, Kenya, and Nigeria for initiating this multicountry, multisite clinical trial.

Milestones achieved

i. **Protocol development workshop**: All sites contributed to protocol development organized in 2020 sponsored by WHO.

ii. **Ethics and other National approvals:** All site IEC approvals have been obtained; Health Ministry Screening Committee (HMSC) approval awaited

iii. **Training:** A structured training on protocol, GCP, study documents, questionnaires, data collection, IP management, safety reporting, outcome measurements conducted for all investigators and coordinators in the trial has been completed. Training of trainers' workshop planned for September 2021 and training and standardization of site study staff in September-October 2021is scheduled

EQUIFINANCE program

EQUIFINANCE aims to measure health gains, financial risk protection, and equity impact of neonatal and child health care interventions in four studies implemented in India. This

program was initiated in collaboration with Bergen Centre for Ethics and Priority Setting (BCEPS)-UiB, CISMAC, and CHRD-SAS. Two of the four work packages will be led and coordinated by **Dr. Wadhwa's** group.

Equity RCT: In one work package they propose to evaluate the equity and financial household impact of the facility-based ACTION 3 trial

Equity high-risk cohort: As another objective, the team will evaluate the equity and financial household impact of high-risk infant follow-up at a secondary health care level hospital.

Augmenting infrastructure for clinical research to create national resources by establishing clinical research units in partner hospitals, data management centers, and a biorepository

A. Clinical and Translational Research Units have been set up at the partner hospitals to enable the ongoing research and as a platform for new programs

B. THSTI Biorepository is an invaluable resource of well-phenotyped biospecimen collected and archived using standardized quality-controlled processes for answering questions of public health importance in the maternal and child health domain.

C. The Data Management Center and Aryabhata Data Science and AI program at THSTI is an aspiring state-of-the-art platform that seamlessly integrates clinical and biological data collection to analytical pipelines with standardized data capture and management workflows.

D. Nutritional biochemistry laboratory has been initiated to perform multiple micronutrient estimation

E. A platform for researching exosome biology and clinical metabolomics

Training programs to develop capacity among young investigators

A. Multiple clinical research methodology courses have been developed and conducted both at THSTI and outside at medical institutions for a diverse group of students

B. Aryabhata Data Science and AI program at THSTI conducts a half-yearly internship program for students and young investigators from diverse backgrounds such as computer science, mathematics, statistics, and medicine to work on the interface of data science and clinical research.

C. Advanced platforms training on placenta biology and metabolomics are periodically undertaken

D. Maternal and child health program at THSTI has successfully implemented young investigators programs such as the Early Career Medical Research Awardee program and supported independent fellowships like the Wellcome-Trust DBT India Alliance fellowship, Dr. MK Bhan young investigator fellowship, and the SERB Research Scientist Grant.

MEMBERS OF GARBH–Ini (in alphabetical order of surnames)

Translational Health Science and Technology Institute, NCR Biotech Cluster, Faridabad, India-Coordinating Institute (Vineeta Bal, Shinjini Bhatnagar (PI), Bhabatosh Das, Mahadev Dash, Bapu Koundinya Desiraju, Pallavi Kshetrapal, Sumit Misra, Uma Chandra Mouli Natchu, Satyajit Rath, Kanika Sachdeva, Dharmendra Sharma, Amanpreet Singh, Shailaja Sopory, Ramachandran Thiruvengadam, Nitya Wadhwa); National Institute of Biomedical Genomics, Kalyani, West Bengal, India (Arindam Maitra, Partha P Majumder (Co-PI) Souvik Mukherjee); Regional Centre for Biotechnology, NCR Biotech Cluster, Faridabad, India (Tushar K Maiti); Clinical Development Services Agency, Translational Health Science and Technology Institute, NCR- Biotech Cluster, Faridabad, India (Monika Bahl, Shubra Bansal); Gurugram Civil Hospital, Haryana, India (Umesh Mehta, Sunita Sharma, Brahmdeep Sindhu); Safdarjung Hospital, New Delhi, India (Sugandha Arya, Rekha Bharti, Harish Chellani, Pratima Mittal); Maulana Azad Medical College, New Delhi, India (Anju Garg, Siddharth Ramji), The Ultrasound Lab, Defence Colony, New Delhi, India (Ashok Khurana); Hamdard Institute of Medical Sciences and Research, Jamia Hamdard University, New Delhi, India (Reva Tripathi); All India Institute of Medical Sciences, New Delhi, India (Alpesh Goyal, Yashdeep Gupta, Smriti Hari, Nikhil Tandon); Government of Haryana, India (Rakesh Gupta); International Centre For Genetic Engineering and Biotechnology, New Delhi, India (Dinakar M Salunke Co-PI); G Balakrish Nair (Rajiv Gandhi Centre for Biotechnology, Trivandrum); Gagandeep Kang (Christian Medical College, Vellore).

List of collaborators

Name of the collaboration	Collaborating institutes
Inter-Institutional Program for Maternal, Neonatal, and Infant Sciences A translational approach –interdisciplinary Group for Advanced Research on BirtH outcomes - DBT INdia Initiative (GARBH-Ini Phase II)	THSTI, Faridabad NIBMG, West Bengal RCB, Faridabad SJRI, Bangalore Gurugram Civil Hospital, Gurugram Safdarjung Hospital, Delhi
Zinc as an adjunct for the treatment of very severe disease in infants younger than 2 months	THSTI, Faridabad KSCH, New Delhi Safdarjung Hospital, Delhi CNBC, Delhi MAMC, Delhi
Understanding human COVID-19 infections: a DBT India Consortium	THSTI, Faridabad ESIC Hospital, Faridabad Loknayak Hospital, New Delhi Regional Centre for Biotechnology, Faridabad National Institute of Immunology, New Delhi
Multi-Omics for Mothers and Infants	THSTI, Faridabad NIBMG, West Bengal RCB, Faridabad SJRI, Bangalore Safdarjung Hospital, Delhi Gurugram Civil Hospital, Gurugram AMANHI, Pakistan AMANHI, Bangladesh AMANHI, Tanzania GAPPS, Zambia GAPPS, Bangladesh University of California, San Francisco, USA Stanford University, USA
CALOPUS - Computer-Assisted LOw-cost Point-of-care UltraSound	THSTI, Faridabad University of Oxford, UK
Garbh-Ini - India Pregnancy Risk Stratification Platform Alignment (GIPA)	THSTI, Faridabad Makunda Christian Leprosy and General Hospital, Bazaricherra, Assam Christian Medical College and Hospital, Vellore Society for Applied Sciences New Delhi
ORCHESTRA: Connecting European Cohorts to Increase Common and Effective Response to SARS-CoV-2 Pandemic	THSTI, Faridabad SJRI, Bangalore

Severe SARS-CoV2 related disease in low-and-middle-income country children aged 0-19 years: a multi-country observational study in a network of hospitals	THSTI, Faridabad ESI Medical College and Hospital, Faridabad Vardaman Mahavir Medical College, New Delhi; Safdarjung Hospital, New Delhi Maulana Azad Medical College, New Delhi St John's Medical College, Bengaluru Asian Hospital, Faridabad
A multi-country randomized clinical trial to 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	THSTI, Faridabad Safdarjung Hospital, New Delhi World Health Organization, Switzerland Other PIs from Ghana, Malawi, Nigeria, and Tanzania
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 a low-resource setting	THSTI, Faridabad Safdarjung Hospital, New Delhi World Health Organization, Switzerland
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	THSTI, Faridabad Tribhuvan University, Nepal University of Bergen, Norway University of Bergen, Norway Safdarjung Hospital, New Delhi Kasturba Hospital, New Delhi Maulana Azad Medical College, New Delhi
ACTION III A multi-country, smulti-center, three-arm, parallel-group, double-blind, placebo-controlled, randomized trial of two doses of antenatal corticosteroids for women with a high probability of birth in the late preterm period in hospitals in low-resource countries to improve newborn outcomes	THSTI, Faridabad Safdarjung Hospital, New Delhi World Health Organization, Switzerland
Equity and financial household impact in randomized controlled trials, implementation research, and cohort studies in India	University of Bergen, Norway THSTI, Faridabad Society for Applied Sciences New Delhi
DBT-Human Placental program	THSTI, Faridabad

	NIBMG, West Bengal RCB, Faridabad
Molecular mechanisms of Vitamin-D on skeletal muscle function	THSTI, Faridabad NII, New Delhi


Gate No. 2


ट्रांसलेशनल स्वास्थ्य विज्ञान एवं प्रौद्योगिकी संस्थान

MULTIDISCIPLINARY CLINICAL AND TRANSLATIONAL RESEARCH

Diagnostics of Infectious diseases

Tropical fevers

Acute febrile illness (AFI) is common in the tropics/ subtropics and is 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 POCT. 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. Gaurav Batra's** team is working on the development of improved POCTs for different tropical febrile illnesses, including Malaria, Dengue, and Scrub Typhus.

Second-generation dengue NS1 antigen detection assays

Commercially available rapid tests for dengue virus (DENV) NS1 antigen have the following problems: 1) Poor sensitivity and specificity compared to lab-based ELISA test; 2) Extremely poor sensitivity in the secondary DENV infection due to the immune-complex formation with human anti-NS1 antibodies from primary infection; 3) Variable sensitivity for different DENV serotypes. Last year, Dr. Gaurav Batra's team reported the development of a dipstick assay, in liquid conjugate format, for the detection of NS1 antigen. As part of this test, additional work was carried out to stabilize all test components in dry form. In comparison, with a reputed commercial NS1 ELISA, the developed rapid NS1 assay showed a sensitivity of 95.24% (95%CI: 86.71% to 99.01%) that is way higher compared to other rapid tests. Moreover, the specificity was found to be 100.00% (95%CI: 96.38% to 100.00%).

Way ahead

Dr. Batra's long-term plan is firstly to develop well-performing singleplex POCTs for the detection of major tropical fevers and then to develop a multiplexed POCT for simultaneous

detection of several tropical fevers. Over the next year, they will finalize an antibody test for scrub typhus.

TB Diagnostics

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

Dr. Sharma and his team developed aptamers against GlcB antigen (also known as Malate synthase), a highly immunogenic TB antigen and it is also an attractive biomarker as it is expressed independently of HIV co-infection in the early stages of TB infection. An aptamer, MS_EPTB10 showed the highest binding to GlcB in the ascitic fluid background and was selected for further studies. Further, the selectivity of MS_EPTB10 was assessed by ALISA with a panel of culture filtrates and whole-cell lysates of various bacterial species that were selected based on their occurrence in various biological fluids during infection. The results showed that the EPTB10 aptamer binds to GlcB with a high level of selectivity. The diagnostic sensitivity of GlcB ALISA ((an aptamer-based assay) was higher at 50% (95% CI: 35;65) compared to that of GlcB ELISA (an antibody-based assay) at a specificity ≥ 93% for both tests. This indicated the superiority of aptamer-based detection over antibody-based detection of GlcB in terms of diagnostic performance for abdominal tuberculosis (aTB).

In parallel, HspX protein was also detected using M6 aptamer and anti-HspX polyclonal antibodies that were generated previously. The sensitivity of HspX ALISA was also superior to that of HspX ELISA (~84% vs. ~18%) (p-value <0.0001), with a specificity of ≥ 93% for both assays. Between the two aptamer-based assays (M6, targeting HspX and EPTB10-targeting GlcB), M6 HspX aptamer exhibited a significantly superior diagnostic performance with a sensitivity of ~84% (95% CI:70;93) as compared to 50% of GlcB targeting EPTB10 aptamer, and a specificity of ~96% (95% CI: 88;99). Amongst the two aptamers developed, M6 HspX aptamer-based ALISA performed the best in ascitic fluid in this study.

Initial validation of these aptamers on ascitic fluid samples gives a highly encouraging result. However, a large-scale validation of GlcB/HspX aptamers will pave the way to a rapid and accurate diagnostic test for aTB.

Way ahead

Dr. Sharma's team has successfully developed aptamers and planning to establish the clinical utility of HspX/GlcB specific aptamers through multi-site validation.

Antimicrobial resistance (AMR) diagnostics

Dr. Niraj Kumar and **Dr. Susmita Chaudhuri** led team has been primarily working on developing rapid diagnostics for AMR. Unnecessary/inappropriate use of antibiotics, due to unavailability of rapid diagnostics for pathogen identification and antimicrobial-susceptibility-testing (AST), has been a significant factor contributing towards emergence/spread of AMR.

Therefore, Dr. Kumar's team has been working to develop syringe compatible, single-use, affordable, and user-friendly combination of devices that can isolate bacteria from whole-blood/urine samples, enrich, culture, identify and establish their antimicrobial susceptibility profile(s) for multiple drugs simultaneously within 90 minutes. His team is also interested in identifying predictive biomarkers for the emergence of AMR among pathogens. As of now, the focus of research has either been on identifying biomarkers for differentiating resistant from sensitive cells or therapeutic targets. This work may enable the marking of antibiotics for their selective regulation before they become completely ineffective for the purpose.

Over the last year, Dr. Kumar's team focused on

i) **Rapid Pathogen capture and culture**: They evaluated the utility of a 1-hour culture of pathogens for their detection using 4 bacterial pathogens grown in native biofluids (human plasma) with PCR based method. The work is ongoing to evaluate it with more pathogens. The work is also ongoing for developing a Minimally Viable Product (MVP) prototype for rapid pathogen capture from biofluids and their 1-hour culture.

ii) **Pathogen identification**: They identified unique gene/protein fragments among ESKAPE pathogens using an *in-silico* workflow. The same has been validated for pathogen-specificity using the PCR-based method. The work is ongoing to make a PCR-based multiplex-test for the identification of ESKAPE pathogens and also to develop diagnostic-grade binders for developing multiplex and point-of-care lateral flow-based diagnostic assay.

iii) **Early-biomarker discovery for AMR**: The team started *in-vitro* provoking blood-stream pathogen (*K. pneumoniae*) to develop antimicrobial resistance against most commonly used antibiotics.

Dr. Susmita Chaudhuri's group adopts a comprehensive approach to address AMR in the form of innovating rapid diagnostics, developing alternative therapies such as anti-biofilm formulations and monoclonal antibodies. Among many extramurally funded endeavors, the innovative project that is at the most advanced stage is the novel eco-friendly anti-biofilm formulation to reduce surgical site infections. Surgical site infections (SSIs) are one of the most common causes of Healthcare-Associated Infections. This issue has acquired greater importance due to the growing concern of antimicrobial resistance (AMR) and so the need for antimicrobial sutures is growing very fast. The team of Dr. Chaudhuri has initiated a multi-disciplinary collaborative project and received a Mission AMR grant in 2019 and has been working on developing biofilm inhibiting coating formulations, which can be deposited on contact lens cases and surgical sutures made of nylon, silk, vicryl sutures to render them antibacterial. The coated substrates were tested for biofilm inhibition using different bacterial strains of clinical importance.

Surface texture modification for reducing physical adherence of bacteria can be a critical alternative to conventional antimicrobials, especially in the case of surgical accessories. Two different nanocomposite coating formulations capable of exhibiting biofilm inhibiting properties were synthesized by Dr. Chaudhuri's team. One of the compositions contained a biocide and could yield a hydrophobic coating. The other composition did not contain any biocide and could yield a coating with improved hydrophobicity. Both the coating compositions were deposited on different substrates like cover glass slips, acrylonitrile butadiene styrene (ABS) coupons, and surgical sutures made of polyglactin 910, nylon, and silk by dip-coating technique. Both the nanocomposite coatings were found to exhibit substantial biofilm inhibition against the different bacteria tested. A durability study with respect to temperature, humidity, body plasma, and cytokine was also carried out for the coated polyglactin 910 sutures. The biofilm inhibition property and durability of the nanocomposite coated suture were compared with the commercially available antibacterial sutures and the performance of coatings was found to be promising. The developed coatings have a promise as an alternative to the existing cytotoxic antibacterial coatings currently available in the market especially for its healthcare application in surgical accessories.

Way ahead

Dr. Kumar plans to develop a Minimally Viable Prototype (MVP) for rapid pathogen capture and culture, develop diagnostic-grade binders against at least 2 pathogens and establish a well-characterized panel of resistant cells, representing early-, mid-, and late-stages of the process of achieving resistance.

Dr. Chaudhuri plans to validate the results obtained with her designed products with respect to other *in vitro* and *in vivo* tests that can make the coated product acceptable in the market. The team has already initiated efforts in this regard to rope in industrial partners and acquire extramural funding for safety and efficacy studies in small animals.

SNAKE BITE DIAGNOSTICS

Snake bite reports on an average ~58,000 deaths annually in India only; the reason is lack of diagnostics for differentiating between poisonous and dry bites and ascertaining the venomous species. As a mainstay of treatment polyvalent antiserum is administrated which are reported to elicit severe immune responses. Hence, there is a need to develop a diagnostic method that can differentiate the snake species. **Dr. Tarun Kumar Sharma's** lab is working to develop Point-of-care (POC) diagnostics to identify the snake bite and biting species so that bi- or monospecific antivenom could be administered to reduce the immune responses. In addition to this, these tests can also contribute to forensic investigation.

A panel of aptamers against crude venom of *Bungarus caeruleus* (Indian Krait) has been developed by his group. A well-optimized SELEX strategy was used for the generation of aptamers followed by next-generation sequencing that led to the identification of a panel of aptamers showing high binding to the venom of *Bungarus caeruleus*. His group assessed the selected aptamer binding against krait venom obtained from three geographically distinct populations (Central, East, and Southern India) using ALISA. The aptamers generated against the crude venom also led to the identification of the specific component of the venom, which is β-Bungarotoxin, a toxin uniquely present in the *B. caeruleus* venom. The best performing aptamer candidates were used as a molecular recognition element on a paper-based device. The developed aptamer-based paper device can be used for potential point-of-care venom detection applications due to its simplicity and affordability.

To detect and discriminate between venomous and non-venomous/dry snake bites, aptamers against Phospholipase A (PLA2), an integral component of snake venom of venomous snake species were also generated. The developed aptamers are a broad spectrum and can detect

crude and purified PLA2 in the venom of snakes. Significantly, aptamers of the present invention bind to the PLA2 with nano-molar affinity. The clinical utility of the developed aptamers will be assessed in serum and swab from the bite site in near future.

Way ahead

After a satisfactory clinical evaluation, Dr. Sharma's team aims to develop an aptamer-based on-site detection device for POC diagnostics. The envisaged POC diagnostic will be evaluated against body fluids for the detection of snake bites.

MONOCLONAL ANTIBODIES AS IMMUNODIAGNOSTICS AND IMMUNOTHERAPY

The early, accurate diagnosis and treatment of pathogens involves the development of reagents/intermediates of high quality. Inadequate diagnosis and antibiotic intake for therapy often contribute to the development of AMR strains. Also, the treatment for such strains needs targeting of these microbes specifically with monoclonal antibodies as an alternate therapy to antibiotics, particularly in compromised patients. **Dr. Chandresh Sharma** has been working towards specific monoclonal antibody generation by targeting the disease etiologies (viruses; CHIKV, and bacteria; ESKAPE group pathogen) for efficient diagnosis and therapy.

Monoclonal antibodies (MAbs) are the pivotal molecules for immunodiagnostic testing. Dr. Sharma's team has generated a pool of MAbs against the *Klebsiella pneumoniae* lipopolysaccharide (O antigen) using the mouse hybridoma technology to help the diagnostic translation through the development of a pathogen-specific binder. At present, they are analyzing clones employing biochemical/biophysical parameters in terms of binding specificity, affinity, and robustness.

