thsti

ट्रांसलेशनल स्वास्थ्य विज्ञान
एवं प्रौद्योगिकी संस्थान

TRANSLATIONAL HEALTH SCIENCE
AND TECHNOLOGY INSTITUTE

ANNUAL REPORT
2015-2016

Our 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.

Our Vision

As a networked organization linking many centers of excellence, THSTI is envisaged 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 developing cohorts and studying the pathogenesis and the molecular mechanisms of disease to generate knowledge to complement the processes of designing interventions and technology development.

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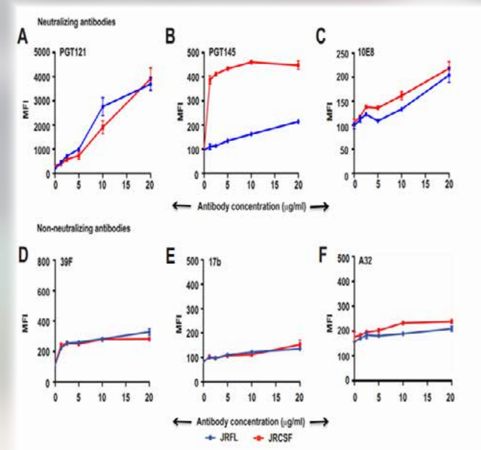

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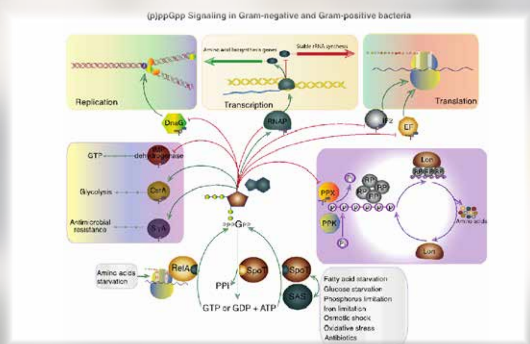

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THSTI
TRANSLATIONAL HEALTH SCIENCE AND
TECHNOLOGY INSTITUTE

Organization

INTRAMURAL CENTRES

Vaccine & Infectious Disease Research Centre (VIDRC)

Pediatric Biology Centre (PBC)

Centre for Biodesign & Diagnostics (CBD)

Centre for Human Microbial Ecology (CHME)

Policy Centre for Biomedical Research (PCBR)

Drug Discovery Research Centre (DDRC)

PARTNERSHIP CENTRE

Population Science Partnership Centre (PSPC)

EXTRAMURAL CENTRE

Clinical Development Services Agency (CDSA)

THSTI Society

1. **Dr. G. Padmanaban**
   Distinguished Professor,
   IISc Bangalore
   President

2. **Dr. K. Vijay Raghavan**
   Secretary,
   Department of Biotechnology
   Govt. of India,
   New Delhi
   Member Ex-officio

3. **Dr. Soumya Swaminathan**
   Secretary, Department of Health Research & Director General ICMR, New Delhi
   Member Ex-officio

4. **Mr. Jagannath Bidyadhar Mohapatra**
   Joint Secretary and Financial Advisor, Department of Biotechnology,
   Government of India,
   New Delhi
   Member Ex-officio

5. **Dr. Alka Sharma**
   Nodal Officer, THSTI, Director, Scientist-F, Department of Biotechnology, Government of India, New Delhi
   Member Ex-officio

6. **Dr. Chandrima Shaha**
   Director,
   National Institute of Immunology,
   New Delhi
   Member Ex-officio

7. **Dr. M. Radhakrishna Pillai**
   Director,
   Rajiv Gandhi Centre for Biotechnology,
   Thiruvananthapuram
   Member

8. **Dr. Ashok Jhunjhunwala**
   Professor,
   Indian Institute of Technology,
   Chennai
   Member

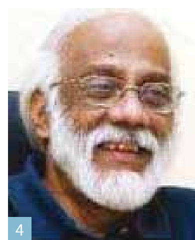
9. **Dr. J. Gowrishankar**
   Director,
   Centre for DNA Fingerprinting & Diagnostics,
   Hyderabad
   Member

10. **Dr. B. Ravindran**
    Director, Institute of Life Sciences, Bhubaneshwar
    Member

11. **Dr. G.C. Mishra**
    Eminent Scientist, National Centre for Cell Sciences,
    Pune
    Member

12. **Dr. G.B. Nair**
    Executive Director, Translational Health Science and Technology Institute,
    Faridabad
    Member

THSTI Governing Body



1. **Dr. K. Vijay Raghavan**
   Secretary,
   Department of
   Biotechnology,
   Government of India,
   New Delhi
   Chairperson Ex-officio

2. **Dr. Soumya Swaminathan**
   Secretary, Department
   of Health Research &
   Director General ICMR,
   New Delhi
   Member Ex-officio

3. **Dr. Subrata Sinha**
   Executive Director
   (additional charge),
   Regional Centre
   of Biotechnology,
   Faridabad
   and
   Director, National
   Brain Research Centre,
   Manesar, Gurgaon
   Member Ex-officio

4. **Dr. G. Padmanaban**
   Distinguished
   Professor, Indian
   Institute of Science,
   Bangalore
   Member

5. **Dr. P. N. Tandon**
   President, National
   Brain Research Centre,
   Manesar, Gurgaon
   Member

6. **Dr. T. S. Balganesh**
   President
   Research and
   Development:
   Gangagen
   Biotechnologies Pvt.
   Ltd, Bangalore
   Member

7. **Dr. Balram Bhargava**
   Professor, All India
   Institute of Member
   Medical Sciences,
   New Delhi
   Member

8. **Dr. K. Srinath Reddy**
   President, Public
   Health Foundation of
   India,
   New Delhi
   Member

9. **Dr. M.S. Ananth**
   Ex-Director,
   Indian Institute of
   Technology, Chennai
   Member

10. **Dr. Ashutosh Sharma**
    Secretary to the
    Government of India,
    Department of Science
    and Technology,
    New Delhi
    Member

11. **Dr. Alka Sharma**
    Nodal Officer, THSTI,
    Director / Scientist-F,
    Department of
    Biotechnology,
    Government of India,
    New Delhi
    Member Ex-officio

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    Joint Secretary and
    Financial Advisor,
    Department of
    Biotechnology,
    Government of India,
    New Delhi
    Member Ex-officio

13. **Dr. Shinjini Bhatnagar**
    Dean, Clinical
    Research,
    Translational Health
    Science & Technology
    Institute, Faridabad
    Member Ex-officio