Chikungunya virus (CHIKV), an alphavirus causes fever and severe arthralgia. Currently, no vaccine or specific therapy is available for human CHIKV infection. However, a virus-like particle vaccine containing CHIKV envelope proteins has recently been produced, offering protection in a simian model. In mice, serum IgG from CHIKV convalescent patients can successfully prevent and cure the infection. CHIKV has a positive-stranded RNA genome that encodes four non-structural proteins (NSP1-4) as well as three structural proteins (capsid,

E1, and E2 envelope proteins). Dr. Sharma and his team, therefore, are attempting to isolate and characterize CHIKV-neutralizing human MAbs against E2 with therapeutic potential. His team has successfully created a pool of antigen-specific-murine-monoclonal antibodies (which includes 40 positive clones) using mouse hybridoma technology by harnessing their robust nature and exalted binding. His team now plans to expand and engineer the positive clones for the production of humanized MAbs with therapeutic potential by neutralizing CHIKV.

Way ahead

Dr. Sharma and his team plan to characterize *Klebsiella pneumonia (Kp)* specific MAbs by sequencing antibody genes and determine their binding affinity. Cross-reactivity with other organisms from the ESKAPE group will also be checked. They plan to develop a prototype assay to demonstrate the proof of concept for improved and specific diagnosis of *Klebsiella pneumonia*. Final evaluation and validation of the prototype assay will be performed utilizing the clinical specimens with *Kp* infection.

His team plans to test the MAb clone specific to the E2 protein of CHIKV for its neutralization potential and plans to sequence the clones. Positive clones with high neutralization titers will be genetically modified for chimerization and humanization. The therapeutic potential will be reassessed post-editing of mAbs by his team. Robust binding clones will be assembled to develop an antigen detection test against the CHIKV infection to provide a proof of concept in RDT. Clinical research will be essentially done to validate the potential and determine the sensitivity and specificity of the assay.

MAMMALIAN BIOPROCESSING

Chinese Hamster Ovary (CHO) cells are the most commonly used host-cell lines for industrial production of high-quality recombinant proteins, accounting for ≥70% of all current therapeutics. However, the cost of such therapeutics is high. To find a solution to this, **Dr. Niraj Kumar** and his team have been working for translating knowledge from natural and professional producers into industrial producers ‘CHO cells’ for improving the yield from mammalian bioprocesses.

Dr. Kumar’s team performed an extensive published literature search and has fetched many potential master-orchestrator gene-of-interest (GOIs). Surprisingly, the expression of a majority of these GOIs has not been explored for protein production in CHO-cell factories to date. Therefore, as a first step, they are evaluating the expression of these GOIs, for the

growth and protein production, over the different phases of batch culture. For this, RNA from cells representing good-grower but bad-producer and bad-grower but good-producer have been isolated. The primers for these GOIs have also been developed. A few of them have been validated for their specificity using DNA-sequencing-based approaches. The work is ongoing to evaluate the expression of GOIs.

Way ahead

Dr. Kumar and the team plans to evaluate the impact of identified GOIs on cell growth and recombinant protein productivity and validate it using inhibition/over-expression assays.

List of collaborators

Dr. Gaurav Batra

Dr. Urpo Lamminmäki, University of Turku, Finland

Prof. Kim Pettersson, University of Turku, Finland

Prof. Rakesh Lodha, AIIMS, New Delhi

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

Dr. Navin Khanna, ICGEB

Dr. Niraj Kumar

Dr. Arti Kapil, AIIMS, New Delhi

Dr. Susmita Chaudhuri

Dr. Subasri Raghavan, ARCI, Hyderabad

Dr. Prashant Garg, LV Prasad Eye Institute, Hyderabad

Dr. Tarun Sharma

Dr. Anita Malhotra, Bangor University, UK

Prof. Jaya S. Tyagi, AIIMS, Delhi

Prof. Neera Sharma, RML Hospital, Delhi

Dr. V.P. Myneedu, NITRD, New Delhi

Dr. Amit Kumar, IIT, Indore

Dr. Robin Doley, Tezpur University, Assam

Dr. M.V. Khadilkar, Premium Serum and Vaccine Pvt. Ltd.

Dr. Guruprasad Medigeshi, THSTI

Dr. Tripti Shrivastava, THSTI

Dr. Chandresh Sharma

Dr. Amit Awasthi, THSTI

Dr. Amit Pandey, THSTI

Dr. Milan Surjit, THSTI

Dr. Niraj Kumar, THSTI

Dr. Susmita Chaudhuri, THSTI

Dr. Shailendra Asthana, THSTI

Dr. Rajesh Kumar, THSTI

NON-COMMUNICABLE DISEASES

INTERPLAY BETWEEN EFFECTOR AND REGULATORY T CELLS IN AUTOIMMUNE DISEASES, INFLAMMATORY BOWEL DISEASE, PSORIASIS & MULTIPLE SCLEROSIS, AND INFECTIOUS DISEASES

The Immuno-biology laboratory at THSTI led by **Dr. Amit Awasthi** focuses to understand the molecular pathways that define the generations and functions of effector and regulatory T cells in autoimmune disease conditions. More precisely, the laboratory is primarily focusing to understand the functions of Th9 and Th17 cells in inflammatory bowel disease, asthma, and cancer immunity. In addition, the laboratory is involved in identifying the cellular pathways that are modulated by intracellular pathogens for their survival. Using *Salmonella* infection as a model, the Immunobiology laboratory identified host-directed therapeutic intervention to control *Salmonella* infection.

Elucidation of molecular pathways leading to the generation and functions of Th9 cells

Cytokines such as TGF-β1 and IL-4 together skews naïve $CD4^+$ T cells to IL-9 producing T helper cells, named as "Th9" cells. Helper T (Th) 9 cells play a critical role in promoting anti-tumor immunity, allergic inflammation, and autoimmune diseases. Dr. Awasthi's team has identified transcription factors that are essential for the development of Th9 cells. Understanding the generation of Th9 cells will allow modulating effector functions of these cells during the pathophysiology of asthma and anti-tumor immune responses. Thus, the identification of biomarkers for Th9 cells is crucial for developing diagnostics and therapy for promoting the clinical outcomes for patients with Th9-mediated diseases. For deciphering the complete identity of a cell, quantitative proteomics is an important tool, as there is a discordance between transcriptomes and proteomes due to post-translational modifications and differential rates of protein synthesis. The mRNA level of a gene is poorly correlated with the protein levels in different conditions due to various above-mentioned reasons. Therefore, despite several transcriptomes analyses, global proteome profiling has gained significant importance for mapping protein signatures at the cellular level to unravel their precise functional characterization. Proteomic analysis identifies post-translational functions of proteins, which remains obscure in transcriptomics. Given the important functions of Th9 cells in anti-tumor immunity, proteome analysis of Th9 cells was undertaken to understand the involvement of proteins that might be crucial for the anti-tumor functions of Th9 cells. A

comprehensive proteomic analysis of murine Th0 and Th9 cells was performed to identify proteins that are enriched in Th9 cells.

Pathway analysis identified an abundance of phosphoproteins in the proteome of Th9 cells as compared to Th0 cells. Among upregulated phosphoproteins, Ppp2ca (catalytic subunit of protein phosphatase, PP2A) was found to be highly enriched in Th9 cells. Although the role of PP2A has been shown to regulate the differentiation and functions of Th1, Th2, Th17, and Tregs, its role in the differentiation and functions of Th9 cells is not identified yet. Here, Dr. Awasthi's team found that PP2A is required for the induction of Th9 cells, as PP2A inhibition leads to the suppression of IL-9 and the expression of key transcription factors of Th9 cells. PP2A inhibition abrogates Th9 cell-mediated anti-tumor immune response in the B16-OVA melanoma tumor model, indicating an essential role of PP2A for the differentiation and anti-tumor functions of Th9 cells.

Role of host cell pathways that are essential for the survival of intracellular pathogens

The global rise of antibiotic-resistant strains of *Salmonella* has necessitated the development of alternative therapeutic strategies. Recent studies have shown that targeting host factors may provide an alternative approach for the treatment of intracellular pathogens. Host-directed therapy (HDT) modulates host cellular factors that are essential to support the growth and survival of intracellular pathogens. Using the proteome analysis of *Salmonella*-infected macrophages, Dr. Awasthi and his team found that EGFR, a host factor, promotes intracellular survival of *Salmonella* via activation of an mTOR-HIF-1α axis. Blocking of EGFR, mTOR or HIF-1α inhibits the intracellular survival of *Salmonella* within the macrophages and in mice. Global proteo-metabolomics profiling indicated the upregulation of host factors predominantly associated with ATP turnover, glycolysis, urea cycle, which ultimately promote the activation of EGFR-HIF1α signaling upon infection. Importantly, inhibition of EGFR and HIF1α restored both proteomics and metabolomics changes caused by *Salmonella* infection. Taken together, these studies identify EGFR and HIF1α as potential HDT against *Salmonella* infection and might be useful for the treatment of other intracellular infections.

IMPLANTABLE MEDICAL DEVICE AND 3-DIMENSIONAL ORGANOID MODELS

Biocompatible placenta-derived materials for soft tissue wound healing

Medical device availability, accessibility, and effectiveness are critical in achieving the highest quality of care within healthcare systems. According to the World Health

Organization (WHO), India has the world's most significant number of road accident fatalities involving craniofacial injuries (World Health Organization 2009). In addition, burn injuries occur at a rate of one million per year in India, with 90% of these being superficial and superficial partial-thickness burns; burn injuries pose a significant challenge for the limited wound dressing materials available. Traditionally, cosmetic/plastic surgeons have repaired various anatomical defects with cryopreserved amniotic membrane, collagen-based scaffolds, and autografts from the skin, bone, and fascia, leaving a significant donor-site deficit. Some of the complications associated with synthetic grafts include chronic infection, implant rupture, and pain.

Dr. Santosh Mathapati's team hypothesized that host dynamic reciprocity-driven biochemical, biophysical, and cellular responses of acellular, cross-linked, and detoxified amniotic membrane may be helpful in soft tissue repair, such as wound healing. His team is investigating tissue biocompatibility using *in vitro* cytotoxicity and full-thickness excisional wound animal models. The extracellular matrix of the placental cotyledon will be used to prepare hydrogels in this study. These hydrogels will be tested for soft tissue repair and *in vitro* disease models for SARS-CoV-2 and Non-Alcoholic Fatty Liver Disease (NAFLD) pathogenesis research. Presently, his team has successfully developed decellularisation techniques for amniotic membrane and cotyledon. The novel strategy would provide new options for wound care products for combat casualty care and wound healing, including the reconstruction of extended acute limb ischemia and battle wounds and facilitating clinical translation in soft tissue engineering.

Placental tissue and 3-dimensional human hepatic organoid model

Cell-based screening has been a vital component of the drug discovery process as it provides a simple, fast, and cost-effective tool for avoiding large and expensive animal testing. Hepatocyte-like cells (HLCs) derived from human pluripotent stem cells (hPSCs) such as embryonic stem cells (ESs) and induced pluripotent stem cells (iPSCs) have shown great promise in meeting this need by providing an infinite source of cells that mimic the genotype of the donor or primary hepatocytes. Dr. Mathapati's team investigated the formation of 3-dimensional (3D) hepatic organoids using placental tissue (amniotic membrane and cotyledon) and hepatic cell line (HepG2), and HLCs derived from hPSCs. In near future, a two-dimensional vs 3D method would be used to examine the long-term functional

maintenance of hepatocytes. His team currently tests different photoinitiators to open up potential avenues for using hydrogel photocrosslinkable systems for *in vitro* applications in disease modeling with enhanced processing efficiency and cell viability. Dr. Mathapati's team has synthesized gelatin methacryloyl (GelMA) hydrogels. In addition, his team also synthesized nanofibers (natural and synthetic polymers) for a 3D tissue culture system. The HLCs derived from hPSCs and hepatic cell line-based organoid models, along with developed materials, may be helpful in the broader disease modeling and drug discovery framework.

Way ahead

Dr. Mathapati plans to develop surgical wound healing materials, cardiovascular implants (conduits and patches), orthopedic implants, and 3-dimensional organoid models for drug discovery and disease modeling.

NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

Non-alcoholic or metabolic fatty liver disease (NAFLD) is induced by excessive accumulation of lipids in the liver, involving oxidative stress, inflammation, apoptosis, and metabolic dysregulation. Non-Alcoholic Steatohepatitis (NASH) is a liver manifestation of NAFLD characterized by liver steatosis, inflammation, and injury of liver cells with or without fibrosis. Currently, the disease is being managed by calorie restriction, physical exercise, lipid-lowering drugs, insulin sensitizers, antioxidants, and bariatric surgery, while there is no approved treatment for NASH and for preventing/halting the progression of fibrosis. Though many drug candidates are currently in clinical trials, none is so far approved by FDA for the treatment of NASH or preventing/halting the progression of fibrosis. NASH is a very challenging disease with >30 % global population being affected with this in one form or another and there is no drug available to date. The lack of validated biomarkers in NASH which can predict the disease development is one of the key difficulties not only for the disease diagnosis purposes but also for patient stratification, assessment of efficacy, and overall process of drug discovery. The presence of comorbidities also increases disease complications.

The team of scientists in the non-communicable disease (NCD) program works in tandem to achieve the following major objectives on fatty liver disease-

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

- Standardization and validation of *in vitro* and *in vivo* models of non-alcoholic fatty liver disease (NAFLD), to evaluate new chemical entities (NCEs) and plant-based extracts to identify therapeutic leads.

The team of scientists for the NCD program broadly work on two dimensions: therapeutics and biomarkers for NAFLD/NASH

Drug discovery for non-alcoholic fatty liver disease (NAFLD)

Development of small molecule-based NCEs and herbal extracts for NAFLD/NASH

One of the keys focuses of the NCD program is the pre-clinical drug discovery with major emphasis on synthetic medicinal chemistry and Distribution, Metabolism, and Pharmacokinetic (DMPK) studies. **Dr. Dinesh Mahajan's** group at THSTI is working on the identification and development of new small molecule-based drugs as well as herbal extracts for NAFLD with a major emphasis on NASH. For herbal drug development for NASH, he is collaborating with an industrial partner and the goal is to validate standardized extracts of Indian herbs for their therapeutic application and safety for NAFLD using modern drug discovery tools. His team has identified a novel series of small molecules and a drug lead, DR62 based on *in vitro* screening assays by exploiting autophagy induction as a possible therapeutic approach for NAFLD. The newly identified compounds were found to be potent autophagy inducers and restricted the triglyceride load and improved the overall cellular health parameters in cell-based assays. Based on medicinal chemistry optimization studies, a potent and orally bio-available drug lead was identified which demonstrated significant therapeutic effect in an animal model by reducing the triglyceride levels in rat liver when dosed orally for four weeks in Proof of Concept (POC) studies based on Choline Deficient High Fat (CDHF) induced NAFLD model. This PoC studies also established a first-in-class therapeutic approach for NAFLD using autophagy inducers based on small molecules. Dr. Mahajan's team working to generate more analogs around DR62 to understand Structure-Activity Relationship (SAR) as well optimizing the pharmacokinetic profile. In a target-specific approach for drug discovery, his team designed, synthesized, and identified a new hit (DR310; IC50=1 µM based on enzymatic assays) based on a novel scaffold as an ASK-1 inhibitor. His group has designed and synthesized second-generation analogs around identified hit DR310 for potency optimization and other *in vitro* evaluation

studies aiming identification of a drug lead. They plan to generate a SAR around DR310 for further optimization.

Targeting FXR and ASK1 proteins for small molecule discovery against NASH using computational approaches

As the pathogenesis of NASH is a complex process involving metabolic disorder and uncontrolled chronic inflammation and fibrosis, the ideal drug target (s) can be a signaling factor(s) that mediate these major pathophysiological pathways. In this regard, **Dr. Shailendra Asthana** and his team are focusing on two targets viz., Farnesoid X receptor (FXR) and Apoptosis signal-regulating kinase 1 (ASK1) to explore for the discovery of small molecules. For FXR, Dr. Asthana's team is working towards mimicking the clinically advanced drugs interaction pattern by identifying the different chemical moieties as an agonist. Furthermore, since full agonism leads towards other major complications such as cardiovascular diseases, therefore, the molecular understanding of partial agonism is also in progress. The hyperactive ASK1 signaling has been recognized as a molecular hallmark in the livers of obese and NAFLD individuals. ASK1 stimulates the deregulation of lipid and glucose metabolism and prompts the inflammatory response in the liver for the most part through a downstream p38–JNK1 and JNK2 (JNK1/2) pathway. Therefore, Dr. Asthana and his team are working to understand the mechanism of various ASK1 regulators at their hyperactive state to explore the ASK1 endogenous regulators via protein-protein interaction approaches for small molecule discovery.

***In vitro* assays**

Dr. Ruchi Tandon's lab is pursuing the drug discovery programs for NASH at THSTI to identify small molecule modulators of key hallmarks of the disease for therapeutic intervention. Her lab is following both targeted and non-targeted approaches to identify potential hits from *in vitro* screening platforms. Dr. Tandon's team is currently working on two potential modulators of disease, Ask-1, and FXR.

- **Small molecule inhibitors of Ask-1**

An *in vitro* enzyme assay using an in-house Ask-1 catalytic domain has been standardized using the catalytic domain of Ask-1 protein by Dr. Tandon's team. Protein was expressed and purified to set up the kinase assay using ADP Glo technology. Two compounds DR0309 and DR0310 were identified with IC50 values in the range of 1 µM.

- **Small molecule FXR agonists**

  Dr. Tandon's lab has set up a luciferase reporter gene assay to identify potential FXR agonists. A preliminary assay has been done using GW4064 as a positive control. Evaluation of test compounds is in the process. Recombinant FXR ligand-binding domain was expressed and purified to identify FXR agonists using *in vitro* assays.

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

***In-vitro* Assays for NASH Using Cell Lines**

The available models to study metabolic and mechanistic processes integral to the pathogenesis of human NAFLD/NASH have their shortcomings and therefore are not very conclusive. Human hepatocytes seem to be the closest model to clinical conditions. However, a limited number of human liver samples and related ethical and logistic issues prevent the widespread use of primary cultures of human hepatocytes. Hepatic cell lines are a reliable substitute over primary culture.

Dr. Ajay Kumar and **Dr. Ruchi Tandon** along with their team have developed phenotypic *in-vitro* assays for NASH based on its key hallmarks (steatosis, inflammation) and fibrosis using human hepatoma cell models viz., HepG2, THP1, and LX2 cell lines.

Dr. Kumar's team used PMA differentiated THP1 cell line, induced with LPS and IFN-γ, for screening compounds/herbal extracts showing anti-inflammatory efficacy. Gene expression of MCP1 in un-induced versus induced conditions was used to establish an inflammation model in the THP1 cell line. Monocyte Chemoattractant Protein-1 (MCP1 or CCL2) MCP1 gene expressions were significantly inhibited in presence of Silymarin, Bhumyamalaki, Guduchi, Haridra, and Kalamegha.