14. **Dr. Sudhanshu Vrati**
    Dean, Academics,
    Translational
    Health Science and
    Technology Institute,
    Faridabad
    Member Ex-officio

15. **Dr. G.B.Nair**
    Executive Director,
    Translational Health
    Science and
    Technology
    Institute, Faridabad
    Member-Secretary
    Ex-officio

THSTI Scientific Leadership

G. Balakrish Nair is the Executive Director of THSTI. His prior assignment, spanning over two decades, was at the National Institute of Cholera and Enteric Diseases (NICED), Kolkata, culminating as the Institute's Director. The tenure at NICED was bridged with a seven year stint as Director, Laboratory Sciences Division, International Centre for Diarrhoeal Diseases Research, Dhaka, Bangladesh. His contributions to the discovery of Vibrio cholerae O139 Bengal, earned him the prestigious Shanti Swarup Bhatnagar award for Medical Sciences in 1998. In his role as the head of the organization, he has brought a unique culture of gentle reshaping of scientific minds towards translational research at THSTI. He also leads the Centre for Human Microbial Ecology at THSTI.

Dr. Sudhanshu Vrati did his MSc in Microbiology from G. B. Pant University of Agriculture and Technology in Pantnagar, DIIT in Biochemical Engineering from Indian Institute of Technology Delhi, and PhD in Biochemistry from the Australian National University in Canberra. He did his postdoctoral research at CSIRO Molecular Sciences in Sydney. Dr. Vrati is the Dean of THSTI responsible for its academic program and the Head of its Vaccine and Infectious Disease Research Centre. He is a virologist with research interests spanning the RNA virus replication, antivirals, and vaccine development. Dr. Vrati has won several distinctions including the National Bioscience Award, the NASI-Reliance Industries Platinum Jubilee Award, the Tata Innovation Fellowship Award, and the fellowship of the Indian Academy of Sciences and the National Science Academy of India.

Professor Shinjini Bhatnagar is the Dean of Clinical Research at THSTI. She served as Senior Research Scientist and Pediatric Gastroenterologist at the Department of Pediatrics, AIIMS. She is the Head of Pediatric Biology Centre and also the co-ordinator of Centre for Biodesign and Diagnostics and the National Biodesign Alliance. In her role as the Clinical research Dean, she has brought in an innovative approach of amalgamating clinical research into biological sciences to enhance translational capacity of THSTI.

Dr. Kanury Rao is the Head of Drug Discovery research Centre at THSTI. He is a faculty in the International Centre for Genetic Engineering and Biotechnology (ICGEB). A Fellow of several academies, Dr. Rao was awarded the Shanti Swarup Bhatnagar Award for Biological Sciences, in 1997. As an Adjunct Faculty in THSTI and the head of a focused product and pipeline development team of scientists, he leads the major drug discovery research program at THSTI.

Dr. Sudhakar Bangera joined CDSA with experience of 21 years in diverse areas of healthcare (Hospitals and Medical Schools), global Contract Research Organisations (CRO), Academic Research Organisation (ARO), Site Management Organisation (SMO), Medical Imaging, Clinical Bioavailability and Bioequivalence (BA-BE), and a global pharmaceutical company. He is a multifaceted, dynamic healthcare and clinical research professional leading the CDSA team at THSTI.

From the Executive Director's desk

Dr. G. B. Nair

The Translational Health Science and Technology Institute (THSTI) is a young establishment located in spanking new premises at Faridabad. The combination of being "young" and "new" makes it unique. Just as the first years of life are crucial for the robust development of a child, muchin the same way, THSTI is at a stage where it requires careful nurturing, relentless coaxing and ultimate care to develop into a healthy, mature and successful establishment. The Institute is bestowed with resounding infrastructure and facilities par excellence. This is because of the vision of Professor Vijay Raghavan and before that Dr. Maharaj Bhan, the present and past Secretary of DBT. Both of them have wisdom of incredible depth and our role today is to execute their foresight into reality.

During the year under report, much has happened. For one thing the Institute with its six intramural centres, one partnership centre and the extramural centre has settled in the new premises. For the first time, all the Centres are functioning under one roof with upcoming flanking facilities. Research continues in full swing after some initial hiccups that are so familiar in any new place. I will not dwell on the research programs of the Centres; the subsequent pages of the Annual Report will give you a detailed account of what is being done and the accomplishments of each centre. You can judge their performance.

But, let us have a quick look at the indicators of achievements at THSTI. An indicator of productivity in research is publications and their impact factors. TSHTI has published reasonably well with 121 publications in the 6 years of its existence, 93% of them published in overseas journals with average impact factor of above 5. Remember, four of the six years were operations from warehouses that were converted into make shift labs in Gurgaon. Sixteen patent applications were filed worldwide during the same time period. THSTI collaborated with 68 Indian Organizations and 80 International Organizations. I am not spewing these numbers to exultin THSTI's accomplishments but these are indicators that yield useful information about the development of a research establishment. The broad conclusion from these indicators, in my opinion, is that THSTI is well grounded to take the next critical step and that is to shape itself into an Institute steeped in translational research, which is its prime mandate.

I still remember vividly, my first meeting with the Faculty in November 2011, few days after joining THSTI. In my talk, I extolled the need for translational research and the importance of transferring knowledge into policy, practice and product. My audience -the faculty - did not hide their skepticism and I

instantly learned that this was not the way to go. Much later into my assignment at THSTI, I realized that the concept of translation is not translated by lectures but by action. And that is what the senior management at THSTI embarked on by arranging hospital visits, inducing colleagues to collaborate and generally began programs that were cross cutting among disciplines. Our philosophy was to show by action. The chemistry began working and almost inadvertently, so to say, there were the commencements of crosstalk. The different centres located in the same premises helped. The basic scientists were enthused by clinicians and the hospital visits left indelible impressions reflected by pale-faced colleagues trooping out of the neonatal intensive care units after seeing pre-term babies struggling to live. We knew for sure that nothing more than this could impact our researchers on the importance of their research in mitigating such problems. Proposals on sepsis and malnutrition developed rather spontaneously as a sequel to that visit.

Much has happened in the six years of existence of THSTI. However, structured well-planned institutionalized research program that addresses priority health needs has not happened. In the definition of World Health Organization, Research translation is the process by which the evidence produced by research is translated into policy, practice and product development[1]. The Institute would need to develop its own agenda for research based on health priorities of the country. The ongoing preterm birth research program is one step in that direction. More such targeted research programs are required. Development of such programs would require teams across centres working together. In organizations of creative accomplishment, I am told that the inherent competitive nature precludes collective work. I am also told that nothing is impossible. THSTI should strive to make this happen.

While all this is gratifying, an often-unrecognized facet is that with all the machines, equipment, contraptions, facilities, technologies and reagents at our disposal, the most important ingredient of success especially in team work is how we care for the human resources working across the Institute. After all these are the people that will really make the Institute and allow us to execute a vision. We need to carefully nurture our human resources may it be scientists, administrators, technicians or just a THSTI and above that we need to nurture our students who would be future leaders of many more Institutes like THSTI. The take-home message should be compassion.

The baton has been passed to Dr. Gagandeep Kang, a friend, colleague and collaborator. I am sure she will take the Institute to new heights. Finally, I would like to thank Professor Vijay Raghavan, Secretary, DBT for his unqualified support and to Professor M. K. Bhan, the Chair of the Scientific Advisory Group of Experts (SAGE), for his continued interest and advice. My gratitude also goes to the Senior Management of THSTI for their guidance and backing. Special words of thanks to our nodal officer from DBT Dr. Alka Sharma; she has this magnificent way of bringing order in chaos. To the Scientists, Staff and Students of THSTI, all I want to say is that it was a privilege working with you.

1 World Health Organization. 2012. The WHO strategy on research for health

Overview of the Annual Report

Dr. Gagandeep Kang

In the past seven years, the THSTI has grown from an idea to an institution that is ready to take its place with leading science institutions in the country, but differentiates itself by being aimed beyond discovery research. Problems in human health cannot be solved by discovery alone. With six centres and an extramural unit, the THSTI's mandate is to maximize the value of science generated by others and by us, through innovation and translation.

The Vaccine and Infectious Disease Research Centre has had a very productive year, with high quality publications and the development of innovative methods to study pathogen biology. Highlights of the year include identification of the correlates of severe dengue disease, showing that primary infections can be as severe as secondary infections in children. Other studies examined the attachment, entry and dissemination of Japanese encephalitis virus in model systems. Other virologists examined replication and pathogenesis of hepatitis viruses, complemented by screening and re-purposing approaches for future therapeutic strategies. The faculty working on Mycobacterium tuberculosis also had a number of notable achievements including development of CRISPRi based methods to identify potential targets for therapy and the application of systems approaches to the study of mycobacterial survival and pathogenesis.

The HIV Vaccine Translational Research group, supported jointly by the International AIDS Vaccine Initiative and the Department of Biotechnology focused on the characterization of the antigenic properties of the HIV-1 envelope proteins and immunogen design, as well as the screening and identification of broadly neutralizing antibodies in clade C infected donors. The collaborations of this group with researchers in northern and southern India and overseas have been critical to advancing its goal of translational research aimed at understanding immune response in chronically infected individuals and non-progressors.

The Paediatric Biology Centre has got one of the most challenging clinical studies ever planned in India off the ground—demanding logistic requirements, rigorous clinical follow-up and recording and the application of cutting edge analytic methods for exploration of biology have been implemented and will yield insights that were not previously possible. The Inter-institutional program for maternal, neonatal and infant sciences is based at the Gurgaon Civil Hospital and involves state of the art imaging and biological analysis, with a real-time data capture and monitoring system. The establishment of this novel cohort of dyads had resource constraints, which are being addressed through complementary projects which support a biorepository and additional transcriptomic and microbiome studies. The Paediatric Biology Centre continues its exploration of the developmental and functional properties of the neonatal immune system and of the role of zinc in sepsis through clinical collaborations across Delhi and the rest of India.

The Centre for Biodesign and Diagnostics will be reviewed shortly, but has attracted funding from the most competitive sources, speaking to the value of the high quality training through

the Indo-Finnish Collaboration with TEKES. Faculty in CBD work on diagnostics for fevers and tuberculosis using a range of approaches. Ongoing work also seeks to develop stable drug delivery systems that can address the issue of compliance when treatment regimens are prolonged. Despite the limited number of faculty and scientists, CBD is undertaking immersion training through the SPARSH program and building its networks with other biodesign initiatives.

The Drug Discovery Research Centre which has an inter-disciplinary team that works together on disease interrogation, target identification and early stage development has demonstrated its ability to take on big challenges by its focus on metabolic syndromes, particularly diabetes. The DDRC moved to THSTI recently and is still in the process of getting all its equipment and personnel in place. With faculty and scientists focused on discovery research, computational proteomics and mass spectrometry, lead discovery and development, mathematical biology and early translation, the DDRC has the potential to inform and support a number of the active and planned clinical programmes at THSTI.

The Centre for Human Microbial Ecology, which is the most recently established THSTI centre, emerged from studies on malnutrition and the gut microbiota, but has expanded to multiple areas, including multi-drug resistance, the role of the microbiome in the pathogenesis of type 2 diabetes, the effect on the immune response and the influence of diet and artificial sweeteners on gut microbial composition. Studies on the pathogenesis of intestinal inflammation examine the role of effector and regulatory T cells in intestinal inflammation and of retinoic acid metabolites in regulation of the inflammatory response. Additional work examines the pathogenesis of Vibrio cholerae, and preparation to support the Paediatric Biology Centre's pre-term birth cohort through studies in the vaginal microbiota association with specific clinical outcomes.

The Policy Centre for Biomedical Research has taken on landscaping analysis in several areas of relevance to the Indian government and to the South East Asian Regional Office of the World Health Organisation, particularly typhoid and cholera. With a small budget and staff, all supported by extramural funding, the Centre has developed documents that have formed the evidence base for policymakers and continues to act as a valuable resource for the government and agencies engaged in public health research and programmes.

The Clinical Development Service Agency has developed its training and clinical research support services into programmes that are well recognized and respected by the academic and research communities and regulatory authorities. Despite limited core staffing, the CDSA has evolved strategies that allow it to deliver effectively on its mission through project-based support that extends through the range of necessary clinical trial activities and training programmes that are tailored to meet the needs of specific audiences. An organization with tremendous potential, CDSA now has the challenge of generating its resources from its programmes, so that it meets the criteria for success that were laid out at its foundation. Given its progress so far, this is feasible and in fact, with the right leadership CDSA may do more than was originally mandated.