Hepatic stellate cells (LX2 cell line), maintained in serum-depleted conditions (2% serum), were activated in presence of TGFβ (4ng/ml) for 24 hours. The fibrotic phenotype was investigated by targeting the gene expression of the collagen (Col1A1) gene. Col1A1 gene expression in hepatic stellate cells reflects their activation to myofibroblast-like cells and has been directly related to experimental liver fibrogenesis, and indirectly to human fibrosis in chronic liver disease. Using this model, Dr. Kumar's team observed a significant reduction in

expression of the Col1A1 gene after treatment with Pippali, Guduchi, Haridra, and Kalamegha.

Dr. Kumar's team shortlisted four herbal formulations (Mustak, Bhumiamalaki, Chirayata, and Haritaki) from lipotoxicity studies while two others (Pippali and Sharpunkha) were prioritized from steatosis assay for further investigations in animal models of NAFLD/NASH.

Dr. Tandon's lab has set up multi-lineage 2D and 3D liver spheroid models of NASH to mimic the liver microenvironment. In a 3D model of NASH, her lab is currently using HepG2 and LX2 cells. The spheroid model has been standardized using high concentrations of free fatty acid to induce steatosis and fibrosis. Test compounds are screened for their anti-steatotic potential using high-content imaging platforms. The anti-fibrotic potential inhibition of mRNA expression of a-SMA, Col1A1, and other relevant fibrotic markers is evaluated using real-time PCR.

To test the anti-inflammatory activity, Dr. Tandon's lab has set up 2D *in vitro* co-culture models using HepG2 and differentiated THP-1 cells (representing Kupffer cells) against the fatty acid challenge in this model. To assess the cell health as a result of the fatty acid challenge and protection by test compounds, her lab is analyzing the mitochondrial health parameters such as oxygen consumption rate (OCR) as well as electron chain acidification rate (ECAR) with the help of SeaHorse XFp analyzer.

Dr. Tandon's lab has also evaluated an autophagy modulator, previously identified by the NCD team to evaluate its anti-steatotic and anti-fibrotic potential.

Way ahead

Going forward, the NCD group will continue working towards making the available NAFLD/NASH disease phenotype models more robust. The group will continue screening new NCEs, designed in-house, using the established phenotypic models in the laboratory. In addition to these models, the team will work toward establishing FFA-induced inflammatory and fibrotic models in THP1 and LX2 cell lines, respectively. Also, the team will establish a macrophage polarization model using PMA differentiated THP1 cell line.

***In vivo* models for NAFLD**

Dr. Madhu Dikshit's team established diet-induced obesity, insulin resistance, and fatty liver models using C57BL/6 and inducible nitric oxide synthase-knockout (iNOS KO) mice, validated the wild type (WT) mice model by using Saroglitizar which is currently being used for the screening (natural products, NCEs), and biomarker studies. iNOS KO mice study was undertaken to understand the role of redox balance and gut microbiome in obesity, insulin resistance (IR), and dyslipidemia. Relevant biochemical, molecular, and lipid parameters were analyzed to assess obesity and IR and NAFLD phenotypes. Additionally, liquid chromatography-mass spectrometry (LC-MS) based lipidomics was also performed to evaluate the changes in the various class of lipids in the plasma and hepatic tissue. Phospholipids (phosphatidyl-cholines, lysophosphatidylcholine, phosphatidyl-ethanolamines, lysophosphatidyl-ethanolamines, plasmanyl-phosphatidylcholines, plasmanyl-phosphatidyl-ethanolamines), diglycerides, and triglycerides were found to be perturbed in the plasma and hepatic tissue after high-fat high fructose diet feeding. Saroglitizar and Hepano treatment though offered protection against IR, however, both the drugs differed in their effect on a different class of lipids.

Antibiotic-induced alterations in microbiome and metabolome rescue insulin resistance and dyslipidemia in iNOS$^{-/-}$ mice

The importance of iNOS was predominantly studied in inflammatory conditions and infectious diseases, and in recent studies, iNOS has emerged as an important metabolic regulator. Previous studies from Dr. Dikshit's lab and others have demonstrated IR, dyslipidemia, and disrupted metabolic homeostasis in iNOS$^{-/-}$ mice fed on chow diet, LFD, or HFD. The group has shown that the IR and dyslipidemia in iNOS$^{-/-}$ mice were partially reversed by enhancing NO bioavailability *via* nitrite treatment suggesting the importance of redox status in host metabolism. To further gain an understanding of the mechanistic insights, a multi-omics approach was used. Gut microbiome analysis in stool samples using 16S rRNA sequencing and serum metabolic profiling using untargeted metabolomics was performed in iNOS$^{-/-}$ and compared with WT (C57BL/6J) mice. They observed significantly reduced bacterial alpha diversity in iNOS$^{-/-}$ mice as compared to WT and differential fecal gut microbiota composition with enhanced Actinobacteria, *Allobaculum,* and *Bifidobacterium*. Next, they modulated the gut microbiome using narrow and broad-spectrum antibiotics (vancomycin and antibiotic cocktail in drinking water) to understand microbial interaction

with host metabolism. Decreased bacterial alpha diversity, enlarged caecum, and increased relative abundance of Proteobacteria were observed in both WT and iNOS$^{-/-}$ mice; and are the signature markers of antibiotics treatment. The depletion of microbiota by antibiotics treatment decreased glucose and insulin levels and improved glucose intolerance and insulin insensitivity in the insulin-resistant iNOS$^{-/-}$ mice. Interestingly, antibiotics treatment decreased serum and liver lipids, liver weight, and adipose tissue weight in both WT and iNOS$^{-/-}$ mice suggesting rescued dyslipidemia *via* decreased expression of genes involved in fatty acid synthesis in the liver, adipose tissue, and intestine, and uptake in adipose tissue and intestine along with enhanced expression of lipid efflux genes in liver and intestine. The correlation analysis revealed positive associations of Actinobacteria, Bacteroidetes, S24-7, *Allobaculum, Bifidobacterium, and Ruminococcus* with glucose intolerance, IR, and dyslipidemia, and negative correlations with *Veillonella* and *Parabacteroides*. Nucleic acids, lipids, carbohydrate, amino acids, and bile acids metabolism were enhanced in insulin-resistant iNOS$^{-/-}$ mice and were reduced upon antibiotics treatment. These points towards the collective role of the gut microbiome and altered metabolome on the host metabolism in iNOS$^{-/-}$ mice. These results, thus demonstrate the role of compositional changes in the gut microbiome, reshuffled metabolic profile, and evolved molecular crosstalk in regulating host metabolic perturbations during altered redox states in iNOS$^{-/-}$ mice

The project jointly funded by AIIMS and THSTI aims to identify stage-specific biomarkers of NAFLD, to distinguish non-alcoholic steatohepatitis (NASH) from no-NASH. Dr. Dikshit's group has been involved in the isolation of various lipoprotein fractions from the plasma of healthy control individuals and the patients of NASH and NAFLD for proteomics and lipidomics analysis.

Way ahead

Dr. Dikshit and group plans to:

(i) Identify plant-based formulations (2) in collaboration with Dabur Pvt Ltd, and potential small molecules (ASK1 inhibitor, FXR agonist, or Autophagy inducer picked up from in vitro protein and cell-based screening) for further studies.

(ii) Select candidate metabolites, lipids (in the lipoprotein fractions separated from plasma following density-based centrifugation) in the plasma and bile acids in plasma/stool samples obtained from NAFLD, NASH patients, and healthy

controls to differentiate/diagnose NASH from no-NASH (in collaboration with Dr. Shalimar from AIIMS, New Delhi)

Identifying potential targets against NASH through computational biology

Dr. Samrat Chatterjee and the team are working to decipher possible mechanisms related to NASH and identify potential targets for the therapy. They curated gene expression data of human tissue samples for control, no-NASH, and NASH from literature. The team got 312 human samples with 47 controls, 116 no-NASH, and 149 NASH. As the disease is associated with a metabolic disorder, genome-scale metabolic models (GSMMs) were used to capture the metabolic perturbation associated with the disease. The team also investigated for changes in different proteins and related pathways through directional protein-protein interaction (PPI) networks. The combination of GSMMs and PPI networks is used to capture the whole system and identify key players and pathways responsible for the disease. The results could lead to possible therapeutic development through potential drug targets identification by understanding the mechanism associated with NASH. The result is expected to enrich the understanding of cellular modulation in NASH. As a future scope, the experimental biologists can explore the results to identify potential novel targets. This can further lead to small molecule identification.

Multiomics to Understand Metabolic disorders and NAFLD

Dr. Amit Kumar Yadav's team works on bioinformatics analysis of omics data to understand Metabolic Disorders and NAFLD Spectrum. His team has devised several tools and algorithms for omics data analysis for searching posttranslational modifications (PTM) which were then applied to liver data for healthy, NAFL and cirrhosis conditions to identify the mod-forms of the proteins expressed and differentially regulated.

Algorithms and tools for PTM data mining

Mining of posttranslational modifications requires better and accurate algorithms that utilize the fragmentation information from mass spectrometry appropriately. Three major modules developed by Dr. Yadav's team are:

A. ModLocator

The team developed a scoring function to capture the correct site of PTM in an identified peptide -

$$LocScore = \frac{\sum_{k=1}^{n} P_k I_k}{e^{\Delta m}}$$

Where,

n = number of matched site determining peaks

P = fragment peaks matched

I = intensity of matching peak

Δm *= mass error of matching peak*

QTOF Data (26019 spectra) from 18Mix (Klimek et al, JPR 2008) was searched for Oxidation as a modification on methionine. At 1% FDR, they identified 4951 spectra and localized the site of oxidation to test the algorithm. Oxidation was found in 1125 spectra, of which 269 were correctly localized while they could re-localize other 855 sites to their correct amino acid. They discovered several localizations of oxidation, apart from the expected ones on methionine, which were validated to be correct (as per the unimod database). Since PTMs control signaling processes in cells and are highly specific to the residues carrying the PTMs for functional output, their analysis depicted the importance of localization of PTM search results for correct biological discovery.

B. PTM Annotator

Finding and localization modification masses also require a follow-up analysis for understanding the biology. This necessitates that these masses are ascribed modification names, called *Annotation*, a process that is manual, laborious, and error-prone due to isobaric modification masses and mass combinations. Using the mass errors from Unimod with amino acid specificity, and the location within a peptide (C-term, N-Term, or within peptide), Dr. Yadav's team developed a probabilistic annotation algorithm that can iteratively find the best match in an automated manner to reduce errors. Using an *in silico* simulated dataset of 3.7 million simulated spectra for 4 PTMs & their combinations for 18 proteins, they evaluated this algorithm and found most annotations (~98%) to be correct.

C. ModQuant

Dr. Yadav's team also devised quantitation algorithms to quantify modification forms (mod-forms) using label-free (using SpC, spectral counts) and labeled data (iTRAQ, TMT, etc) to distinguish the modified and non-modified peptides of a protein.

$$SpC_{Mod/NonMod} = \frac{\sum_{k=1}^{n} PSM_k^{Mod/NonMod}}{L}$$

Some proteins were identified only in mod-form and different proteins are present in different ratios of their modified to non-modified forms which are generally missed in data analysis.

For labeled mass spectrometry data-

$$I_{Mod/NonMod} = \left(\frac{\left(\sum_{k=1}^{n} I_{condition}^{Mod/NonMod}\right)/n}{\left(\sum_{k=1}^{n} I_{control}^{Mod/NonMod}\right)/n} \right)$$

A public phosphoproteomics dataset with ~81000 spectra in 1:1:1 TMT labels (Hogerebe et al, Nat. Comm. 2018) was analyzed and the team found that the ratios of modified to non-modified peptides differ across proteins, which were expected to be 1:1.

NAFLD and NASH studies from omics and data mining

Dr. Yadav's team then applied these algorithms to a public dataset for mining the modifications in liver disease. To achieve this, they downloaded clinical mass spectrometry proteomics RAW data (PXD011839) for comparing healthy, NAFLD, and cirrhosis patients (Niu et al, 2019. Mol Syst Biol.). The original study generated a plasma proteome dataset that was analyzed for proteins expressions using mass spectrometry but modifications were not analyzed and a reanalysis can discover hidden information in the large-scale dataset. The data was converted into MGF format which contained 399 files with ~6.2 million spectra. This dataset was searched with phosphorylation, methylation, acetylation along with common artifacts methionine oxidation and deamidation as variable modifications, as well as carbamidomethyl as fixed modification using the MSGF+ search engine. The team then processed this output using the PTM tools developed earlier, to analyze the proteins and PTMs using a comprehensive workflow. They found that a large fraction of proteome is overlapping between the comparison groups and these were best suited for differential mod-form analysis. The team compared the proteins with a list of the known human liver proteome (2558 proteins) and found that most of the proteins captured in the experiment did not fully overlap with the liver proteome. This indicated that most of the proteins were not solely originated from the liver (many organs could secrete proteins into blood plasma). It could

also indicate that some liver-related proteins may not have been found in plasma in earlier studies. The team is investigating further for details on potential molecular signatures to differentiate the conditions based on their mod-forms.

Way ahead

Dr. Yadav plans to analyze apo-lipoproteins and other proteins important for liver disease progression in detail for their different mod-forms and targeted studies will be planned to monitor their use as biomarkers in biopsy-proven test samples, in collaboration with AIIMS. These samples will also be used to analyze and integrate omics data, towards achieving sensitive markers for proteomics, metabolomics, and lipidomics studies, for biomarker panel development.

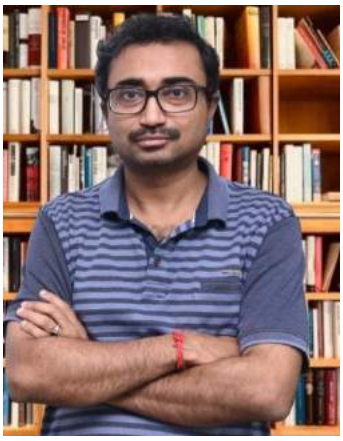
Development of biomarkers for NAFLD using high-resolution mass spectrometry and multivariate data analysis

Dr. Yashwant Kumar's lab is working on understanding the different stages of fatty liver disease using high-resolution mass spectrometry and multivariate data analysis approaches to identify the metabolites and lipids involved in the development of the disease.

On a small sample size, using high-resolution LCMS-based lipidomics, Dr. Yashwant Kumar's team found that during the development of the fatty liver disease the flux of lipid synthesis in the liver is diverted to triglyceride synthesis and lipids such as acylcarnitine content and different phospholipids were downregulated.

Dr. Yashwant Kumar's group is also studying the role of bile acids in the progression of NAFLD. Bile acids are an important molecule that regulates energy and lipid metabolism in humans. In a preliminary study, they found that conjugation of bile acids with glycine and taurine are affected as the disease progresses. They observed that taurine conjugation is more abundant in fatty liver conditions as compared to glycine conjugates. They are validating this finding on a larger cohort and trying to understand the role of the taurine conjugate in fatty liver disease. This study could be a way forward to understanding the mechanism of fatty liver development and identifying novel drug targets.

MATHEMATICAL MODELLING AND SYSTEMS BIOLOGY



Dr. Samrat Chatterjee's lab (Complex analysis group) focuses to understand the mechanism of a biological phenomenon, disease progression, and identification of potential drug targets using mathematical and computational tools. His team also works on the identification of molecular signatures associated with a disease and its progression.

Genome-scale metabolic models (GSMMs)

Research on new cancer drugs is performed either through gene knockout studies or phenotypic screening of drugs in cancer cell lines. These approaches are costly and time-consuming. GSMMs, a computational framework, is a good alternative to find potential drug targets. Dr. Chatterjee and his team investigated the applicability of gene knockout strategies to find drug targets using GSMMs.

Single-gene knockout studies were performed on existing GSMMs of the NCI-60 cell lines. The metabolic genes responsible for the growth of cancerous cells were identified and gene ranking was done to identify potential drug targets. Dr. Chatterjee's team identified 13 potential targets that significantly reduced the growth rate in cancer cell lines. It was found that the drugs mitotane and myxothiazol inhibited the growth of at least four cell lines of the NCI-60 database.

A high throughput proteomics data for the response of macrophage-like THP1 cell line to *Mycobacterium tuberculosis* infection was also analyzed by Dr. Chatterjee's team. THP1 cells were infected with H37Ra, H37Rv, BND433, and JAL2287 strains of *Mycobacterium tuberculosis*, and host response was studied after infection. His team developed a modified flux balance analysis (FBA) to find the fluxes of metabolic reactions in different strains and stages of infection. They also established a method of rewiring using GSMMs to change the flux state of virulent *Mycobacterium tuberculosis*-infected macrophages. The accuracy of the results was validated with gene knockout experimental data. The group found that more than one reaction has to be rewired simultaneously to alter virulent to an avirulent response. These results show that these reactions could be therapeutically targeted.

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

To maintain a healthy cardiac function, systematic plasma glucose transportation into

cardiomyocytes and in other cells is essential. In the transportation mechanism, a transportation delay could be crucial to maintain normal cardiac function. Dr. Chatterjee's team built a four-dimensional model to mimic the cross-talk among plasma glucose, plasma insulin, intracellular glucose, and cytoplasmic calcium of a cardiomyocyte. Extensive numerical computations were performed to validate the analytical results. Sensitivity study of the system parameters using the LHS-PRCC method revealed that some rate parameters, which represent the input of plasma glucose, absorption of glucose by non-cardiac cells, and insulin production, are sensitive and may cause a significant change in the system dynamics. It was observed that the time taken for transportation of extracellular glucose into the cell through GLUT4 plays an important role in maintaining physiological oscillations of the state variables. Parameter recalibrations showed that reduced input rate of glucose in the blood plasma or an alteration in transportation delay may be used for therapeutic targets in diabetic-like conditions for maintaining normal cardiac function.

Way ahead

Dr. Chatterjee plans to expand these studies to pancreatic beta cells using larger metabolic models like GSMM with online clinical data. This will give a global picture of the system for better exposure.

Konnect2prot: A database of protein information

A crucial step to study the role of protein in a biological system involves the use of protein-protein interaction (PPI) database. There are various PPI databases developed covering several features of protein interactions, however, covering most information in one place is still not reported. Henceforth, Dr. Chatterjee's team searched the databases and literature to curate essential information in one place and developed Konnect2prot. This web interface executes queries to visualize functional directional networks between different proteins and includes information on proteins involved in different diseases, specific pathways, molecular functions, biological processes, PTMs, and localization. The website also contains structural information like available inhibitors, activators, and types of 3D structures. The content will be launched as open-source. The work is copyrighted under Indian Copyright Diary No.: 14303/2020-CO/SW.

Studying Input-Output relation in signaling networks under random perturbation

Cell signaling plays a very important role in cell activities and the coordination of multiple-cell actions. The role of network motifs in controlling signaling error is being studied by Dr. Chatterjee's group. They are now working on the importance of bistability in the input-output

(I/O) relation. The study aims to capture and emphasize the role of motif structure influencing the I/O relation between two nodes in the context of bistability. A model-based analysis is used to investigate the critical conditions responsible for the emergence of different bistable PPI motifs and their possible applications to find the potential drug targets.

COMPUTATIONAL BIOPHYSICS AND STRUCTURAL BIOINFORMATICS

Discovery of Autophagy inducers

Autophagy induction is well reported to have a therapeutic effect in multiple diseases. Autophagy is a highly regulated and complex process of destroying damaged proteins and organelles under stressful conditions. The dysregulation of the autophagy process has been established to play a role in various diseases such as neurodegenerative diseases and cancers. Consequently, the discovery of novel therapeutic agents targeting various stages along this process has emerged as a promising approach for small molecule discovery.