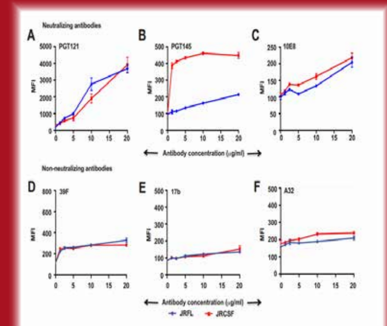
In India, we have human health challenges that are unique to developing countries. It is our responsibility to develop a translation and innovation strategy that can maximize the value that can be generated from the science at THSTI and elsewhere to improve the lives of people in India. Opportunities for new and improved therapies, for diagnosis and prevention strategies informed by our understanding of human and population biology offer routes to innovation that can and must be seized by THSTI.

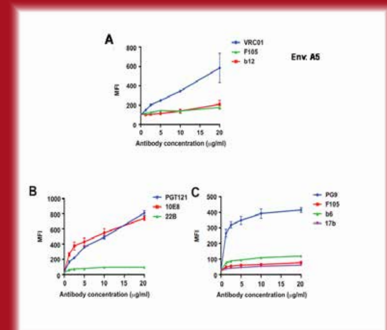
We expect to see significant innovation emerge over time, and for the necessary technology development and clinical translation, we need partnerships with the government, academia and industry. An environment that enables innovation and drives the translation of research into clinical settings and commercial enterprises is the mandate of THSTI and we will work to facilitate its creation.

Vaccine and Infectious Disease Research Centre

HIV Vaccine Translational Research

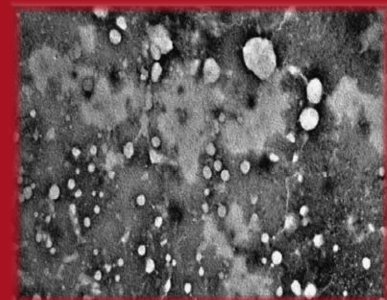
- Screening and identification of broadly neutralizing antibodies in Indian donors chronically infected with HIV- 1 clade C 19
- Purification and characterization of antigenic properties of HIV-1 trimeric envelope proteins obtained from broadly neutralizing plasma of Indian donors 20
- Identification of neutralizing antibody epitopes on Indian and South African HIV-1 subtype C viruses for HIV vaccine design 21
- HIV vaccine immunogen development 22
- Stabilization of 4-2.J41 ENV in a native-like trimeric structure for immunogen design 22
- Role of C-terminal domain in restoration of wild type conformation for 4.2.J41 ENV with deleted cytoplasmic tail at protein level 24
- Identification and characterization of HIV envelop surface exposed areas to be used as immunogen for successful vaccine development 24
- Stabilization and characterization of soluble immunogen of Clade C ENV 4-2.J41 in a near native trimeric conformation 25
- A comparative immunogenicity study in rabbits of cleavage competent Indian clade-C ENV (4-2.J41) or with clade-B JR-FL ENV or in combination of these two by using DNA priming followed by soluble trimeric protein boost immunization format 25
- To stabilize the cleaved native-like conformation of cytoplasmic deleted Indian clade-C 4-2.J41 ENV at cell surface level 26





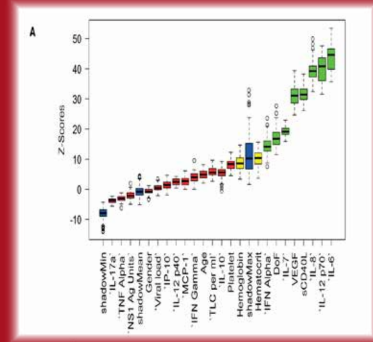
Development of vaccine/gene delivery vectors

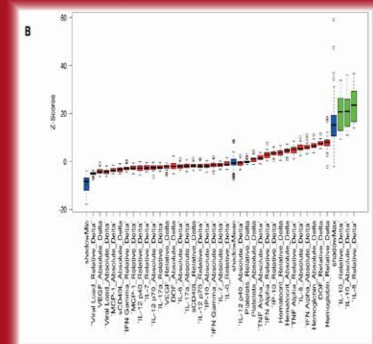
- Characterization of animal adenoviruses isolated from field animals for their suitability as vaccine/gene delivery vectors 27
- Development of therapeutically viable delivery systems for DNAzyme-based antiviral therapeutics against flaviviruses 28
- Mycobacterial membrane-derived vesicles: Role in pathogenesis and exploration as a novel subunit vaccine vehicle against tuberculosis 28



Biology of medically important viruses and the viral infections

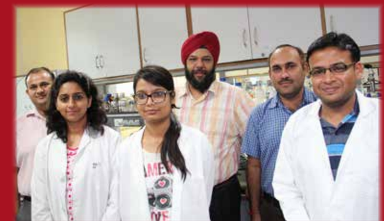
- Identification of correlates of severe dengue disease 30
- Role of tyrosine kinases in dengue virus and Japanese encephalitis virus life-cycle 31
- Investigating the effect of viral infections on zinc homeostasis as a cause of permeability barrier disruption in polarized epithelial and endothelial cells 33
- Functional significance of neuron/microglia-derived exosomes in Japanese encephalitis virus-induced in flammation and virus dissemination 34
- Use of designer microRNAs against 3’UTR of JEV to inhibit viral replication 35
- Transcriptome analysis and identification of novel biomarkers of disease progression in dengue patients focusing on noncoding RNAs 35
- Antiviral signaling during Japanese encephalitis virus infection of neuronal cells 37
- Identification of the Japanese Encephalitis Virus Attachment and Receptor System 38
- Cellular Entry Mechanisms of Japanese Encephalitis Virus 39
- Interactions between Japanese encephalitis virus and host cellular pathways: implications for pathogenesis 40
- Mechanism of thrombocytopenia during Dengue virus infection 41
- Investigation of the mechanisms dictating poor replication efficiency of genotype-1 Hepatitis E virus 42
- Understanding the functions of uncharacterized proteins of Hepatitis E virus 43
- Expression and purification of recombinant Hepatitis E virus-like particles in Pichia pastoris 44
- Interference of innate immunity response by hepatitis E virus 44
- Characterization of hepatitis E virus RNA-dependent RNA polymerase and its associated proteins in the replicase complex 45
- Screening of small molecule compounds to identify inhibitor(s) of hepatitis C virus (genotype 3a) RNA-dependent RNA polymerase 45





Biology of Mycobacterium tuberculosis

- Understanding the role of polyphosphate kinases and polyphosphatases in physiology of Mycobacterium tuberculosis 48
- Integrative approaches to understand function, stability and structure of bacterial toxin-antitoxin systems 49
- Identification of novel scaffolds and drug targets to combat tuberculosis 50
- CRISPRi-based genetic screen to identify therapeutic targets of TB-causing human pathogen Mycobacterium tuberculosis 52
- To characterize the role of putative preprotein translocase in Mycobacterium tuberculosis 52
- A systems approach to analyze changes in global phosphorylation status of proteins in macrophages infected with Mycobacterium tuberculosis complex bacteria and their repercussions on mycobacterial virulence 54
- Deciphering Mycobacterium tuberculosis artillery 54
- Functional characterization of mycobacterial aminopeptidase 55
- Carbon metabolism in Mycobacterium tuberculosis and its implications on mycobacterial pathogenesis 57
- Integrative genomics of host-pathogen interaction to identify new drug targets against persistent Mycobacterium tuberculosis 58
- Cholesterol utilization pathway genes as therapeutics target 58
- Genetic essentiality study of Mycobacterium tuberculosis under various growth and stress conditions 59



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An Overview

Dr. Sudhanshu Vrati

Globally, infectious diseases remain the leading cause of death, but these are particularly pernicious in developing economies like ours. In the context of India, the ever-increasing population density, migration of population to urban areas and associated changes in environment and ecology provide fertile ground for emergence of newer infectious agents. Vaccines, anti-virals, novel antibiotics, and antibody-based strategies for passive immunization remain the most cost-effective means to combat the infectious organisms. Knowledge of pathogen biology, and population based protective immune response are important components of strategies for combating infectious diseases. Vaccine and Infectious Disease Research Centre (VIDRC) has the mission to study infectious diseases and pathogens relevant to India to produce knowledge that could be translated to novel prophylactics and therapeutics.

A large number of viral infections are reported from different parts of India either sporadically as the virus has become endemic, or in the form of an epidemic. HIV/ AIDS continues to be a major challenge for India and an effective vaccine is urgently needed. In this context, we have a joint research program between THSTI and the International AIDS Vaccine Initiative (IAVI) aimed at developing HIV vaccine candidates. The other viral diseases that are currently studied at VIDRC are those associated with the widespread unhygienic conditions in many parts of the country. These are rota- and hepatitis A viruses transmitted feco-orally though contaminated drinking water. VIDRC is partnering the Society for Applied Studies (SAS) through the Population Science Partnership Centre (PSPC) of THSTI for the clinical development of rotavirus vaccine candidates. The other viruses that are studied at VIDRC include Dengue and Japanese Encephalitis viruses, which are widespread in India and are transmitted through mosquitoes. As for the bacterial infections, tuberculosis has been a major medical challenge for India and VIDRC is studying Mycobacterium tuberculosis, the pathogen responsible for the disease, tuberculosis (TB) with a view to identify novel genes/proteins/ pathways that could be potential drug targets or vaccine candidates.

Provided below are some of the salient scientific achievements of VIDRC made during the reporting period. A more detailed account of the progress in various ongoing programs and projects at VIDRC is provided on the subsequent pages.

Vaccine research and development at VIDRC

The human immunodeficiency virus (HIV) envelope protein (Env) is the main target of the broadly neutralizing antibodies (bNAbs) and our strategy is to design multiple near-native-like Env trimmers for the elicitation of those types of antibodies. It has been demonstrated that in comparison to protein

prime/protein boost, DNA prime followed by protein boost elicits durable and qualitatively better immune response in animals. The desirable property of HIV-1 Env expressed from plasmid is its efficient cleavage as this type of HIV Env on the cell surface selectively binds to neutralizing antibodies. Therefore, for developing a suitable native trimeric soluble candidate vaccine for India and across the world, identification of an efficiently cleaved Env is desirable. Previously, we reported the isolation of a naturally occurring, efficiently cleaved clade C Env 4-2.J41 isolated from an Indian patient that displayed almost all of the known bNAb epitopes. We have tested its immunogenicity in a plasmid DNA prime - nonnative trimeric protein boost regimen in rabbits. The sera from clade C 4.2.J41 Env group showed a strong autologous neutralizing antibody response in rabbits. Neutralizing antibody response to the virus pseudotyped with Env isolated from Indian primary isolates suggested it to be a promising candidate for designing soluble form of immunogen.

Designing of a soluble native trimeric Env protein that mimics the Env on the viral surface based on an efficiently cleaved HIV-1 Env is one of our strategies for HIV immunogen design. However, the HIV Env protein is inherently metastable in nature and has a tendency to disintegrate into gp120 and gp41 subunits. We have stabilized the Indian clade C Env 4-2.J41 in a native-like, trimeric form using a unique domain swap strategy. The purified protein could bind to a panel of conformation-dependent bNAbs. Additionally, this Env was poorly recognized by non-neutralizing antibodies. The presence of trimeric Env was confirmed by electron microscopy. The immunogenicity of the trimeric protein will now be studied in animals.

Furthermore, a successful preventive vaccine to HIV-1 should induce broadly neutralizing antibodies (bNAs) capable of preventing infection with HIV-1 of substantial genetic diversity. Our scientists had previously isolated Envs from a patient infected with HIV-1 clade B/C recombinant virus. It is to be noted that the broadly neutralizing activity in that patient's serum was detected. Now, near-native-like trimeric envs were constructed using an HIV-1 clade B/C recombinant and the soluble form of the native trimeric Env has been purified. The antigenicity studies demonstrated that the trimeric Env from the B/C recombinant displayed minimal change in its conformation in the CD4-bound state, a property that qualifies this protein as an excellent antigen. The immunogenicity of this Env will now be examined in rabbits.

Research on medically important viruses and viral infections

VIDRC scientists have been studying the correlates of disease severity in pediatric dengue patients and attempting to define the early biomarkers to predict the dengue disease progression. These studies are being conducted on dengue patients in collaboration with clinicians from the All India Institute of Medical Sciences, New Delhi; School of Tropical Medicine, Kolkata; and the GTB Hospital, Delhi. We found that dengue viremia was indistinguishable between patients with dengue infection, dengue with warning signs, or severe dengue either in primary or in secondary infection, but all the secondary infection cases had a significantly higher viremia as compared to primary infections. These data suggest that Dengue virus infects by both antibody-dependent and -independent mechanisms, however, antibody-dependent enhancement may contribute to prolonged viremia observed in secondary infections. As reduced interferon levels seem to correlate with severe disease in both primary and secondary infections, therapies designed to transiently restore interferon levels to a threshold level required for recovery may prove to be useful in addition to antivirals and vaccine development.