Dr. Shailendra Asthana and his team is working to understand the signaling cascade responsible for biological function in form of autophagy induction in protein-protein interaction (PPI) manner. This group is focusing to inhibit the GAPR, which is a –ve regulator of Beclin-1 (a marker for autophagy induction). They incorporate flexibility, conformational change, and dynamical motion which is an intrinsic property of the proteins to map the interaction interfaces of GAPR and Beclin-1. In absence of GAPR, the beclin-1 is overexpressed and its overexpression is sufficient to induce autophagy (Kwata S. et. Al. Nature, 2013). They are focusing to explore the interaction sites localized at PPI interfaces to design the peptides (stable and permeable fused with HIV-tat) including the various ensembles of proteins. The computationally active peptides were selected for autophagy induction assays by monitoring the LC3 and p62 levels. The other essential *in vitro* and *in vivo* experiments are being carried out to characterize the lead peptide.

Targeting Sirtuins for metabolic disorder

Sirt1–3 are the most studied sirtuins, playing a key role in caloric-dependent epigenetic modifications. Since they are localized in distinct cellular compartments and act differently under various pathological conditions, Dr. Asthana and the team are looking at selective

inhibition as a promising strategy to understand their biological function and to discover effective therapeutics.

Immunological target focusing on PD-1/PD-L1

The dynamics and plasticity of the programmed cell death protein 1/ Programmed death-ligand 1 (PD-1/PD-L1) axis are the bottlenecks for the discovery of small-molecule antagonists to perturb this interaction interface significantly. Understanding the process of this PPI is of fundamental biological interest in structure-based drug designing. FDA-approved anti-PD-1 monoclonal antibodies (mAbs) are the first-in-class with distinct binding modes to access this axis clinically; however, their mechanistic aspects remain elusive. Dr. Asthana and his team investigated the native plasticity of PD-1 at global (structural and dynamical) and local (residue side-chain orientations) levels. They found that the structural stability and coordinated Cα movements are increased in the presence of PD-1's binding partners. Based on intra-/inter-residues' contact networks and energetics, the hot spots were identified which are essential to arrest the dynamical motions of PD-1 significantly. The results will help in the design of therapeutic agents by mimicking the mAbs mechanism.

List of Collaborators

Dr. Amit Awasthi

Dr. Vineet Ahuja, AIIMS, Delhi

Dr. Madhu Dikshit

Dr. Shalimar, AIIMS, Delhi

Dr. Amit Awasthi, THSTI

Dr. Bhabatosh Das, THSTI,

Dr. Dinesh Mahajan, THSTI

Dr. Shailendra Asthana, THSTI

Dr. Amit Kumar Yadav, THSTI

Dr. Ruchi Tandon, THSTI

Dr. Yashwant Kumar, THSTI

Dr. Renu Goel, THSTI

Dabur India Pvt Ltd

JIVA

BogaR Labs

Dr. Samrat Chatterjee

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

Prof. Nandadulal Bairagi, Jadavpur University, Kolkata

Prof. Joydev Chattopadhyay, ISI, Kolkata

Dr. Animesh Ray, AIIMS, Delhi

Dr. Ramray Bhat, IISc, Bengaluru

Dr. Sanjay Kr. Banerjee, NIPER Guwahati

Dr. Arup Banerjee, RCB

Dr. Milan Surjit, THSTI

Dr. Santosh S. Mathapati

Dr. Dinesh Kalyanasundaram, IIT-Delhi, New Delhi.

Dr. Anil Kumar Pandey, ESIC Medical College and Hospital, Faridabad.

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

Dr. Mukta Pujani, ESIC Medical College and Hospital, Faridabad.

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

Dr Shinjini Bhatnagar, THSTI

Dr Madhu Dikshit, THSTI

Dr Milan Surjit, THSTI

Dr Amit Awasthi, THSTI

Dr. Dinesh Mahajan

Dr. Sanjay Kr. Banerjee, NIPER Guwahati

Dr. N. C. Talukdar, IASST, Guwahati

Dr Jagat Bora, IASST, Guwahati

Dr Madhu Dikshit, THSTI

Dr. Ramandeep Singh, THSTI

Dr Shailendra Asthana, THSTI

Dr. Ajay Kumar, THSTI

Dr. Yashwant Kumar, THSTI

Dr Ruchi Tandon, THSTI

Dabur India Pvt Ltd

Dr. Shailendra Asthana

Dr. B. Ravindran, ILS Bhubaneswar

Dr. C. K Mukhopadhyay, JNU

Dr. Santosh Chauhan, ILS, Bhubaneswar

Dr. Deepak Nair, RCB

Dr. Prasenjit Guchhait, RCB

Dr. Manjula Kalia, RCB

Dr. Ambadas Rode, RCB

Dr. Nitish Mahapatra, IIT, Chennai

Dr. Amit Awasthi, THSTI

Dr. Samrat Chatterjee, THSTI

Dr. Ramandeep Singh, THSTI

Dr. Amit Pandey, THSTI

Dr. Milan Surjit, THSTI

Dr. Sankar Bhattacharyya, THSTI

Dr. Rajesh Kumar, THSTI

Dr. Ajay Kumar

Dr. Madhu Dikshit, THSTI

Dr. Dinesh Mahajan, THSTI

Dr. Shailendra Asthana, THSTI

Dr. Samrat Chatterjee, THSTI

Dabur India Pvt. Ltd.

Dr. Amit Kumar Yadav

Dr. Shalimar, AIIMS, Delhi

Dr. Debasis Dash, IGIB

Dr. Sanjay Banerjee, NIPER, Guwahati

Dr. Manjula Kalia, RCB

Dr. Madhu Dikshit, THSTI

Dr. Nisheeth Agarwal, THSTI

Dr. Bhabatosh Das, THSTI

Dr. Amit Awasthi, THSTI

Dr. Krishnamohan Atmakuri, THSTI

Dr. Yashwant Kumar, THSTI

Dr. Ruchi Tandon

Dr. Shalimar, AIIMS, Delhi

Dr. Ramesh Babu Boga, BogaR Laboratories, Hyderabad

Dr. Dinesh Mahajan, THSTI

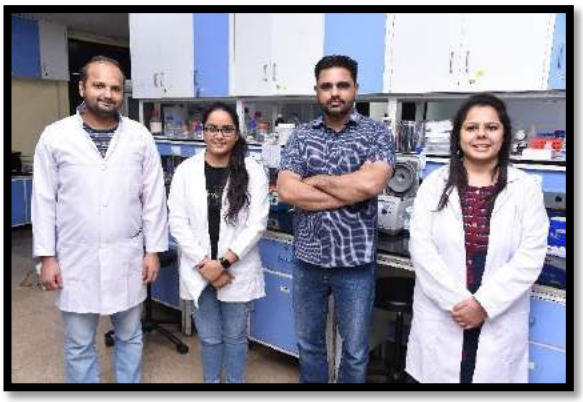
Dr. Shailendra Asthana, THSTI

Dr. Yashwant Kumar

Dr. Shalimar, AIIMS, Delhi

Dr. Chittaranjan Yajnik, KEM hospital, Pune


ट्रांसलेशनल स्वास्थ्य विज्ञान एवं प्रौद्योगिकी संस्थान



RESEARCH RESOURCE PLATFORMS

BIOASSAY LABORATORY

The Bioassay laboratory (BL), THSTI was established by the Ind-CEPI grant of DBT in 2018 and is a translational laboratory (BSL-3 facility) for the development of assays to measure clinical immunogenicity. The mandate of BL is to provide validated assays for vaccine development that are on par with global standards. It is now a NABL accredited (17025:2017) - cGLP laboratory with trained manpower and a robust quality management system. The laboratory has currently established molecular and serological assays for Dengue, Chikungunya, and COVID-19 and is intended to serve as a national resource platform for the clinical development of vaccines and biological.

BL responded to the COVID-19 pandemic by proactively partnering with ESIC hospital, Faridabad to initiate diagnostic testing in Faridabad. The lab has also contributed to training manpower and establishing COVID-19 testing labs in many hospitals in the Haryana region namely ESIC, Nuh, Al Falah, and BK Hospital. It is also an ICMR-recognized COVID-19 testing facility, the first one in the Faridabad-Palwal region, and has processed more than 80,000 clinical samples to date for diagnosis from several nearby districts to date. BL is also managing the mobile testing laboratory for COVID-19, the only one of its kind in India. This mobile lab has processed samples from Nimka jail, Palwal, Dhudoola, and Mujesar districts of Haryana- for more than 15,000 samples to date. BL is an ICMR-recognized COVID-19 diagnostic kit validation facility, the first one in the Faridabad-Palwal region, and has performed beta validation for over 20 kits from various companies all over India. In this short period, BL has also generated multiple in-house assays namely the THSTI SARS-CoV-2 real-time RT-PCR Detection kit, SARS-CoV-2 RNA DNAzyme Sensor, quantitative ELISAs to detect and estimate SARS-CoV-2 antibodies.

BL is the only Indian lab (one of seven centralized network labs), to be selected by CEPI (Coalition for Epidemic Preparedness Innovations) for facilitating COVID-19 vaccine development. The primary objective of this collaboration is to establish a common platform that employs the same protocol, assays, and data analysis methods, to ensure that vaccine candidates are assessed in a manner that is acceptable to regulators. Through this partnership, vaccine manufacturers are working through CEPI and will have access to validated assays to measure the efficacy of vaccines from Phase I through Phase IIb trials in these labs. Under the CEPI Global network, BL will be equipped to measure the immune response of multiple vaccine candidates under clinical trial, thereby enhancing the selection of the most effective candidate. To date, BL has completed the Tech transfer process for two assays- namely

Micro-Neutralization Assay and Pseudovirus neutralization Assay. BL is proud to be the only lab amongst the centralized 7 labs worldwide to have achieved this milestone for 2 assays. As a result, it was able to start testing Phase I/II samples from different vaccine developers across the globe, through the CEPI network.

In collaboration with Foundation for Innovative New Diagnostics (FIND), BL provided 4-day hands-on-training, at THSTI, in 6 batches, to 55 lab personnel on "Real-time PCR for SARS-CoV-2 detection". BL technical team, in collaboration with FIND, has also significantly contributed to the development of a questionnaire for the development of an online Chatbot app to address queries with regards to technical and operational issues of laboratory personnel engaged in COVID-19 testing. This app is expected to be released by the end of July 2021. It was also involved in the validation of diagnostic kits for FIND. Further, in appreciation of the effort that the BL team has been putting in with regards to all FIND collaborations, FIND has provided BL with diagnostic kits and Hi-Media high throughput RNA extractor (amounting to Rs 40 Lakhs) for enhancing the existing testing capacity of the COVID Wing.

BL has been actively involved in supporting various sero-surveillance studies across India. BL is currently processing service inquiries and collaborating with renowned national and international companies/institutes namely IIT Hyderabad, IIT Delhi, ICGEB, Panacea Biotech, TATA MD, J.Mitra and company, NCBS, INCLEN, and Hangzhou Biotech.

Thus, BL aims to provide clinical assay services of the highest quality and standard to industry and academia and is working relentlessly for vaccine development in the country by not only providing CGLP assays but also by training manpower and forging partnerships and collaborations with industry and academia.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RAPID RESPONSE MOBILE LABORATORY
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BIOREPOSITORY FACILITY

The Biorepository Facility (BRF) at THSTI is a biobank that has expanded not only in the area, infrastructure, and capacity but has been able to implement guidelines and SOPs as per the best practices in the field of biobanking. BRF is now a registered member of the International Society for Biologicals and Environmental Repositories (ISBER), which has increased the visibility of BRF globally.

Biorepository (BRF) organogram and structure

BRF provides scientific and technical support as services under various activities through its core team of five members. The management oversight of BRF is provided by THSTI Professor of Eminence, Faculty in charge, and Scientists who report to the Executive Director of THSTI. Cohesive teams reinforce high-quality research and have built a health care platform that augments the fundamental and translational research work by coordination with the clinical and data management teams.

During the past year, BRF strengthened its institutional platform and governance structure. Scientists and Technical Staff were recruited on Study Programs/Projects, infrastructure was upgraded and team capacity of collecting and processing infectious samples viz., SAR-CoV-2 biospecimen was initiated. Technical and scientific support to several ongoing projects within the institute and around the NCR cluster was provided either in a collaborative or service mode. Skill development training to all the BRF staff on Good Clinical Laboratory Practice (GCLP) and ISO 9001:2015 implementation and internal auditor training were imparted by CDSA and British Standard Institution (BSI) training academy respectively.

Currently, BRF has more than ten lakh biospecimen stored at different temperatures and is in process of facilitating the rational use of these unique biospecimen. The team plans to expand its capacity to harbor additional specimen collected under cohorts of various human diseases.

Biorepository Team Steps forward towards tackling the pandemic situation

During the COVID-19 pandemic waves, the biorepository team was abreast in taking up the challenges of setting up a COVID-19 Bioresource center via co-ordinations with hospital sites and their collaborators for samples collection, transport, and storage. The institutional biorepository was notified as a National COVID-19 bioresource of well phenotyped clinical samples by Govt. of India. This bioresource is supporting researchers to conduct seroepidemiology, immunology, diagnostic studies, production of monoclonal antibodies, and vaccine efficacy studies using well-characterized clinical samples. Currently, samples are

being collected from two different hospitals following the standard protocol detailed by the ICMR, by trained technicians/ health care workers while maintaining all necessary personal precautions as recommended by the WHO.

The biorepository team has undergone hands-on training for "Handling Infectious biological materials with special reference to COVID-19" jointly organized by the Department of Biotechnology (DBT), Centre for Disease Control and Prevention (CDC), and Indian Association of Medical Microbiologists (IAMM). This helped in streamlining the collection and transportation of COVID-19 samples. The data management team at the institute is taking care of data entry and curation along with the clinical and biorepository team.

The following milestones were achieved by BRF in the past year:

(i) COVID-19 Biospecimen Collection (Successfully Ongoing)

The collection and storage of clinically well-phenotyped samples of SARS-CoV-2 positive, suspected and negative participants post written informed consent is being carried out. An extensive questionnaire is being executed to collect the history and post COVID longitudinal follow-up clinical data and samples from the enrolled participants as a longitudinal cohort study with planned home visits. Participants are enrolled under two arms of the COVID-19 Consortium study: (a) COVID Testing Cohort: enrolled at COVID testing centers as suspected COVID-19 patients and are being tested as per National testing criteria; (b) COVID Positive Cohort: enrolled from COVID hospitals after being tested positive at the clinical site.

(ii) Development of various panels for validation of antigen and antibody kits (Completed)

The samples collected in the COVID-19 Consortium have been further characterized using in-house ELISA methods (in collaboration with Bioassay Lab, THSTI). A set of standardized nasopharyngeal/oropharyngeal swabs seeded with the inactivated virus for use in the development & validation of molecular & antigen diagnostics has been prepared. A standardized serum panel for testing the presence of SARS-CoV-2 antibodies and for qualitative and quantitative validation of various serodiagnostic kits have also been designed and developed as a bioresource at THSTI. Micro- and macro-pooled samples with known IgG titer have been used to develop reference standards/controls calibrated to NIBSC calibrants for use in serology and neutralization assay platforms at THSTI. A total of six (06)

antigen/antibody kits are either at the manufacturing stage or available in the market wherein the samples from biorepository have been utilized by external requesters.

(iii) Sharing of COVID-19 Bioresources with the Academia and Industry (Successfully ongoing)

The COVID-19 bioresources developed at THSTI biorepository are accessible to both academia and industry nationwide. These bioresources are being successfully used for the discovery and validation phase of diagnostics and vaccine development. The repository has developed a well-structured process of receiving requests and getting them evaluated by domain experts. The sample and data access requests for these bioresources have been streamlined by creating SOPs and pipelines of sharing the same could be accessed through the institutional website (https://thsti.res.in/newthsti/en/research-details/3/biorepository). To date, biorepository has shared more than 16000 biospecimen/aliquots developed as COVID-19 Bioresources at THSTI with various academia and industries through an Access Control Committee nominated by DBT.

THSTI's indigenous anti-SARS-CoV-2 IgG ELISA was developed using the bioresource at the biorepository and has been transferred to the industry partner Xcyton Diagnostics, Bengaluru. This kit has been successfully used for a serosurvey study for the region of Karnataka.

The BRF plans to extend the services to the NCR cluster and beyond to tackle pandemic situations by integrating into existing preparedness plans with developing infrastructure at biorepository. Further, it is planned to extend existing COVID-19 biorepository facilities towards COVID-19 pre- and post-vaccination effects and augmenting efficacy studies.

Way ahead

For the upcoming year, the team plans to upgrade the current biorepository to a national facility along with

(i) Consolidation of the standard processes established in the first phase (which are already in process);

(ii) Procurement of Cloud-based biorepository management database with limited access (FDA 21CFR part 11 complaint) for audit regulated archival and retrieval of samples;

(iii) Expansion of Biorepository space at two locations (within institute) and procurement of additional equipment.

In addition to the abovementioned, the Biorepository team is extending its work towards various certifications like ISO 9001:2015 for Quality Management and Quality Assurance by employing Quality Management systems at the facility, and international accreditations (ISO 20387) as per the International Society for Biologicals and Environmental Repositories (ISBER) guidelines.

In the coming years, BRF plans to provide consultancy support to other organizations that wish to develop new biorepository setups and to develop biorepository-related training courses.

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

The Data Management Centre (DMC) has established robust systems/processes to ensure data management services are to the highest standards of quality. After setting up the IT and clinical data management systems required as per GCP and best practices and enhancing the core expertise of the data management team, the DMC expanded its portfolio of projects to include clinical studies being done not only in the MCH domain but also within THSTI. The center has supported 9 completed studies and is actively supporting 12 (8 within MCH, 4 outside MCH, and within THSTI) more studies. DMC is currently supporting 3 Ph.D. thesis for data cleaning and statistical analysis. It has already established the data management process for both paper-based data capture and electronic data capture using an in-house clinical data management platform with good quality control measures. Three of our large programs the young infant sepsis program, GARBH-Ini and the DBT research consortium for COVID-19 study are using EDC platforms ensuring reliable data quality, audit trail, a quicker turnaround in addition to data security, storage, and backup. DMC has also adopted REDCap software, which is a 21 CFR part 11 compliant, secure data collection tool that meets compliance with widely used data management standards for clinical research. The advantages of REDCap are its global acceptability and community support. We have initiated this with a study global study supported by WHO on pediatric COVID-19.

Capacity for multidimensional data management

Video data capture and management for an international collaborative study (CALOPUS): DMC & 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 Delhi 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 & ADAPT enables the processing of microbiome, proteomics, and metabolomics data from the GARBH-Ini program. The programmatic automation by ADAPT has led to

efficient, error-free, and reproducible data analysis. They have also started the analytics support with the recently initiated DBT-COVID-19 consortium and WHO pediatric COVID-19 study. They have built automated study report generation systems to monitor important information like enrolment status and an overview of baseline characteristics of participants and sample availability. An automated pipeline based on R programming language was developed for quality control and to generate a daily update on the recruitment numbers for the WHO pediatric COVID-19 study. These scripts were sent to WHO and are being used by the other study sites to generate similar reports. In the future, DMC proposes to build interactive real-time dashboards to monitor study progress. These dashboards can be incorporated into institutional/project websites enabling real-time monitoring of the study progress.