Targeting host factors required for the virus life cycle presents an alternate approach to antiviral development. Tyrosine kinases regulate a diverse range of cellular processes and many viruses have been shown to exploit them at various stages of viral life-cycle. VIDRC scientists have identified the role of c-terminal Src kinase (CSK) and Protein Kinase A (PKA) in mosquito-borne flavivirus replication providing a new perspective into the role of SFK signaling in flavivirus infection. Further efforts to characterize the exact role of CSK in Dengue virus replication may provide novel targets to develop dengue antivirals.

Understanding the receptor system and the cellular entry mechanism will greatly help in designing novel antivirals. Our studies have identified GRP78 as a receptor for Japanese encephalitis virus on mammalian cells. Additionally, this protein was found to be important for the replication complex formation and had a role in virus replication post-entry. The crucial role of GRP78 in important steps of virus infection, that is virus uptake and replication, make this an attractive target for designing novel antivirals.

Hepatitis E virus (HEV) is one of the most common causes of acute and sporadic viral hepatitis. It is a positive strand RNA virus whose replication is poorly understood due to the lack of an efficient cell culture system. Despite sharing similarity in genome organization and encoding similar proteins, genotype-3 HEV replicates more efficiently in cell culture than the genotype-1 virus. Our studies have revealed that the endoplasmic reticulum stress inducers promote genotype-1 HEV replication by initiating a cap-independent, internal translation-mediated synthesis of a novel viral protein, which we named ORF4. The ORF4 protein is expressed only in genotype-1 virus and it acts by interacting with multiple viral and host proteins such as eEF1α1 to enhance the activity of viral RNA-dependent RNA polymerase. Moreover, a proteasome-resistant ORF4 mutant significantly enhanced viral replication. These studies have thus helped identify the optimal conditions for replication of genotype-1 HEV and would be instrumental in developing an efficient system for studying the virus replication

Tuberculosis research at VIDRC

A number of studies are underway at VIDRC to characterize the essential genes and pathways of Mycobacterium tuberculosis (MTB) and evaluate them as potential drug targets or for developing attenuated mutants as potential vaccine candidates. PolyP is a linear polymer of inorganic phosphate that is upregulated in bacterial pathogens upon exposure to various stress conditions. MTB genome harbors enzymes involved in both PolyP synthesis (PPK-1) and its utilization (PPK-2). We we have constructed both PPK-1 and PPK-2 mutants of MTB. Using these bacterial strains, we show that PolyP deficiency is associated with increased susceptibility of MTB to front-line TB drugs. We have also demonstrated that any dysregulation of PolyP levels impairs the ability of MTB to cause disease in guinea pigs. The animals infected with these mutant strains have significantly less bacterial loads and gross pathology in comparison to parental strain infected guinea pigs, and thus these mutants present potential vaccine candidates.

Analysis of MTB genome sequence together with a series of transposon mutagenesis studies have led to identification of various metabolic pathways, which are essential for MTB growth in vitro and therefore are potential targets for anti-tubercular therapy. Numerous studies have validated enzymes involved in cell wall biosynthesis, DNA replication, central carbon metabolism,

amino acid biosynthesis, stringent response pathways, two component systems, sigma factors and co-factor biosynthesis as drug targets. Since mammals are unable to synthesize most of these amino acids we are trying to validate various amino acid biosynthetic enzymes as drug targets. Using CRISPRi approach VIDRC scientists have established essentiality for some of these enzymes for MTB growth in vitro. Experiments are in progress to screen small molecule libraries against these targets. The identified sacffolds would then be evaluated for their ability to inhibit MTB growth in macrophages and mice.

Very little is known about the nutritional requirements of MTB while replicating inside the host cell. Various studies have demonstrated that cholesterol is required for the persistence of MTB. We hypothesize that this carbon switch is very critical for MTB to slow down its replication and metabolic rate thereby activating a more latent form of infection. VIDRC scientists are attempting to generate an interactome map of the regulatory pathways of cholesterol utilization in MTB. The goal here is to identify critical cholesterol catabolic pathway genes as novel target for developing a live-attenuated vaccine candidate against tuberculosis. Additionally, studies are in progress to understand the MTB virulence mechanisms. To this end VIDRC scientists are developing tools to identify MTB protein effectors that access macrophages. Besides the effector molecules, mycobacteria are known to shed in their surroundings membrane vesicles packed with proteins that may be pathogenic or have important physiological functions. Studies are underway to identify these proteins from pathogenic and non-pathogenic mycobacteria.

HIV Vaccine Translational Research Program

The HIV envelope (ENV) protein is responsible for cell entry and it is also the target of neutralizing antibodies. In its active form, it is composed of GP120 and transmembrane GP41 polypeptides, derived from the cleavage of GP160 precursor protein. The cleaved envelope proteins, which form a trimer by noncovalent association on the viral membrane, bind to the primary receptor CD4, followed by co-receptor, to mediate entry into host cells. A key strategy of HIV vaccine design is to identify immunogens that elicit antibodies that recognize the native ENV and thus block viral entry into target cells. The recent isolation of several broadly neutralizing monoclonal antibodies (MAbs) demonstrates that the human B cell repertoire can generate broadly neutralizing antibodies targeting ENV. However, the target of these inhibitory antibodies, the HIV ENV, displays a high degree of genetic and structural variability, requiring the elicitation of broadly reactive antibody responses to functionally conserved elements. Recent progress in isolating potent, broadly neutralizing MAbs from HIV-infected individuals and characterization of their cognate epitopes has increased the number of potential Env antibody targets. Several of these new targets recognize trimeric ENV, suggesting that in some cases it is the functional trimer that drives the elicitation of broad neutralization during natural infection.

The mission of this program is to identify candidate immunogen that elicits broadly neutralizing antibody responses against HIV-1 by establishing an innovative discovery program employing high throughput technology. This joint program between THSTI and the International AIDS Vaccine Initiative (IAVI) also envisages developing a unique set up for accelerating the effort for vaccine development for IAVI and other R&D partners, and integration of this unique facility in other labs of IAVI and appropriate R&D partners globally.

The program is currently organized to pursue the following projects through the various projects detailed below.

- Screening for cleaved functional ENVs
- Development of rapid and high-throughput screening of ENV immunogens
- Isolation and characterization of Broadly Neutralizing Antibodies from Indian Patients

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Screening and identification of broadly neutralizing antibodies in Indian donors chronically infected with HIV-1 clade C

HIV-1 ENV glycoproteins are targets of neutralizing antibodies and represent a key component for immunogen design. The mapping of epitopes on viral ENV vulnerable to immune evasion will aid in defining targets of vaccine immunogens. We identified novel conformational epitopes on the viral ENV targeted by broadly cross-neutralizing antibodies elicited in natural infection in an elite neutralizer infected with HIV-1 clade C. Our data extend our knowledge on neutralizing epitopes associated with virus escape and potentially contribute to immunogen design and antibody-based prophylactic therapy.