Building a skilled team for methodologically advanced data analysis for intramural and extramural studies

To support advanced analytics including artificial intelligence and machine learning, DMC 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 harmonization and clinical research workshops. Apart from this, interns of ADAPT are an active and integral part of the automation and programming aspects of data management. The analytical support is currently for the studies within the Maternal and Child Health in THSTI. DMC plans to provide consultation on statistical analysis if requested on a case-to-case basis for other studies.

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

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

Rising 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 the COVID-19 pandemic. A program to establish 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. Real-time data monitoring, prompt analysis, and sharing of data with multiple industry and academic partners are being done actively. This was enabled by the programming skills and computational power developed at DMC & ADAPT.

SMALL ANIMAL FACILITY

The Small Animal Facility (SAF) at THSTI breeds and maintains quality laboratory animals. The facility provides support to the scientific community of NCR Biotech Science Cluster and presently supports the animal-related requirements of THSTI and the Regional Centre for Biotechnology (RCB). The SAF has been established in compliance with the guidelines of the Committee for 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. All the animal experiments are performed only after taking prior approval from the Institutional Animal Ethics Committee (IAEC). Training on animal care, handling, and experimental techniques for research staff and students is conducted at regular intervals.

At present, the facility houses one hamster and one guinea pig stock, and 41 mouse strains that include inbred, transgenic, knock-out, knock-in, and immunodeficient strains. In the current year, five mouse strains were added to the breeding colony. Additionally, K18-hACE2 a genetically modified mouse strain, required for establishing an animal model platform for COVID-19 research, was imported from an overseas animal supplier. Currently, the researchers are actively using the above platform for designing appropriate intervention strategies against COVID-19. The facility has also acquired AG129, a genetically altered mouse for establishing an animal model platform for research on the dengue virus. As a part of the expansion plan, additional biosafety cabinets, cage changing stations, and individually ventilated caging systems for mice were procured and installed in the facility to increase the housing capacity. To comply with the latest CPCSEA advisory, CCTV surveillance monitoring, and access control system were installed in the facility.

To keep up with the requirement of the research community, SAF is planning to upgrade the current facility to increase the quality of animals and build up additional support and infrastructure. This includes the establishment of new Quality Control, Histopathology, and GLP laboratories. SAF is also planning to create both transgenic and germ-free facilities. The vision is to make the SAF a nodal center for various animal model platforms to be used for both communicable and non-communicable diseases by researchers and various other stakeholders in a public-private partnership model.

Establishment of Hamster and ACE2 mice model of SAR-CoV2 infection

During the COVID-19 pandemic, SAF contributed towards establishing the hamster and K18-hACE2 mice model platform for SAR-CoV2 infection. This platform was used by both public and private partners that include national and international vaccine manufacturing companies for testing the efficacy of these products (vaccine, anti-virals, antibodies, etc) to design better intervention strategies in our fight against the pandemic.

Establishment of Ferret model for research on respiratory viruses

To support the research on respiratory viruses such as Influenza viruses, SAF has helped design and commission a new BSL-3 ferret facility at THSTI. Currently, SAF is working on providing all the logistical support required for ferret holding and experimentation. The ferret facility, an integral part of the small animal facility at THSTI, is the first such facility in India. This facility will help support preclinical studies to identify future intervention strategies against human respiratory viruses of clinical importance.

Infectious Disease Research Facility (IDRF)

IDRF, a specialized Animal Biosafety Level 3 (ABSL-3) containment facility is available to research infectious diseases caused by a pathogen that requires a biosafety level 3 (BSL3) facility for their handlings such as COVID-19, Tuberculosis, and HIV. SAF supports all animal-related activity inside this facility. All the staff working in this facility are imparted mandatory BSL3 training.

Plans

THSTI is in process of implementing a plan of up-gradation of this existing small animal facility to achieve the national and international standards related to animal research and animal facility management. THSTI aimed to develop and establish SAF as a national resource and nodal center for national collaborative work required in the field of small laboratory animals within India. THSTI is working on making the ferret facility operational soon. It is also planned to establish a mouse genome engineering laboratory to conserve the germplasm of important mouse strains. For maintaining the quality of animals, SAF is planning to implement molecular-based diagnostic screening for pathogens in due course of time. Further, in association with the SAF, THSTI is working to establish histopathology, transgenic, GLP, and germfree facility at the NCR Biotech Science Cluster.

CLINICAL DEVELOPMENT SERVICES AGENCY (CDSA)

Clinical Development Service Agency (CDSA) has a mandate to support and nurture clinical product development and clinical research capacity in India. It fills a unique niche in the publicly funded clinical research and product development landscape in India and is the only such agency established by the Department of Biotechnology, Government of India.

The objectives of CDSA are:

1. To function as an Academic Clinical Research Unit (A-CRU)

   Undertake and provide end-to-end clinical study support for investigators, sponsors, and SMEs in study planning, set-up, conduct: project management, monitoring, data management, safety reporting, analysis and report writing
2. Training

   Build research capacity and capability through high-quality training in the area of clinical development/trials and regulation
3. Support and strengthen clinical research environment in the country
4. Regulatory science and policy support by providing tools and approaches to support researchers, regulators, health policymakers & industry

Academic Clinical Research Unit (A-CRU)

Team science underpins high-quality health research. The multidisciplinary teams comprising domains like clinical science (medical affairs) & regulatory science, clinical portfolio management (operations), data science, and biostatistics at CDSA, each with their distinct role and competence, work cohesively to support investigators and sponsors for the successful delivery of a high-quality program.

Clinical Portfolio Management (CPM)

The CPM team has been leading the A-CRU activities with support from the other teams/ verticals within CDSA and have established a track record in providing regulatory guidance, study start-up support, clinical data, and laboratory monitoring, project management, data management, safety management, medical writing and training, and closeout support to investigators/ sponsors for effective implementation of clinical trials/studies. They have now expanded this portfolio to include trial designs/conducts. During the past year, CDSA set up robust clinical trials/studies IT infrastructure, like clinical data management system (CDMS), electronic trial master file (eTMF), clinical trial management system (CTMS) to provide clinical study and data management support services for large multicentre trials/studies, with the ability to easily accommodate a growing portfolio of trials/studies stretching over many years. CDSA is building competent

manpower resources within the core clinical trials teams: clinical science, data science, clinical portfolio management, regulatory science, and biostatistics.

Key achievements

- Successfully supported 18 studies of varying risk, number of sites, participants. Many of the studies are large multicentre studies with sites across India.
- Played a critical role in monitoring a large multicentre TB vaccine clinical trial evaluating 2 indigenous TB vaccines, enrolling 12000 healthy volunteers, and coordinated by ICMR.
- Supported the project management unit at National Biopharma Mission to prepare 11 DHS sites to set up a population cohort database and initiate activities for COVID-19, Dengue, and Chikungunya sero-epidemiology studies
- Supported the project management unit at National Biopharma Mission to establish five clinical trial networks in specific disease areas and facilitating readiness of the 36 hospitals that are part of the networks, to conduct clinical trials
- Awarded two seed grants viz., SURAKSHA and IMAPRT by CRUK -DBT-India Alliance on Affordable approaches to cancer.
- Awarded two grants where CDSA was a co-applicant for project management: HORIZON 2020 grant on 'Effective and Affordable Flu vaccines to the world' and An academic clinical trial in partnership with AIIMS with funding from ICMR where CDSA is supporting site set up, clinical operations, project management, IP management, quality management, and safety reporting and monitoring.

Training

The training vertical works on a national mandate of capacity and capability building in the area of clinical development and clinical research in India. The focus is on designing and developing short term training programs on GxPs like Good Laboratory Practice (GLP), Good Clinical Laboratory Practice (GCLP), Good Clinical Practice (GCP), regulatory requirements for various types of product development (new drug, medical device, *in vitro* diagnostic kits, phytopharmaceuticals, biopharmaceuticals, vaccines, etc.), ethics, clinical research methodology, etc.

Major achievements of training domain

- **Online short-term training courses**

CDSA was the first to develop and launch online courses on drug regulations. With the release of the New Drugs and Clinical Trials (NDCT) rules in March 2019, the team revised the course and launched version 2.0 in 2020. Around 5000 learners enrolled in these online courses.

- **International engagements**

  CDSA in collaboration with Ind-CEPI (India Coalition for Epidemic Preparedness Innovations), worked to strengthen the capacity and capability development in clinical research for India's friendly countries through a series of e-courses conducted in two rounds. The programs were conducted as weekly modules over 10 weeks. 12 countries participated with more than 1300 participants. Successful completion of the training programs and interactions led to several research collaborations.

- **Online training programs for National Biopharma Mission (NBM)**

  CDSA designed and conducted well-structured comprehensive training programs on GCP and GCLP for the five sero-surveillance sites established by NBM.

- **Wednesday webinars**

  The training team launched a series of 'Wednesday Webinars with CDSA' as a new medium to connect with the learners/stakeholders. Launched in May 2020, ten webinars were conducted by the CDSA training team, where more than 3000 participants have attended. The team has launched its YouTube channel. (https://www.youtube.com/channel/UCgqiDzkwRHhrtjG-Kv1Yrtg)

- **Institutional Bio-Safety Training**

  A bio-safety training (IBS) was organized for THSTI research staff in December 2020.

- **GCP professional certification scheme (GCPPCS): a new flagship initiative**

  Having developed a niche in training in GCP, CDSA leveraged its strength to create an ecosystem for enhancing the quality of GCP professionals (clinical trials/research) at the national level. CDSA has designed and developed a scheme for certifying training institutes as well as individual professionals using international best practices for assessment and certification like ISO 17024. This certification scheme is the *first-in-the globe* and is based on a growing global trend of evaluation of competence of professionals. To gain international acceptance, the scheme relies on accreditation of third-party personnel certification bodies by the national accreditation body, the National Accreditation Board for Certification Bodies (NABCB), a constituent Board of the Quality Council of India (QCI), who have attained international equivalence for its accreditation.

The certification scheme is in the process of being launched and will bring in a paradigm shift on how human resources are trained and certified in clinical research/trials.

Support and strengthen clinical research environment in the country

CDSA is working closely with FERCI and PATH to develop an integrated electronic research application platform for clinical research approvals. As a first step, they have developed and operationalized an online workflow management software that helps ethics committees track submissions, generate queries, assign reviewers and ensure the security of study documents. The common ethics review form has been incorporated into the software for filling and submission by investigators.

List of collaborators

National Collaborations

i. Department of Biotechnology (DBT)
ii. National Biopharma Mission (NBM), BIRAC
iii. Indian Council of Medical Research (ICMR)
iv. Central Drugs Standard Control Organization (CDSCO)
v. National Programme for Technology Enhanced Learning (NPTEL)
vi. AIIMS, New Delhi
vii. VMMC & Safdarjung Hospital, New Delhi
viii. National Institute of Mental Health and Neurosciences (NIMHANS)
ix. Christian Medical College, Vellore

International Collaborations

i. Medical Research Council (MRC), Clinical Trials Unit (CTU), University College London (UCL), UK
ii. World Health Organization
iii. Public Health Research Institute (PHRI, Canada).
iv. USAID
v. Bill and Melinda Gates Foundation

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.	GarbhINI: 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, General Hospital Gurgaon VMMC & SJH, CDSA, MAMC	• Study start-up support • Monitoring: Study processes, data, laboratory and biorepository	• GCP-compliant study documents, ICD, CRF, SOPs, and data collection tools. • Site set-up as per project requirements • GCP and GCLP trained the 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 CISMAC)	Dr. N.Wadhwa, THSTI	• Study start-up support • Monitoring: data and laboratory • Support safety reporting (verbal autopsy)	• 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, VMMC & Safdarjung Hospital, Delhi & Dr N. Wadhwa, CDSA	• Co-applicant/ Co-PI • Study start-up support • Internal quality management • Data management	• Successfully supported Training of trainers & 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	• GCP-compliant study documents, ICD, CRF, SOPs, and data collection tools.

S. No	Project Title (Funding Agency)	Principal Investigator / Institute	Role of CDSA	Contribution
			reconciliation	• Study execution as per Protocol, GCP, and CDSCO guidelines
5.	Accelerating the application of stem cell technology in human disease – ADBS Study (DBT)	Dr. S. Jain, NIMHANS, Bengaluru	• Study start-up support • Monitoring: data and study documents	• 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. Padmapriyadarsini, 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. Padmapriyadarsini, National Institute for Research in Tuberculosis (NIRT), Chennai (05 sites across India)	• Regulatory guidance • 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,	Dr. AM Khan	• Study start-up	• GCP and

S. No	Project Title (Funding Agency)	Principal Investigator / Institute	Role of CDSA	Contribution
	Double-blind, Three arms 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 (18 sites across India)	support • GCP training • Project management support • Safety and data monitoring • Data management support	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
9.	The 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. Chandele, ICGEB; Dr. N. Wadhwa, CDSA (03 clinical sites across India; 4 research institutes)	• Program management support • Monitoring: Clinical sites including sample collection and shipment, laboratory, biorepository • Clinical data management	• Clinical 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	• The study was conducted 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	• Co-PI • 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	Dr. G. Karthikeyan,	• Site start-up support	• Study start-up ongoing as per

S. No	Project Title (Funding Agency)	Principal Investigator / Institute	Role of CDSA	Contribution
	randomized placebo-controlled trial (ICMR)	AIIMS, Delhi	• Quality management • IP Management	ICMR requirements • GCP training of project team.
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	• Project management • **Lead on** a systematic review of breast cancer risk factors in South Asian populations • Contribute to protocol development • Support submission of PPI objective proposal to site IECs • Contribute to **Co-PPI work on 'Creating People-Centered Clinical Research Experience in SURAKSHA'**	• Project coordination • Protocol development for PPI objective • Supported clinical sites in the 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 of site feasibility questionnaire • Support submission of PPI objective proposal to clinical site IECs • Collaborated on PPI work
16.	Sero-prevalence study for COVID-19 in Mumbai (BMGF)	Dr G Kang, THSTI & Dr U. Kolthur TIFR, Mumabi	• Independent quality monitoring	• Contribute to the delivery of high-quality research

S. No	Project Title (Funding Agency)	Principal Investigator / Institute	Role of CDSA	Contribution
17.	Effective and Affordable Flu Vaccines for the World (INDIGO)	Dr, G.Kang, CMC Vellore; Dr. Remko, AIGHD.	• Dissemination services, Clinical trial project management, monitoring regulatory	Contributed to the development of Dissemination Tools. (Website, Newsletters, Press release)
18.	Sepsis-related mortality in neonates in India: A multi-disciplinary, multi-institutional research program for context-specific solutions (DBT)	Dr. M Jeeva Sankar, AIIMS, Delhi	• Clinical Trial Monitoring • Clinical site management: financial management; resources for site: manpower, equipment • Data Management	• Manpower recruitment, Equipment procurement, Study plans. • Site set-up as per project requirements GCP and GLP training of project team.
19.	Mission COVID Suraksha (NBM-DBT)	Dr. Nitya Wadhwa, CDSA	• Support capacity enhancement at sites • Clinical trial monitoring	Site development and evaluation activities

Consultancy Services

Project Title (Funding Agency)	Principal Investigator / Institute	CDSA Role
Clinical Trial Regulatory Advisory and Data Safety Consultancy Services	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

Details of training programs conducted in 2020-21 by CDSA

S.NO.	DATE	WORKSHOP/COURSES	FUNDING	PLACE & CITY	FACULTY	PARTICIPANTS	ATTENDEE INSTITUTIONS
1	August 07-September 04, 2020	Good Clinical Practice online training program	NBM, BIRAC	Online (virtual)	10	68	7
2	August 27-September 25, 2020	Good Clinical Practice online training program	NBM, BIRAC	Online (virtual)	10	87	7
3	October 14 & 21, 2020	Good Clinical Laboratory Practice online training program	NBM, BIRAC	Online (virtual)	4	82	7
4	October 09-October 29	GCP_Ind-CEPI_Strengthening Clinical Trial Research Capacity in Neighbouring Countries	Ind-CEPI, BIRAC	Online (virtual)	23	161	51
5	November 06-November 13	Ethical Considerations In Clinical Research _Ind-CEPI_Strengthening Clinical Trial Research Capacity in Neighbouring Countries	Ind-CEPI, BIRAC	Online (virtual)	9	216	55
6	November 20-November 27	GCLP_Ind-CEPI_Strengthening Clinical Trial Research Capacity in Neighbouring Countries	Ind-CEPI, BIRAC	Online (virtual)	8	136	33
7	December 04-Decembe	Novel vaccine development and immunization policy in	Ind-CEPI, BIRAC	Online (virtual)	9	123	32

	r 11	a pandemic_Ind-CEPI_Strengthening Clinical Trial Research Capacity in Neighbouring Countries					
8	December 30-December 31	THSTI-IBSC online biosafety training course	CDSA	Online (virtual)	6	119	1
9	February 05-February 26, 2021	GCP_Ind-CEPI_round 2_Strengthening Clinical Trial Research Capacity in Neighbouring Countries	Ind-CEPI, BIRAC	Online (virtual)	14	266	65
10	February 16-March 02, 2021	GCP training program_CTN Oncology	NBM	Online (virtual)	10	55	7
11	March 12 - March 19, 2021	Ethical considerations in clinical research_Ind-CEPI_round 2_Strengthening Clinical Trial Research Capacity in Neighbouring Countries	Ind-CEPI, BIRAC	Online (virtual)	8	189	43
12	February 16-March 02, 2021	GCP training program_CTN Rheumatology	NBM	Online (virtual)	7	47	7
				Total	**118**	**1549**	**315**

ACHIEVEMENTS

List of Publications for the year 2020-2021

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Books and Chapters

Singh, R., Kumar, P., and Tahlan, K. Drugs against *Mycobacterium tuberculosis*. Book Chapter in Drug discovery targeting drug-resistant bacteria. Elsevier Press. 2020.

Patents filed in the year 2020-2021

S. No.	Title	Application No.	Filing Date	Inventors
1.	Novel DNA aptamers against spike protein of SARS-CoV-2 and uses thereof	202011017852	27/04/2020	Tarun Kumar Sharma, Anjali Anand, Ankit Gupta
2.	DNA construct expressing receptor binding domain of SARS-CoV-2 protein, recombinant protein, and the process to produce the same and its uses thereof	202011018845	02/05/2020	Tripti Shrivastava, Sandeep Goswami
3.	A DNAzyme-based colorimetric detection of SARS-CoV-2 and its uses thereof	202011020560	15/05/2020	Guruprasad R. Medigeshi, Tarun Kumar Sharma, A. Anantharaj, Soon Jyoti Das
4.	SARS-CoV-2 Spike domain Immunogenic peptide-based vaccine candidates and uses there of	202011028760	06/07/2020	Sweety Samal, Shubbir Ahmed, Rajesh Kumar, Priti Vishwakarma, Naveen Yadav, Adarsh Chiranjivi
5.	Novel silver nano-based aqueous sanitizer against pathogens	202011030085	15/07/2020	Sumit Kumar Pramanik, Sanjay Pratihar, Susmita Chaudhuri, Sankar Bhattacharya, Manisha Yadav, Niraj Kumar, Shailendra Mani
6.	Novel DNA aptamers against nucleocapsid protein of SARS-CoV-2 and uses thereof	202011034641	12/08/2020	Anjali Anand, Ankit Gupta and Tarun K. Sharma
7	Immunoassay for detection of coronaviridae.	202011039696	14/09/2020	Gaurav Batra, Farha Mehdi, Souvick Chattopadhyay, Ramachandran Thiruvengadam
8.	Biofilm inhibiting sol-gel composition for coating on substrates and process of preparing the same	202111001104	11/01/2021	R. Subasri (ARCI), Ramay Patra (ARCI), K. R.C. Soma Raju (ARCI), Susmita Chaudhuri (THSTI), Prashant Garg (LVPEI), B. Bhaskar (LVPEI), Debrupa Sarkar

				(THSTI).
9.	Antimicrobial biofilm containing silver nanoparticles	2021011928	01/01/2021	Ananta Dey, Susmita Chaudhuri, Debrupa Sarkar, Subhash Tanwar, Anik Kumar Dey, Amitava Das, Sumit Kumar Pramanik
10.	A novel process for generation of Nitric Oxide by cells in vitro and endogenous induction of Nitric Oxide in vivo and blockade of Endotoxemia	202031050500	20/11/2020	B. Ravindran, DK Singh, Shailendra Asthana, S. Gaikwad, D. Vasudeven and N. acharya
11.	Novel Fenton reagent-based sanitizer protect against toxi c pathogens	2021089351	06/11/2020	Sanjay Pratihar, Sumit Kumar Pramanik, Susmita Chaudhuri, Sankar Bhattacharya, Manisha Yadav, Niraj Kumar, Shailendra Mani

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   Authors: Dr. Samrat Chatterjee, Dr. Shailendra Asthana, Mr. Dipanka Tanu Sarmah, Mr. Shivam Kumar, Mr. Ekant Sharma, Ms. Komal Sharma, Mr. Krishan

List of extramural projects sanctioned during the Financial Year 2020-21

S. No.	Project Title	Principal Investigator	Funding Agency	Duration of sanction	Amount in INR
1.	Developing broadly neutralizing monoclonal antibody mediated prevention and treatment strategy by assessing their effectiveness in neutralizing HIV- 1 subtype C circulating in India across different regions and distinct risk groups	Dr. Jayanta Bhattacharaya	DBT/Welcome Trust India Alliance	2020-2025	₹ 3,84,00,000
2.	Developing HIV broadly neutralizing antibodies as a prevention product for global access through antibody half-life extension engineering	Dr. Jayanta Bhattacharaya	Council of Norway	2020-2022	₹ 6,00,81,321
3.	To study host cell regulation under influence of *Mycobacterium tuberculosis* using mathematical models	Dr. Samrat Chatterjee	DBT	2020-2022	₹ 24,95,460
4.	Diagnostic solutions for SARS-CoV-2	Dr. Gaurav Batra	DBT	2020-2021	₹ 2,78,98,980
5.	Preclinical and pharmacokinetics evaluations of select Ayush herbal extracts/ formulations for mitigating SARS-CoV2 associated pathologies	Dr. Madhu Dikshit, Dr. Amit Awasthi	DBT and Ministry of AYUSH	2020-2022	₹ 4,96,28,000
6.	Understanding regulation and function of complex network of TA systems in *Mycobacterium tuberculosis*	Dr. Ramandeep Singh	DBT/Welcome Trust India Alliance	2020-2025	₹ 4,49,80,511
7.	Preterm birth risk in pregnant women and prediction using machine learning models (ki data challenge for MCH)	Dr. Shinjini Bhatnagar	BIRAC	2020-2022	₹ 38,95,000
8.	Towards development of a potent antiviral against the SARS-CoV-2 by targeting the interaction between nucleocapsid protein and viral RNA	Dr. Milan Surjit	SERB	2020-2023	₹ 48,70,240
9.	Multi-Omics Signatures of Human Placenta: Real time assessment of underlying mechanisms for prediction of birth outcomes and	Dr. Pallavi Kshetrapal	DBT	2020-2023	₹ 1,56,87,612

	development				
10.	Design, Validation and development of novel peptidomimetic therapeutics targeting SARS-CoV-2 replication	Dr. Amit Awasthi, Dr. Sweety Samal	SERB	2020-2023	₹ 31,23,040
11.	Development, Characterization and evaluation of protective efficacy of self-amplifying mRNA vaccine candidates against the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	Dr. Milan Surjit Co-PI - Dr. Tripti Shrivastava	BIRAC	2020-2021	₹ 94,60,000
12.	Development and Evaluation of diagnostics and Candidate VACacines for emerging SARS-Coronavirus-2 (DEC-VAC-SARS)	Dr. Amit Awasthi	SERB	2020-2023	₹ 47,79,240
13.	COVID-19 Bioresource at the NCR Biotech Science Cluster	Dr. Pallavi Kshetrapal	DBT	2020-2022	₹ 5,83,94,133
14.	This multi-country observational study will be used to understand the clinical characteristics of SARS-CoV-2 related disease in neonates, children and adolescents aged 0-19 years presenting to hospital in LMIC	Dr. Shinjini Bhatnagar	WHO	2020-2021	₹ 1,07,62,180
15.	Adaptive Molecular Diagnostics	Dr. Bhabatosh Das	Welcome Trust, UK	2020-2022	₹ 9,88,06,519
16.	INCENTIVE: Indo-European Consortium for next generation Influenza vaccine innovation	Dr. Amit Awasthi	DBT	2020-2025	₹ 3,93,44,880
17.`	ENDFLU: Evaluation of rationally designed Influenza vaccines	Dr. Sweety Samal	DBT (Horizon 2020 with EU)	2020-2025	₹ 6,66,30,520
18.	INDIGO: "Effective and affordable flu vaccine for the world"	Dr. Sweety Samal	DBT (Horizon 2020 with EU)	01st January 2021 to 01st January 2026	₹ 14,63,76,720
19.	Identification of early-biomarkers for emergence of antimicrobial resistance among bacterial pathogens due to inadequate antimicrobial dosing and duration	Dr. Niraj Kumar	ICMR	2021-2024	₹ 19,43,593

20.	Development of a recombinant vaccine against the Hepatitis-E virus and immunological characterization of Hepatitis-E immune cohort and potential vaccine recipient cohort	Dr. Milan Surjit	BIRAC	2021-2023	₹ 2,21,00,000
21.	Cellular Assay Platform for determining the immunogenicity of vaccine candidates	Dr. Amit Awasthi	BIRAC	2021-2022	₹ 4,86,01,200
22.	Hunt for PANACEA (Pan-Anti-Coronavirals) against coronavirus of the past, present and the future	Dr. Guruprasad R Medigeshi	DBT	2021-2022	₹ 37,67,720
23.	Pre-clinical evaluation of combination adjuvants for Humoral and T cell immunity to Respiratory Syncytial Virus	Dr. Guruprasad R Medigeshi	DBT	2021-2023	₹ 67,48,640
24.	DBT City/Regional Clusters for COVID-19 testing-Phase-II: Scaling up of COVID-19 testing by hub and spoke model	Dr. Pramod Garg	DBT	2021	₹ 84,91,200
25.	Sepsis-related mortality in neonates in India: A multi-disciplinary, multi-institutional research program for context-specific solutions	Dr. Krishnamohan Atmakuri, Dr. Bhabatosh Das, Dr. Tarun Kumar Sharma, Dr. Pallavi Kshetrapal	DBT	2021-2026	₹ 13,95,21,562
26.	MOMI Biorepository Local analysis – India	Dr. Shinjini Bhatnagar	BIRAC	2020-2022	₹ 4,45,63,874
27.	ORCHESTRA: Connecting European cohorts to increase common and effective response to SARS-CoV-2 pandemic	Dr. Shinjini Bhatnagar	DBT	2021-2024	₹ 2,35,52,080
28.	GARBHINI – India Pregnancy Risk Stratification (IPRS) Platform Agreement (GIPA)	Dr. Pallavi Kshetrapal	BIRAC	2021-2024	₹ 5,93,15,794
Total					₹ 1,04,42,20,019

Honors and Awards for the year 2020-2021

Dr. Madhu Dikshit delivered the prestigious Daulat Singh Kothari Memorial Lecture, Indian National Science Academy in 2020.

Dr. Ramandeep Singh was awarded the prestigious DBT-Wellcome Trust India Alliance Senior Fellowship in May 2020.

Dr. Amit Awasthi was selected for the prestigious S. Ramachandran-National Bioscience Award for Career development 2020-21 for his research contributions in understanding the induction and effector functions of T cell subsets in inflammatory diseases.

Dr. Bhabatosh Das was awarded the Young Scientist Award by the International Life Science Institute, India for Improving Public Health in the Areas of Food Safety, Nutrition, and Wellbeing.

Dr. Pallavi Kshetrapal is a committee member on the Regional Prospective Observational Research in Tuberculosis (RePORT) India initiative under the Indo-US Vaccine Action Programme, Department of Biotechnology.

Dr. Suchitra D Gopinath is on the Institutional Committee for Stem Cell Research for Regional Center for Biotechnology (RCB).

Dr. Nitin Hingankar was awarded a scholarship to participate in HIV research for prevention- An IAS Virtual Conference (HIVR4P /VIRTUAL Conference).

Invited talks for the year 2020-2021

S. No.	Name of the Faculty member/Scientist	Title of the talk	Title of the event	Date
1	Dr. Shinjini Bhatnagar	A module on "Study Designs": a) Cohort Studies b) Randomized Controlled Trials	ESI Endocrine Research Methodology Workshop – Fun Research 2020 Module 2, ESI Faridabad	10th Oct 2020
2	Dr. Shinjini Bhatnagar	"inspire data challenge grantees ..…… on the potential impact of data-driven approaches on policies particularly in LMICs settings"	Plenary speaker invitation: Oct 22 ki Grand Challenges MNCH Data Science Meeting	22nd Oct 2020
3	Dr. Shinjini Bhatnagar	How to get the most from your mentor?	Final Program of the Research Methodology Workshop, AIIMS, New Delhi	27th Oct 2020
4	Dr. Shinjini Bhatnagar	DBT Initiatives in Clinical research during COVID-19 pandemic	FERCICON 2020 – Talk "Challenges in Research Ethics during COVID-19 Pandemic"	27th Nov 2020
5	Dr. Madhu Dikshit	Honesty, Integrity, and Ethics in Science	NEIST Foundation day, NEIST Jorhat	18th Mar 2021
6	Dr. Madhu Dikshit	Women in Science and Technology: From Education to Employment	NCERT, Shilong	8th Mar 2021
7	Dr. Madhu Dikshit	Women in Science and Technology	IITR, Lucknow	8th March 2021
8	Dr. Madhu Dikshit	Traditional Indian Medicines as potential therapeutic options for SARS-CoV-2/COVID-19: *Withania somnifera*/Ashwagandha	Global Bio-India	2nd March 2021
9	Dr. Madhu Dikshit	Scientific Temper and Societal Needs - Drivers of Innovation: CSIR Efforts in New Drug Discovery	Excellence in Leadership, CSIR-HRDC New Delhi	15th Jan 2021
10	Dr. Madhu Dikshit	Neuronal NOS - from Parkinson's disease to neutrophil differentiation: *Our Journey*	IAN (US Chapter)-Indo-US meeting	21 Dec 2020
11	Dr. Madhu Dikshit	Ashwagandha-Human Health and Covid-19	NASI symposium	16th Oct 2020
12	Dr. Madhu Dikshit	Biological activities of Ashwagandha (*Withania somnifera*): Indian regulations for Nutraceuticals	SBSU Dehradun Symposium on Nutraceuticals	14th Oct 2020
13	Dr. Madhu Dikshit	Natural Products for Human Health	NEIST Jorhat, Natural Product symposium	25th July 2020
14	Dr. Madhu Dikshit	New Drug Discovery: Challenges in India	ELSEVIER Symposium	16th July 2020
15	Dr. Guruprasad R Medigeshi	Coronavirus disease: lessons from the past, living in the	Webinar "Current Trends in Life Sciences"	5th Aug 2020

		pandemic and future perspectives	organized by Bangalore University	
16	Dr. Milan Surjit	Developing a potent vaccine against the SARS-CoV-2: Feasibility and challenges	National webinar on developing a potent vaccine against the SARS CoV2, Maitreyi College, Delhi University	Oct 2020
17	Dr. Milan Surjit	Host-pathogen interactions in SARS-CoV-2: Insights from virus-host RNA-protein interaction network analysis	National conference on host-pathogen interactions: present and future perspective, NIT Rourkela	Sep 2020
18	Dr. Milan Surjit	How to treat COVID-19? Towards identifying potent antiviral(s) against the SARS-CoV-2	Indo-Italian meeting on COVID-19, SERB, DST	July 2020
19	Dr. Milan Surjit	Potential prophylactic and therapeutic strategies against the SARS CoV2	Invited seminar, Pathways International School, Noida	May 2020
20	Dr. Milan Surjit	How to treat COVID-19? focus on developing potent antivirals against the SARS-CoV-2	Invited seminar, Apeejay Stya University, Gurugram	April 2020
21	Dr. Milan Surjit	Potential prophylactic and therapeutic strategies against the SARS-CoV-2	Invited seminar, Pathways International School (IB curriculum), Noida	April 2020
22	Dr. Amit Awasthi	IIS-FIMA online Immunology Course	IIS-FIMA online Immunology Course	8^{th}-10^{th} Oct 2020
23	Dr. Samrat Chatterjee	COVID-19 transmission dynamics during unlocking phase	Mathematical and Statistical modeling of infectious diseases relevant to COVID-19, Department of Mathematics, Assam University, Silchar	8^{th} July 2020
24	Dr. Samrat Chatterjee	Bistability in cell signaling and network motifs	International Web Conference on Advance Research in Science, Humanities and Social Science (IWCARSHSS 2020), Department of Mathematics, Maharaja Bir Bikram University	9^{th}-10^{th} July 2020
25	Dr. Samrat Chatterjee	Understanding the role of calcium dynamics in different disease conditions through mathematical models	Two-day National Level Webinar on Mathematics and It's Recent Trends, Department of Mathematics in collaboration with IQAC, Maulana Azad College and Biomathematical Society of India, Kolkata	28^{th}-29^{th} Sep 2020
26	Dr. Bhabatosh Das	Molecular insights into COVID-19 and its pathogen SARS-CoV-2	National Symposium	8^{th} June 2020
27	Dr. Bhabatosh Das	Genetic nature and dynamics of antibiotic resistance traits in the	University Faculty Recharge Programme	1^{st} March 2021

		commensal and pathogenic enteric bacteria		
28	Dr. Bhabatosh Das	Genomics of Extensively Drug-Resistant Enteric Bacterial Pathogens Isolated from India	International Symposium "Biocrest".	26th February 2021
29	Dr. Bhabatosh Das	Understanding the role of the human gut microbiome in the emergence and spread of antibiotic-resistant enteric bacterial pathogens	DBT Star Seminar	2nd February 2021
30	Dr. Bhabatosh Das	Homeostasis and Dysbiosis of the Human Gut Microbiome in Health and Diseases	Departmental Seminar	18th August 2020
31	Dr. Bhabatosh Das	Human Microbiome in Health and Diseases	International Symposium	6th August 2020
32	Dr. Bhabatosh Das	Human Microbiome in Health and Diseases	National Symposium	12th April 2020
33	Dr. Bhabatosh Das	Gut Microbiome: Associations, functions, and implications for health and disease	University Seminar	6th July 2020
34	Dr. Krishnamohan Atmakuri	One of the panelist members: Talk on "Protein secretion systems: a conduit of natural transformation	Vaibhav Summit session on AMR	17th Oct 2020
35	Dr. Pallavi Kshetrapal	Translational Medical Sciences	Webinar session organized by Initiative for Research and Innovation in Science Program, DST, Government of India, and the Indo-US Science & Technology Forum (IUSSTF)	31st July 2020
36	Dr. Pallavi Kshetrapal	BioRepository (BRF), NCR Biotech Science Cluster A DBT India Initiative	"Digital solutions for clinical trials/studies in the pandemic world" organized by CDSA and MRC, UK Session 2: Digital tools for participant engagement	16th Dec 2020
37	Dr. Sweety Samal	Virology and Animal & Human Challenge Models	IVR SME Consultation: Influenza Vaccines Research and Development (R&D) Roadmap Consultation, Wellcome Trust, UK	10th Sep 2020
38	Dr. Shubbir Ahmed	Protein-subunit Based Immunogen Design in HIV-1	Host-pathogen interaction: present and future perspective, NIT Rourkela	24-25 Sep 2020
39	Dr. Amit Kumar Yadav	The post-translational modification hotspots in human sirtuin interactors- mining and integrating proteomics data resources for studying cardiovascular diseases	"Proteomics in Agriculture and Healthcare", organized by the School of life sciences, University of Hyderabad, in association with Proteomics Society,	13th Mar 2021

			India (PSI). March 13-14, 2021 celebrating the "Proteomics Day", University of Hyderabad	
40	Dr. Amit Kumar Yadav	Proteomics and Mass Spectrometry – from peptides to proteins	Bioinformatics approaches in Genomics & Proteomics. NITTE University Centre for Science Education and Research, NITTE University, Mangalore	10th Dec 2020
41	Dr. Amit Kumar Yadav	Protein post-translational modifications (PTMs)-emergent properties, disease associations, and identification from proteomic data	12th Annual Meeting of Proteomics Society, India (PSI 2020)- International Virtual Symposium on "Integrated Omics Approaches in Health and Agriculture", NCL, Pune	22nd Nov 2020
42	Dr. Tripti Shrivastava	COVID-19; where are we today	Science Setu talk for Shaheed Rajguru College of Applied Sciences for Women, Delhi University	19th Oct 2020
43	Dr. Suchitra D Gopinath	Nutrient signaling in lean muscle development and disease	Conference title "Muscle and Diseases", Asoka University	6th-7th Nov 2020
44	Dr. Suchitra D Gopinath	The COVID-19 pandemic: Epidemiology and Current diagnostics in India	National Level Webinar, Vidyasagar College for Women, Kolkata	5th Jun 2020
45	Dr. Ruchi Tandon	Frontiers Editorial Board Webinar	Frontiers Editorial Board Webinar	28th May 2020

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 also affiliated with the Regional Centre for Biotechnology (RCB), Faridabad, Manipal Academy of Higher Education (MAHE), Karnataka, and Jadavpur University (JU), Kolkata for the Ph.D. program. The thematic areas for research are under the broader areas of Infection & Immunology, Maternal & Child Health, Non-Communicable Disease, Multidisciplinary Clinical & Translational Research, and Mathematical and Computational biology. As of 31st March 2021, 83 students have been enrolled in the THSTI doctoral programs.