				G37080 Plasma	
	Envelope	Accession No.	Clade	Visit-1	Visit-2
	MuLV	S53043.1	NA	<20	<20
	HIV-2 (7312A)	JX235925.1	A	<20	<20
India Clade C	16055-2.3	EF117268	C	23.69	25.95
	16936_2.21	EF117270	C	293.39	358.14
	25710-2.3	EF117271	C	887.84	1355.96
	25711-2.4	EF117272	C	454.97	851.38
	00836-2.5	EF117265	C	845.18	860.15
	2-5.J3	GU945311.1	C	604.09	106.68
	4.J22	EU908219.1	C	1557.8	848.91
	4-2.J41	GU945316.2	C	2939.22	1308.11
	3-5.J25	GU945314.1	C	3262.97	785.12
	5-4.J16	GU945326.1	C	2201.58	969.68
	5.J41	EU908221.1	C	3401.76	4851.8
	7.J16	EU908222.1	C	418.44	288.59
	7.J20	EU908223.1	C	838.77	765.86
	11-3.J3	GU945330.1	C	3449.22	2279.27
	11-5.J12	GU945333.1	C	1413.06	3081.77
	LT-1.J1	JN400529	C	444.8	580.9
	LT1.J3	JN400534	C	350.71	201.48
	LT5.J3b	JN400538	C	378.23	125.45
	LT5.J7b	JN400540	C	323.37	157.48
	93IN905	AY669742.1	C	2389.65	8533
	Median ID50			841.975	817.015
Africa Clade C	CAP45.G3	DQ435682.1	C	42.95	66.58
	CAP84	EF203963.1	C	533.03	604.23
	CAP88	EF203972.1	C	149.1	80.32
	CAP239.G3	EF203983.1	C	642.06	520.14
	Du422.1	DQ411854.1	C	274.86	304.31
	Du151.2	DQ411851.1	C	1485.89	355.89
	DU156.12	DQ411852.1	C	1400.78	1573
	DU172.17	DQ411853.1	C	672.8	195.63
	ZM109F.PB4	AY424138.2	C	235.75	291.1
	ZM197M.PB7	DQ388515.1	C	317.87	296.25
	IAVIC22	--	C	1836.31	1592.26
	Median ID50			533.03	304.31
Clade B	JRFL	U63632.1	B	229.36	122.15
	PVO.4	AY835444.1	B	118.64	274.91
	TRJO4551.58	AY835450	B	192.61	122.76
	AC10.0.29	AY835446	B	721.69	963.76
	QH0692.42	AY835439	B	199	187.2
	REJO4549.67	AY835449	B	205.79	51.7
	SC422661.8	AY835441.1	B	161.41	21.05
	6535.3	AY835438	B	2238.72	1541.11
	RHPA 4259.7	AY835447.1	B	584.69	491.46
	HO61.14	EF210730	B	805.49	414.5
	92BR020	AY669718.1	B	847.48	3508.16
	JRCSF	M38429.1	B	387.56	571.68
	Median ID50			486.125	414.5
Clade A	Q769.ENV.b9	AF407157.1	A	191.25	42.05
	Q461.e2	AF407156	A	29.68	<20
	Q842.d12	AF407160.1	A	851.19	1542.05
	Q23.17	AF004885	A	1853.63	2328
	Q259.d2.26	AF407152	A	3060.32	693.17
	BG505	DQ208458.1	A	574.02	206.79
	94UG103	AY669705.1	A	102.12	247.46
	Median ID50			574.02	470.31
Others	92TH021	AY669775.1	A/E	88.43	206.11
	LT5.J12	FJ515876	B/C	341.4	130.44
	CH038.12	EF042692	B/C	945.18	562.2
	CH114.8	EF117264	B/C	343.17	314.47
	CH120.6	EF117260	B/C	182.69	75.71
	CRF 02AG_235	EU513195	A/G	445.06	319.86
	191727_D1_12	HM215267.1	D	761.79	298.55
	Median ID50			343.17	298.55

Table-1: Neutralization breadth of Protocol G G37080 plasma samples collected at two different time points tested against panel of 57 Envpseudotyped viruses.

Broadly neutralizing antibodies isolated from infected patients who are elite neutralizers have identified targets on HIV-1 ENV glycoprotein that are vulnerable to antibody neutralization; however, it is not known whether infection established by the majority of the circulating clade C strains in Indian patients elicit neutralizing antibody responses against any of the known targets. We examined the specificity of one broad and potent cross-neutralizing plasma obtained from an Indian elite neutralizer infected with HIV-1 clade C. This plasma neutralized 53/57 (93%) HIV pseudoviruses prepared with ENV from distinct HIV clades of different geographical origins (Table 1). Mapping studies using GP120 core protein, single-residue knockout mutants, and chimeric viruses revealed that G37080 broadly cross-neutralizing (BCN) plasma lacks specificities to the CD4 binding site, GP41 membrane-proximal external region, N160 and N332 glycans, and R166 and K169 in the V1-V3 region and are known

Env-pseudotyped Virus Panel	Fold reduction in neutralization (ID_{50}) by depleted G37080 plasma: gp140 Trimer (BG505-SOSIP.664)	gp120 Monomer (4-2.J41)	MPER (C1C peptide)
25710-2.3	> 10.30	1.3	0.83
25711-2.4	>8.52	1.4	1.44
3-5.J25	>7.85	0.9	0.84
4-2.J41	12.11	1.1	1.04
IAVI_C22	>15.92	1.2	1.18
92BR020	>35.08	1.1	1.34
93IN905	3.41	1.2	0.94
JRCSF	>8.75	0.5	0.93
Q23.17	>23.28	1.0	0.98
Du156.12	>15.73	0.8	1.61
HVTR-PG80v1.eJ7	>10.03	0.9	1.12
HVTR-PG80v1.eJ19	>15.60	0.5	1.18
HIV-2 7312A-C1C	-	-	>10

Table-2: Degree of shift in sensitivity of ENV-pseudotyped viruses to G37080 BCN plasma depleted with monomeric and trimeric ENVs as well as a clade C MPER peptide (C1C).