List of Ph.D. Students joined in 2020-21 under the THSTI-JNU Ph.D. program

Name of the Student	Supervisor	Funding Agency
Ms. Himadri	Dr. Pallavi Kshetrapal	CSIR
Mr. Lovnish Thakur	Dr. Niraj Kumar	DBT
Ms. Meenal Chawla	Dr. Bhabatosh Das	CSIR
Mr. Ralzon Rosalia R	Dr. Gaurav Batra	CSIR
Mr. Chandan Kumar Verma	Dr. Guruprasad Medigeshi	UGC
Mr. Vishawjeet Barik	Dr. Amit Kumar Pandey	CSIR
Ms. Samriddhi Mehta	Dr. Milan Surjit	UGC
Ms. Ruchi	Dr. Krishnamohan Atmakuri	CSIR
Ms. Lovely Sisodiya	Dr. Krishnamohan Atmakuri	UGC
Ms. Bhawana	Dr. Santosh Mathapati	CSIR
Ms. Mrinali Paradkar	Dr. Shailaja Sopory	DBT
Ms. Saloni Sainger	Dr. Nisheeth Agarwal	CSIR
Mr. Diwakar Rathour	Dr. Guruprasad Medigeshi	DBT
Ms. Tanshi Mehrotra	Dr. Bhabatosh Das	UGC
Mr. Hardik Grover	Dr. Amit Awasthi	THSTI
Mr. Abhay Mishra	Dr. Amit Kumar Pandey	DBT
Ms. Umang Berry	Dr. Sankar Bhattacharyya	DBT
Ms. Divya Pillai	Dr. Nitya Wadhwa	THSTI
Ms. Neha Khan	Dr. Ramandeep Singh	CSIR
Ms. Imran	Dr. Nisheeth Agarwal	DBT

Ms. Taruna	Dr. Nitya Wadhwa	DBT
Mr. Sevaram Singh	Dr. Niraj Kumar	DBT
Mr. Shivam Singh	Dr. Guruprasad Medigeshi	DBT
Ms. Ranjana Mishra	Dr. Gaurav Batra	CSIR
Mr. Sunil	Dr. Santosh Mathapati	THSTI

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 Ph.D. students alike. Last year THSTI hosted and trained 45 students in the fields of Infection & Immunology, Maternal & Child Health, Non-Communicable Disease, Multidisciplinary Clinical & Translational Research, and Mathematical and Computational biology.

Students' Achievements:

- Seven of our Ph.D. students were conferred with doctoral degrees in 2020-21: Dr. Eira Chaudhary, Dr. Shilpi Sehgal, Dr. Bugga Paramesha, Dr. Mohammed Soheb Anwar, Dr. Hina Lateef Nizami, Dr. Parmeshwar Bajirao Katare, Dr. Kiran Bala

Eira Chaudhary

Shilpi Sehgal

Bugga Paramesha

Mohammed Soheb Anwar

Hina Lateef Nizami

Parmeshwar Bajirao Katare

Kiran Bala

EXTERNAL RELATIONS AND INSTITUTIONAL DEVELOPMENT OFFICE

External Relations and Institutional Development Office (ERID)

External Relations and Institutional Development (ERID) office supports researchers at THSTI with grants management, regulatory compliance for ethics committees, intellectual property management, scientific communications, and outreach. Ms. Vidhya Krishnamoorthy is the Technical Manager handling grants and ethics at ERID. At the time of this reporting, ERID welcomed Dr. Soma Patnaik as Professional Expert in scientific communications and intellectual property rights.

ADMINISTRATION

The Administration of THSTI performed relentlessly during the financial year 2020-21, to provide the scientific functions of the Institute with unstinted support for smooth functioning. The personnel in administration comply with the Government of India Rules and related guidelines issued by the Govt. of India from time to time in their functioning.

The THSTI Administration comprises of several functional sections, namely General Administration, Human Resources and Legal, Finance & Accounts, Stores & Purchase, Information Technology, Engineering & Estate Management, and Intellectual Property Management. The essential activities performed by various sections are detailed below.

General Administration

General Administration section broadly deals with the constitution of committees, the conduct of meetings of THSTI society, Governing body, Finance Committee, Scientific Advisory Committee, and other internal committees and wherever required, follow-up and implementation with the decisions of these committees, a commemoration of important days, hostel management, front office management, RTI and grievances handling, official language implementation functions, logistics arrangement for the staff, security management, house-keeping services management and any other functions as required by the Executive Director.

THSTI Governance: THSTI conducted one Society, two Governing Body, two Finance Committee, and one Scientific Advisory Committee meetings during the FY 2020-21. Recommendations made by the concerned 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. The various committees and the members of the same may be seen in the latter part of this report.

RTI: In compliance with the provisions of the Right to Information Act, 2005, THSTI has nominated Public Information Officer and Appellate authority to provide information to the citizens who seek information. 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 is minimized. During the period from 1st April 2020 to 31st March 2021, a total of 49 applications and 10 appeals were received by the institute. All the applications and appeals have been disposed of as per the provisions of the RTI Act, 2005 within the prescribed time limit.

Public Grievances: Public Grievances received offline and through CPGRAMS are monitored and disposed of on regular basis. During the FY 2020-21, 3 public grievances were disposed of within the prescribed time limit.

Measures taken to support the employees during the spread of COVID-19: The administration was working on all the days during the pandemic even when there was strict lockdown all over India. This was to support the COVID work that was happening in THSTI. To contain the spread of COVID, several measures were taken by the institute. A COVID Response Team (CORT) was constituted to provide support to the employees with a coordinated approach at the Institute level. Hostel and housing were provided free of cost to all the employees who were considered essential and called for duty for COVID-19 research activities and clinical work. Contact tracing was done for more than 200 employees and students who were infected with COVID and appropriate action was taken as per COVID protocol issued by the Ministry of Health and Family Welfare. To enable the movement of employees, we were in constant touch with the District Administration to provide permission for the same. Additional cabs and buses were introduced to transport employees since the other means of transport were not functional. Hand sanitizers were made available at every access point. N95 Masks were provided to all the employees free of cost. Sanitization of the common use areas and other places like doors, taps, lift buttons, etc. were done regularly. Awareness of the COVID-19 pandemic was made through emails from time to time to all the employees to comply with COVID appropriate behavior and to get themselves vaccinated.

Implementation of the official language policy: The Institute made efforts to promote the use of Hindi in official work to ensure proper implementation of the Official Language Policy of the Government. An official language implementation committee is functioning in the institute which had two meetings during the FY 2020-21. Suggestions were given to the employees to promote the use of Hindi in official work.

Emails were issued from time to time from Head-Administration to all officers/sections emphasizing the need for proper implementation of the official language policy of the Government. All correspondence received in Hindi was replied to in Hindi.

Hindi *Saptah* was organized from 7^{h} to 14^{th} September 2020 in the institute duly following the COVID appropriate behavior. Various Hindi competitions were organized, and successful participants were given cash awards.

Intellectual Property Protection and Collaborations

During the FY 2020-21, THSTI has made significant achievements 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 8 patent applications, 1 patent granted, and developed 4 technologies. The institute was a primary part of the 33 research collaborations/MoUs which were executed with different agencies during the FY 2020-21

Main events organized during the year 2020-21

THSTI observed all the important days as directed by the Govt. of India such as Sadbhavana Diwas, Constitution Day, International Yoga Day, Hindi Saptah, etc. in a low-key manner due to the pandemic duly following the COVID protocols.

- **International Yoga Day (21st June 2020)**

  Due to COVID-19 restriction, no mass gathering was advisable and hence an email was circulated to all students, staff, officers, and faculty/scientists of the institute to encourage people to practice yoga at their homes along with their family members and send their pictures so that the same can be uploaded on our social media handles.

- **Sadbhavana Diwas**

  Sadbhavana Diwas was observed on 20th August 2020. All students, staff, officers and faculty/scientists of the institute took the pledge virtually.

- **हिन्दी सप्ताह समारोह (7 सितम्बर 2020 से 14 सितम्बर 2020)**

  हिन्दी सप्ताह को मनाते हुए संस्थान में 14 सितम्बर 2020 को हिन्दी सप्ताह के समापन समारोह मे विभिन्न कार्यक्रम आयोजित किए गये| वैज्ञानिकों, अधिकारियों, कर्मचारियों एवम् विद्यार्थियों ने उत्साह से आयोजन में भाग लिया | कविता पाठन, हिन्दी मे बनाई गयी विडियो का प्रदर्शन आदि प्रतियोगिताओं का आयोजन किया गया | हिन्दी सप्ताह के समापन समारोह में मुख्य अतिथि डॉ यश गर्ग, आई.ए.एस, आयुक्त, नगर निगम, फरीदाबाद ने वैज्ञानिकों, अधिकारियों, कर्मचारियों एवम् विद्यार्थियों को संबोधित किया और सफल प्रतियोगियों को पुरस्कृत किया |

- **Constitution Day/Samvidhan Diwas**

Constitution Day was observed on 26th November 2020. The Preamble of the Constitution was displayed on the landing page of the institute's website All students, staff, officers, and faculty/scientists of the institute read the preamble in English and Hindi from their respective sections/labs.

- **Road Safety Week**

  THSTI observed Road Safety Week between 10th-17th February 2021. Pamphlets/banners at prominent locations were placed on the campus to spread road safety awareness. The week ended with a pledge administered by the Executive Director and taken by all students, staff, officers, and faculty/scientists of the institute took the pledge.

- **Swachhta Pakhwada**

  As a part of the Swachta Pakhwada celebrations, the institute took the following steps:

  - Civil engineering and biosafety departments spearheaded housekeeping activities covering restrooms, all building corridors, and roofs, hostel premises, cleaning drinking water coolers/dispensers, etc.
  - Signages directing people to avoid smoking, littering was installed on campus premises.
  - Posters and stickers depicting safety and cleanliness practices that should be adopted by everyone, especially scientific and technical people working in the labs have been put up on all floors.
  - Students and staff were regularly sensitized on the importance of maintaining cleanliness and adopting safety practices in the labs while working.
  - All investigators and administrative heads were directed to develop zero garbage offices, engage in e-waste management, de-clutter spaces, recycle paper waste and ensure working stations in labs are cleaned. They were asked to direct the staff to use different dustbins to collect different kinds of waste for effective waste management.

- Cafeteria of the institute and the hostel kitchen strictly adhere to the 'No plastic' policy and therefore usage of plastic cups, spoons, etc. is prohibited in these places.

Human Resources

The Human Resource Section of the institute deals with all the employees' matters, such as recruitment, promotion, training, probation, travel, employee benefits, discipline, employee welfare, exit, and other related matters. HR section deals with service matters of more than 700 employees. HR section also deals with the legal cases about the establishment and other matters. During the FY 2020-21, the section dealt with 2 cases, one each in the High court and District court.

Recruitment: 318 positions were filled up through 27 recruitment notices and many rolling recruitment notices. Rolling recruitment notices are issued to fill up vacancies of JRF/PA-I/SRF/PA-II//RA and clinical positions twice every month to cater to the requirements, which would arise regularly.

Promotion/Upgradation: Under the Modified Flexible Complementing Scheme (MFCS), Dr. Krishnamohan Atmakuri and Dr. Amit Kumar Pandey were promoted from the position of Assistant Professor to Associate Professor; Dr. Ramandeep Singh and Dr. Nisheeth Agarwal were promoted to the position of Professor. Eight regular employees were upgraded under the Modified Assured Career Progression Scheme during the FY 2020-21 from their entry grade to the next higher grade.

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 and family get-togethers and sports activities are organized once a year.

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 non-governmental funds. These awards are distributed every year during the Foundation Day celebrations. During the year 2020-21, the following personnel/teams were awarded:

S.No	Name of the award	Cash award (in Rs)	Name of the employee/team
1	Dr. M.K. Bhan Group Award for the most impactful collaboration	Rs. 50000/-	Clinical, Biorepository, Diagnostic and Bioassay team for COVID-19 cohort
2	Award for a faculty for the best-published paper	Rs. 15000/-	Dr. Nisheeth Agarwal
3	Award for a Ph.D. student for the best-published paper	Rs. 15000/-	Ms. Jyoti Verma, Ph.D. Student
4	Award for a Ph.D. student for the 5 years all-round performance	Rs. 15000/-	Dr. Hina Lateef, PhD Student
5	Award for a faculty for overall contribution to the institutional development during the previous financial year	Rs.10000/-	i. Dr. Guruprasad R. Medigeshi, Professor ii. Dr. Ramandeep Singh, Professor
6	Award for an administrative staff for overall contribution to the institutional development during the previous financial year	Rs.10000/-	i Mr. Vikash Kumar, Clerical Assistant ii. Mr. Narender Kumar, Technical Officer II
7	Award for technical staff for overall contribution to the institutional development during the previous financial year	Rs.10000/-	i. Mr. Roshan Kumar, Senior Technical Officer ii. Ms. Anjali Anand, Technical Officer I

Stores and Purchase

The day-to-day purchase work is managed by the Standing Purchase Committee, a team of officials and Section Officers 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 from 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 2020-21 is provided below:

Procurement for Financial Year 2020-2021				
Particular	**Total Orders**		**Total Order Value (INR)**	
	Non-GeM	**GeM**	**Non-GeM**	**GeM**
Consumable	3272	216	1,944,38,150.00	22,19,813.00
Equipment	167	85	75,812,488.84	5,038,414.00
Total	3439	301	27,02,50,638.84	72,58,227.00

Finance and Accounts

The F&A section advises on the financial matters, receipt of funds from various funding agencies and attends to the day-to-day payments to contractors/ suppliers, payment of salaries to staff duly taking into account all tax matters, etc. The section is also responsible for preparing the annual statement of accounts which is provided in the latter part of this report.

For the FY 2020-21 the brief financial highlights are mentioned here under:

As on 31.03.2021

(Rs in Lakhs)

Head	**Op. Balance 01.04.20**	**Grant Receipt**	**Other receipts**	**Total Receipt**	**Expenditure up to 31.03.21**	**Interest deposited back to the GoI**	**Balance as on 31.03.21**
1	2	3	4	5=2+3+4	6	7	8=5-6-7
GIA	127.70	750.00	0.00	877.70	668.54	0.00	209.16

Manpower							
GIA General	0.00	1800.00	0.00	1800.00	1800.00	0.00	0.00
GIA Capital	4.50	1000.00	0.00	1004.50	1004.10	0.00	0.40
Bank Interest & other receipt	201.30	0.00	126.66	327.96	0.00	201.30	126.66
Total	**333.50**	**3550.00**	**126.66**	**4010.16**	**3472.64**	**201.30**	**336.22**

The section has adopted various digital methods for payment disbursements/collections to avoid cash transactions.

The section has implemented the PFMS EAT, TSA modules, and also implemented ERP partially.

Information Technology

The IT section of the institute is responsible for the complete network infrastructure, all the hardware maintenance, purchase of IT equipment, website maintenance, employee ethsti portal, operation and maintenance of CCTVs, and any other matter dealing with IT.

During the financial year 2020-21, the ERP's Finance module was made live, set up for CCTV in SAF with LAN infrastructure was made operational, various arrangements were made to improve the quality of online meetings and events with the latest technology and as a standby to the NKN network, BSNL internet connectivity through optic fiber cable was also taken to ensure uninterrupted internet connectivity for the campus.

Civil Engineering

The civil engineering section is responsible for the civil engineering works and the estate management functions for the institute. During the FY 2020-21, the section was instrumental in the completion of infrastructural modification for the COVID-19 testing laboratory, Immunology core lab, Influenza lab, and BSL-3 laboratory building.

Within the NCR BSC campus, they carried out various horticulture work plans, construction of stone boundary wall for the 85 acres of land, construction of Office of Connectivity, and completed operationalization of newly constructed floors of hostel building among other assignments. Besides, many ecological developmental works were carried out as required by the IBSC and EHS committees to ensure sustainable development with minimum impact on the environment. The section has ensured 100% recycling of used water and produces compost from the waste generated from the cluster. The section has initiated the tender activities for the construction of Phase-III works of NCR-BSC, Faridabad through Project Management Company.

Electrical Engineering

The Electrical Engineering section is responsible for the maintenance of scientific and other electrical equipment, electrical sub-station activities including operation of various diesel generator sets, grid connectivity from the Pali sub-station of DHBVN, electrical installation in the campus, solar panels, fire extinguishing equipment, and statutory requirements to prevent the risk of fire, lift maintenance and BSNL/Airtel telephone connectivity.

During the FY 2020-21, the section was instrumental in the completion of infrastructural modification for Bioassay laboratory, Biorepository, Aryabhata Data science center, AI Program center, and Infectious Disease Research Facility (IDRF). Moreover, the electrical installation for the additional hostel rooms as a result of the vertical extension of the hostel and office of connectivity were completed

Within the NCR BSC campus, the section successfully executed an energy audit and took action to save electricity worth Rs. 20 lakh per annum by various measures such as replacement of the existing electrical fixtures with LED, effective utilization of HVAC, lifts, and so on. A detailed project report was prepared for the installation of a solar power system of 500 KW capacity and a 66KV electric grid within the campus and action is on hand to implement the same subject to approvals from the concerned authorities.


ट्रांसलेशनल स्वास्थ्य विज्ञान एवं प्रौद्योगिकी संस्थान

Mehra & Sistani

Chartered Accountants

New Delhi

AUDITORS' REPORT

To
Executive Director
TRANSLATIONAL HEALTH SCIENCE AND TECHNOLOGY INSTITUTE
FARIDABAD

1. We report that we have audited the Balance sheet of "**TRANSLATIONAL HEALTH SCIENCE AND TECHNOLOGY INSTITUTE** "as at 31 March 2021 and the relative Income & Expenditure Account and Receipt & Payment Account for the period ended on that date, annexed there to. These financial statements are responsibilities of the society management. Our responsibility is to express an opinion on these financial statements based on our audit.

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

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

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

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

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

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

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

.....Contd/2

Apptt. 101, I-22, Jangpura Extn. New Delhi-110014, Tel. : 24324085, 24316479, 43580293 Fax : 24326339
E-mail :info@mehrasistani.com , Web-site:mehrasistani.com

I. In the case of the Balance sheet, of the state of affairs of the Institute as at 31'march 2021;and

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

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

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

(Sanjiv Rai Mehra)
Partner
Membership No.080402
Date : 29/07/2021
UDIN: 21080402AAAAET3060

TRANSLATIONAL HEALTH SCIENCE AND TECHNOLOGY INSTITUTE,FARIDABAD

BALANCE SHEET AS AT 31ST MARCH, 2021

Amount (In Rs.)

CORPUS/CAPITAL FUND AND LIABILITIES	Schedule	31.03.2021	31.03.2020
Corpus / Capital Fund	1	1,90,05,51,697	1,92,33,67,334
Reserves and Surplus	2	11,98,24,778	10,07,82,346
Earmarked/Endowment Funds	3	-	-
Secured Loans and Borrowings	4	-	-
Unsecured Loans and Borrowings	5	-	-
Deferred Credit Liabilities	6	-	-
Current Liabilities and Provisions	7	73,48,02,179	59,34,99,641
TOTAL		**2,75,51,78,654**	**2,61,76,49,321**
ASSETS			
Fixed Assets	8	1,68,90,75,598	1,72,17,91,391
Investment From Earmarked/Endowment Funds	9	-	-
Investment-Others	10	2,700	2,700
Current Assets, Loans, Advances etc.	11	1,06,61,00,356	89,58,55,230
Miscellaneous Expenditure (to the extent not written off or adjusted)		-	-
TOTAL		**2,75,51,78,654**	**2,61,76,49,321**
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.PRAMOD KUMAR GARG)
EXECUTIVE DIRECTOR

(SANJIV RAI MEHRA)
PARTNER
M. No. 80402

TRANSLATIONAL HEALTH SCIENCE AND TECHNOLOGY INSTITUTE FARIDABAD

MEHRA & SISTANI Chartered Accountants

Place: Faridabad
Date: 29/07/2021

TRANSLATIONAL HEALTH SCIENCE & TECHNOLOGY INSTITUTE (THSTI)
Faridabad

CONSOLIDATED RECEIPTS AND PAYMENTS ACCOUNT FOR THSTI,PROJECTS & FELLOWSHIP FOR THE YEAR ENDED 31ST MARCH, 2021

AMOUNT-IN-RUPEES

RECEIPTS	31.03.2021		31.03.2020	
OPENING 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	
Grant-in Aid Received:-				
Fellowship	1,49,41,736		2,34,24,252	
Projects	59,77,22,209		53,17,08,569	
THSTI	35,50,00,000		54,50,00,000	
Other Receipts -THSTI				
Application Fees	-		51,775	
Guest House Receipt	18,83,372		6,85,437	
HRA Recovery	22,00,681		24,48,652	
Income from Sales and Services	2,43,75,403		1,53,41,391	
Interest Received from Banks	1,26,65,521		1,61,15,378	
Miscellaneous Receipts	23,10,292		4,61,254	
Penalty Receipt	79,257		1,11,712	
Receipt from STTP	5,02,500		3,37,000	
Recruitment Fee	11,77,416		12,34,507	
RTI Receipt	110		478	
Sales of Scrap	63,277		51,486	
Tender Fee	51,884		66,700	
Vendor Registration Fee	68,973		1,10,000	
Donation	1		-	
Accrued Interest Received	20,56,981		9,77,921	
Advance Receipt From Debtors	4,44,000		4,248	
Building Contribution From Constituents	8,18,53,056		7,56,00,000	
Decrease in advances	1,46,25,428		85,75,331	
Earnest Money Deposit	34,61,372		41,00,379	
Govt. Dues Payable	23,13,974		5,65,759	
Other Liabilities/Payable	3,14,54,682		2,66,12,050	
Security / Hostel Deposit Received	27,90,967		5,72,240	
TOTAL		**1,75,16,23,651**		**1,70,76,23,420**

AMOUNT-IN-RUPEES

PAYMENTS Particulars	31.03.2021		31.03.2020	
Fellowship Paid	**1,75,83,245**		**2,29,49,928**	
Projects Expenditure	**54,96,44,643**		**43,16,42,494**	
THSTI Expenditure:-				
Fixed Assets	5,91,69,502		6,61,66,252	
Patent WIP	38,19,985			
Work -in- Process- Building	94,26,236		28,18,850	
Consumables	3,20,19,358		4,46,92,973	
Manpower	6,68,54,053		7,83,09,701	
Administrative Expenses	14,78,63,669		14,95,43,299	
Advances , Receivables & Liabilities	11,58,14,825		31,19,19,364	
Closing Cash & Bank Balance				
Fellowship	**(92,36,699)**		**(65,95,190)**	
Projects	**54,60,10,129**		**49,79,32,562**	
THSTI	**21,26,54,707**		**10,82,43,187**	
TOTAL		**1,75,16,23,651**		**1,70,76,23,420**

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

(MANOJ KUMAR)
SECTION OFFICER (F & A)

(NAVNEET)
HEAD ADMINISTRATION

(DR. PRAMOD GARG)
EXECUTIVE DIRECTOR

(SANJIV RAI MEHRA)
PARTNER

PLACE: Faridabad
DATE: 29/07/2021

TRANSLATIONAL HEALTH SCIENCE AND TECHNOLOGY INSTITUTE
FARIDABAD

SCIENTIFIC EVENTS AND OUTREACH

As a part of spreading awareness about COVID-19, THSTI conducted many webinars and podcasts. In April 2020, THSTI partnered with DBT/Wellcome Trust India Alliance, IAVI, and Nature India to bring together a series of webinars "COVID-19 Ask the Experts" where panelists answered questions about COVID-19, scientific underpinnings, public health questions, and the role of communication. The webinar series was primarily targeted at journalists writing health and science stories in the regional media.

THSTI participated in the webinars of COVID-19 Preparedness - National Cancer Grid Initiative with Dr. Gagndeep Kang as a speaker along with Dr. Randeep Guleria, Daniel Vanderende in the 2nd webinar, and with Dr. K. VijayRaghavan, Dr. Shruti Tandon, and Dr. Soumitra Pathare in the 4th webinar.

THSTI conducted COVID-19 Ask the Experts Part -7: Building bridges – Engaging communities during and beyond the pandemic on 20th May 2020 which was attended by 100 participants. In a webinar entitled "strengthening research capacities remotely to empower COVID-19 research" organized by DBT and Elsevier, Prof. Kang spoke about COVID-19 testing, vaccines, and drug development. During a Podcast by Bangalore International Centre in May 2020, Dr. Gagandeep Kang spoke about the challenges of developing antivirals. She also participated in IndSciCOVID's Live Q&A on 17th May 2020.

In June 2020, Webinar on "Ethical considerations for clinical research during COVID-19 pandemic" was conducted by CDSA (https://www.youtube.com/watch?v=wFWse-2G5Io&feature=youtu.be). THSTI co-organized the 8th webinar of COVID-19 Ask the Experts titled "Dealing with the Unknown – Mental health challenges during the pandemic and beyond" with DBT/WT India Alliance, IAVI, and Nature India (https://www.youtube.com/watch?v=xj0GE2sYj4Y). In June 2020, CDSA organized two webinars viz., "New medical device regulations in India and commonly used medical devices in COVID-19: Questions & answers" and "Pharmacovigilance during COVID-19 Pandemic." (https://www.youtube.com/watch?v=uZYQ_ObeJn8&feature=youtu.be).

In July 2020, a series of online training courses were rolled out for the BIRAC-National Biopharma Mission (NBM). Module 1 of this online course on Good Clinical Practice (GCP) was organized by CDSA.

In August 2020, CDSA, THSTI conducted a webinar on ''Pharmacoeconomice: Is your drug therapy value for money?'' CDSA-THSTI contributed to the Faculty Development Program

of the University of Delhi. CDSA completed its Online Good Clinical Practice (GCP) Course (Series I). The course was conducted from Aug 07-Sept 04, 2020.

THSTI and RCB co-organized a session of the VaibhaV summit 2020 of the Government of India, themed antimicrobial resistance on 17th October with experts from India and overseas. The session was titled "Insights into the emergence and spread of clinically relevant antimicrobial-resistant bacterial pathogens in India."

In collaboration with Ind-CEPI (India centric Coalition for Epidemic Preparedness Innovations) - Biotechnology Industry Research Assistance Council (BIRAC) organized the following series of online programs under DBT's initiative "Strengthening clinical trial research capacity in neighboring countries''

- Good Clinical Practice (Oct 09 – 29, 2020)
- Ethical considerations in clinical research (Nov 06-13, 2020)
- Good Clinical Laboratory Practice (Nov 20 – 27, 2020)
- Novel vaccine development and immunization policy in a pandemic (Dec 04-11, 2020)

To address gaps in understanding the method and for acquiring critical skills in testing, THSTI in collaboration with Foundation for Innovative New Diagnostics (FIND) conducted a series of training workshops on "Real-time RT-PCR method of SARS-CoV-2 detection". This workshop was organized by the Bioassay laboratory (BL), THSTI. It has provided training to more than 50 personnel.

CDSA conducted the online course on "Current regulatory requirements for conducting clinical trials in India for investigational new drugs/new drug (Version 3.0)". This course is developed by CDSA in partnership with the Central Drugs Standard Control Organisation (CDSCO) and National Programme on Technology Enhanced Learning (NPTEL). The course is hosted on the SWAYAM platform and accessed by anyone, anywhere at any time.

On 26th February, THSTI celebrated National Science Day virtually with participants from different colleges across Delhi-NCR. Virtual Science Setu was conducted on 16th-17th March 2021. Faculty members and scientists from THSTI and teachers and students from Ram Lal Anand College, Delhi University, Deen Dayal Upadhyaya College, Delhi University, Acharya Narendra Dev College, DU, and St. Aloysius College, Jabalpur participated in the webinar.

THSTI COMMITTEES

S.No	Committee	Members
1	**Scientific Advisory Committee**	Dr. Partha Majumder Dr. Raghavan Varadarajan Dr. Rajesh Gokhale Dr. Ashok Venkataraman Dr. Judi Allen Dr. Balachandran Ravindran Dr. Jaya S. Tyagi Dr. B.V. Ravi Kumar **Chairperson - Dr. Partha Majumder**
2	**THSTI Management Committee**	Executive Director and Heads of all the Centres **Chairperson - Executive Director**
3	**Finance Committee**	Financial Advisor, DBT (**Chairperson)** Executive Director, THSTI Deputy Secretary (Finance), DBT Scientific Coordinator-THSTI Executive Director, RCB Dr. B. Ravindran, Former Director, ILS, Bhubaneshwar Dean, THSTI Administrative Officer (F & A), 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**
5	**Purchase Committee**	Dr. Amit Awasthi Dr. Shailaja Sopory Dr. Nisheeth Agarwal Dr. Dinesh Mahajan Dr. Gaurav Batra Dr. Shubbir Ahmed Mr. Manoj Kumar Mr. Satish Kumar **Chairperson - Dr. Amit Awasthi / Dr. Shailaja Sopory**
6	**Specification subcommittee**	Dr. Jayanta Bhattacharya Dr.Milan Surjit Dr. Gaurav Batra Mr. Satish Kumar **Chairperson – Dr. Jayanta Bhattacharya**

7	**IT & 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**
8	**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 Ms Amandeep Kaur Ahuja Dr. Ujjayini Ray Ms. Jasmine Singh Dr Shailaja Sopory Prof. Arti Kapil Dr. Bhabatosh Das Ms. Vidhya Krishnamoorthy **Chairman - Prof. Satinder Aneja**
9	**Institutional Animal ethics committee**	Dr. Sudhanshu Vrati Dr. Niraj Kumar Dr. Krishnamohan Atmakuri Dr. Amit Awasthi Dr. Amit Pandey Shri. M.T. Sambandam Mr. Ranvir Parashar Prof. Harbans Lal Dr. J.P. Mittal **Chairperson - Dr. Sudhanshu Vrati**
10	**Institutional Committee for Stem Cell Research (IC-SCR)**	Prof. Narinder Mehra Prof. Sujata Mohanty Dr. Sam Mathew Dr. Prasad Abnave Prof. Prasenjit Guchhait Dr. Sivaram Mylavarapu Prof Nalin Mehta Dr. Ujjayini Ray Ms. Jasmine Singh Mr. Munawwar Naseem **Chairman - Prof. Narinder Mehra** **Coordinator/Member Secretary - Ms. Vidhya Krishnamoorthy**
11	**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**
12	**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**
13	**RTI Act**	Dr. Krishnamohan Atmakuri – PIO Dr. Nisheeth Agarwal – Appellate Authority Mr. M.V. Santo – Nodal Officer Executive Director – Public Authority
14	**Complaints Committee (to enquire into complaints of sexual harassment)**	Dr. Shinjini Bhatnagar Dr. Nita Bhandari Dr. Nisheeth Aggarwal 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**
15	**Student & Employee Welfare, Sports and Hostel Committee**	Dr. Sankar Bhattacharyya Dr. Susmita Chaudhuri Dr. Tripti Srivastava Dr. Nagender Rao Rameshwaram Dr. Santosh Sadashiv Mathapati Mr. M.V. Santo **Chairperson – Dr. Sankar Bhattacharyya**
16	**Tender Opening Committee**	Mr. Manoj Kumar Mr. Satish Kumar Mr. Gopal Kishan Chauhan Ms. Rajni Verma **Chairperson – Mr. Manoj Kumar / Mr. Satish Kumar**
17	**Vigilance Officer**	Dr. Ramandeep Singh
18	**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, Bangalore Dr. Partha Majumder, Former Director, NIBMG

		Dean Clinical Research, THSTI **Chairperson - Dr. V.S. Chauhan**
19	**SC/ST Grievance Redressal Committee**	Dr. Niraj Kumar Dr. Milan Surjit Mr. M. V. Santo **Chairperson – Dr. Niraj Kumar**
20	**Environmental Health & Safety Committee**	Dr. Dinesh Mahajan Dr. Nisheeth Aggarwal Dr. Sushmita Chaudhuri Mr. Vishal Gupta Dr. T. Ramachandran Dr. Sanjay K. Banerjee Dr. Milan Surjit **Chairperson – Dr. Dinesh Mahajan**
21	**ERP committee**	Dr. Amit Kumar Yadav Dr. Nisheeth Aggarwal Dr. Ramandeep Singh Dr. Guruprasad R. Medigeshi Mr. M. V. Santo **Chairperson – Dr. Amit Kumar Yadav**
22	**IDRF Committee**	Dr. Ramandeep Singh Dr. Prasenjit Guchhait Dr. Avinash Bajaj Dr. Guruprasad R. Medigeshi Dr. Nisheeth Aggarwal Dr. Padmakar Tambare Mr. Ramesh Kumar Rathore Mr. G. R. Aggarwal **Chairperson – Dr. Ramandeep Singh / Dr. Prasenjit Guchhait**
23	**Science Setu Committee**	Dr. Krishnamohan Atmakuri Dr. Pallavi Kshetrapal Dr. Tarun Kumar Sharma Dr. Siuli Mitra **Chairperson - Dr. Krishnamohan Atmakuri**
24	**Ecological Committee**	Dr. Shinjini Bhatnagar Dr. Ramandeep Singh Dr. Sankar Bhattacharya Dr. Tushar K. Maiti Dr. Feroz Khan Suri Mr. Narender Sharma Mr. M. V. Santo Mr. Ramesh Kumar Rathore **Chairperson - Dr. Shinjini Bhatnagar**
25	**Sports Committee**	Dr. Amit Awasthi Dr. Samrat Chatterjee Dr. Pallavi Kshetrapal Dr. Niraj Kumar Dr. Amit Kumar Yadav

		Chairperson - Dr. Amit Awasthi
26	**Radiation Safety Committee**	Dr. Milan Surjit Dr. Guruprasad R. Medigeshi Dr. Dinesh Mahajan Dr. Bhabatosh Das Dr. Krisnamohan Atmakuri Dr. Susmita Chaudhuri Mr. Vishal Gupta **Chairperson - Dr. Milan Surjit**
27	**Official language implementation committee**	Dr. Amit Kumar Pandey Dr. Amit Awasthi Dr. Pallavi Kshetrapal **Chairperson** - Dr. Amit Kumar Pandey
28	**Memorandum of Understanding (MoU) committee**	Dr. Ramandeep Singh Dr. Amit Awasthi Dr. Krishnamohan Atmakuri Mr. M. V. Santo **Chairperson - Dr. Ramandeep Singh**
29	**Staff Welfare Fund Managing body**	Executive Director – ex-officio member Dr. Amit Kumar Pandey Head-Admin – ex-officio member Administrative Officer (F&A) – ex-officio member Mr. Vishal Gupta Ms. Rajni Verma **Chairperson - Executive Director**
30	**Peer Review Committee (PRC)**	Dr. Guruprasad R. Medigeshi Dr. Nitya Wadhwa Dr. Susmita Chaudhuri **Chairperson - Dr. Guruprasad R. Medigeshi**
31	**THSTI-RCB Day-Care committee**	Dr. Pallavi Kshetrapal Dr. Susmita Chaudhuri Dr. Divya Chandran Dr. Sivaram V. S. Mylavarapu Dr. Deepti Jain **Chairperson - Dr. Pallavi Kshetrapal**

List of Seminars & Conferences in the year 2020-2021

S. No.	Name of the participant	Meeting title	Venue	Date
1	Dr. Shinjini Bhatnagar	SEHAT Extraordinary General Meeting	Virtual	1st April 2020
2	Dr. Shinjini Bhatnagar	Schedule of Mentorship: CRU, AIIMS	Virtual	29th April 2020
3	Dr. Shinjini Bhatnagar	Interactive meeting with Hon'ble Minister	Virtual	28th April 2020
4	Dr. Shinjini Bhatnagar	MOMI Harmonized Variable Workshop- Option 1 of 2	Virtual	5th-7th May 2020
5	Dr. Shinjini Bhatnagar	Interview for the post of ED, NHSRC	New Delhi.	16th May 2020
6	Dr. Shinjini Bhatnagar	Invitation for Faculty Development Program on Clinical Research	Sharda University, Noida	1st-6th June 2020
7	Dr. Shinjini Bhatnagar	India-EU-co-funding under Horizon 2020-Expression of interest-Coronavirus Research	Virtual	2nd June 2020
8	Dr. Shinjini Bhatnagar	Expert Committee Meeting: IIBC Proposals	Virtual	5th June 2020
9	Dr. Shinjini Bhatnagar	Follow-up discussion meeting of Secretary, DBT with Directors of DBT Autonomous Institutes on COVID-19 research efforts	Virtual	6th June 2020
10	Dr. Shinjini Bhatnagar	BIRAC interviews Selection Committee Meeting for NBM Positions	Virtual	27th July 2020
11	Dr. Shinjini Bhatnagar	DBT AIs and PSUs meeting with the Hon'ble Minister	Virtual	1st Aug 2020
12	Dr. Shinjini Bhatnagar	WLGH 2020 Day 1: Spotlight on South Asia	Virtual	13th-14th Oct 2020
13	Dr. Shinjini Bhatnagar	Research Methodology Workshop	Sharda University, Noida	6th Nov 2020
14	Dr. Shinjini Bhatnagar	The First Prof. Maharaj Kishan Bhan Memorial Lecture	Virtual	9th Nov 2020
15	Dr. Shinjini Bhatnagar	SRF-RA Selection committee for MEDIC-11 - Invitation from Director, CSIR-IICT	Virtual	17th Dec 2020
16	Dr. Shinjini Bhatnagar	Concluding remarks Medic - 11 virtual interview CSIR-SRF/RA 18th Dec	Virtual	18th Dec 2020
17	Dr. Shinjini Bhatnagar	SUN PHARMA RESEARCH AWARDS 2020 - JURY MEETING	Virtual	19th Dec 2020
18	Dr. Shinjini Bhatnagar	Research Council Meeting	Virtual	7th Jan 2021
19	Dr. Shinjini Bhatnagar	The first meeting of the Expert Group of the Vaccine Expert Committee	Virtual	21st Jan 2021
20	Dr. Shinjini Bhatnagar	2nd meeting of the Technical Monitoring and Advisory	Virtual	22nd Jan 2021

		Committee for periodic monitoring of the progress of the "GenomeIndia" and "Human Microbiome" initiatives		
21	Dr. Shinjini Bhatnagar	Reconstitution of Steering Committee MEETING	THSTI	8th March 2021
22	Dr. Shinjini Bhatnagar	AIIMS: DBT Wellcome Trust India Alliance Clinical and Public Health fellowship – Grantsmanship Workshop	Virtual	10th March 2021
23	Dr. Tripti Shrivastavav	Global Bio-India 2021	Virtual	1st-3rd March 2021
24	Dr. Suchitra D Gopinath	Muscle and Diseases	Virtual	6th-7th Nov 2020
25	Dr. Lovejeet Kaur	Comprehensive National Nutrition Survey: Data User Workshop	Institute of Economic Growth, University of Delhi	21st Jan 2020
26	Dr. Lovejeet Kaur	7th Anemia Research Consortium Meeting	Virtual	3rd Sep 2020
27	Dr. Lovejeet Kaur	Pediatric Anemia in India: Current Scenario, Challenges, and Solutions	Virtual	28th Jan 2021
28	Dr. Suprit Deshpande	HIV Research for Prevention (R4P)	Virtual	27th-28th Jan and 3rd-4th Feb 2021
29	Dr. Ranajoy Mullick	HIV Research for Prevention (R4P)	Virtual	27th-28th Jan and 3rd-4th Feb 2021
30	Dr. Nitin Hingankar	HIV Research for Prevention (R4P)	Virtual	27th-28th Jan and 3rd-4th Feb 2021
31	Ms. Jyoti Sutar	HIV Research for Prevention (R4P)	Virtual	27th-28th Jan and 3rd-4th Feb 2021