predominant targets for BCN antibodies. Depletion of G37080 plasma with soluble trimeric HIV-1 clade A BG505-SOSIP.664 Env protein (but with neither monomeric gp120 nor clade C membrane-proximal external region peptides) resulted in significant reduction of virus neutralization (Table 2), suggesting that G37080 BCN antibodies mainly target epitopes on cleaved trimeric Env. Further examination of autologous circulating Envs revealed the association of mutation of residues in the V1 loop that contributed to neutralization resistance. In summary, we report the identification of plasma antibodies from a clade C-infected elite neutralizer that mediate neutralization breadth via epitopes on trimeric GP120 not yet reported and confer autologous neutralization escape via mutation of residues in the V1 loop. Taking cue from this study, we have initiated isolation of broadly neutralizing monoclonal antibodies by antigen specific memory B cell sorting by flow cytometry. Avi-tagged BG505-SOSIP.664 (clade A) and HVTR-PG80.eJ19-SOSIP.664 trimeric proteins are being utilized to sort single memory B cells from the PBMC of the elite Indian neutralizers. Heavy and light (both lambda and kappa) variable IgG chain sequences from single B cells have been amplified by PCR and cloned into mammalian expression vectors and further characterization are under progress.

Purification and characterization of antigenic properties of HIV-1 trimeric envelope proteins obtained from broadly neutralizing plasma of Indian donors

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A successful preventive vaccine to HIV-1 would induce broadly neutralizing antibodies (bNAbs) capable of preventing acquisition of HIV-1 with substantial genetic diversity. Our primary objective has been to identify primary viral Env sequences from BCN plasma that present the maximum number of epitopes recognized by neutralization antibodies elicited in natural infection particularly in Indian epidemic. Our goal is to utilize such Envs to prepare trimeric Env protein antigens and assess their immunogenicity in suitable animal models and also to use as baits for sorting antigen-specific memory B cells towards isolation of bnAbs.

The viral Env is the main target of the bnAbs and one of the very important strategies being taken by several investigators is to design multiple near native like Env trimers based on sequences representing distinct clades associated with diverse geographical epidemic. Recently, near native Env trimers have been reported based on HIV-1 clade A, B and C sequences. It has been postulated based on recent studies that inclusion of near native HIV-1 Env trimers would constitute a pool of immunogens which would likely elicit neutralizing antibodies with considerable breadth. In the present study, we selected HIV-1 primary Envs (a clade C and a B/C recombinant) obtained from BCN plasma from Indian donors to prepare soluble HIV-1 Env proteins. We constructed near native like trimeric Envs: a B/C recombinant (LT5.J4b12C-SOSIP.664 prepared between the sensitive and resistant autologous Envs) and a clade C (HVTR-PG80.eJ19-SOSIP.664) and examined their antigenic properties by D7324-capture ELISA. We found that in contrast to the other Env trimers that have so far been examined and documented in literature, the

LT5.J4b12C-SOSIP.664 displayed minimal change in its conformation in the CD4-bound state, a property that qualifies this protein as an excellent antigen to be examined for its immunogenic property. We next plan to examine the immunogenic properties of these protein antigens in rabbit model.

Identification of neutralizing antibody epitopes on Indian and South African HIV-1 subtype C viruses for HIV vaccine design

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Clade C is the predominantly circulating strains of HIV-1 both in India and South Africa. The primary objective of the present study was to determine the extent of intra-clade C neutralization by cross neutralizing antibodies elicited in chronically infected Indian patients and to map the specificity of the broadly neutralizing serum antibodies.

Glycan supersites in the V3 region of HIV-1 envelope form vulnerable targets that are exploited by potent and bNAbs such as PGT121 and PGT128. The glycan N332 residue in the V3 base has been demonstrated to represent an important supersite of vulnerability for comprehensive antibody mediated virus neutralization and is currently aiding design and development of an effective vaccine. In the case of mAbs PGT121 and PGT128 that target the V3-glycan supersite, loss of the glycan at position 332 is often associated with resistance. Recently, Goo et al. and Sok et al. reported that some viruses despite the presence of key N301 and N332 V3 glycans were found to be resistant to the potent and bNAbs, PGT121 and PGT128. They suggested that altered conformation of Env due to unknown mechanisms resulted in neutralization resistance of viruses to these mAbs. In the present study, we examined the basis of resistance of HIV-1 clade C Envs to contemporaneous broadly cross-clade neutralizing plasma (INDO-SA 2007) obtained from a slow progressing Indian patient whose specificity mapped to the N332 at the V3 base. By examining HIV-1 Envs obtained from a patient who was known to have developed broadly neutralizing antibodies with specificity for the N332 glycan in the V3 base, we found that longer V1 loop length hinders the bnAbs such as PGT121 and PGT128 to access the N332 glycan epitope. Our observation provides information that explains the basis of resistance of HIV-1 variants that are naturally resistant to bnAbs targeting N332 glycan epitope.

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HIV vaccine immunogen development

Development of HIV-1 vaccine is dependent on generating immunogens that mimic the viral Env glycoprotein; the target of bNAbs that develop in about 15-30% of infected patients; which then can induce the humoral immune response to elicit bNAbs. The enormous diversity of HIV-1 suggests that these immunogens need to be developed from multiple subtypes. Clades A, B and C make up about 75% of globally circulating strains with the latter being predominant in India. Our goal is to identify Envs from these subtypes which are suitable for developing immunogens both for genetic vaccination as well protein vaccination.

The HIV-1 Env glycoprotein, which is the target of bNAbs, is expressed as a gp160 polypeptide that undergoes proteolytic processing to form the gp120 transmembrane (TM) and gp41 soluble (SU) subunits that undergo rearrangement to form a trimer of heterodimers that form the viral Env spikes. It has been demonstrated that efficient cleavage of the gp160 precursor is co-related with preferential binding to bNAbs and not to non-neutralizing antibodies (non-Nabs) and neutralizing capacities of viruses pseudo-typed with such Envs. Thus, efficiently cleaved Envs are the closest mimics of native Envs, suitable for immunogen design. However, such Envs are relatively rare in nature. Prior to our studies, only one reported naturally occurring, efficiently cleaved Env JRFL (clade B) had been isolated. Previously, we reported the isolation of a naturally occurring, efficiently cleaved clade C Env, 4-2.J41, isolated from an Indian patient that displays majority of the bNAb epitopes. We have now shown that the clade B Env JRCSF and the clade A Env A5 are also efficiently cleaved Envs that display desirable antigenic properties. JRCSF, which is a relative of the well characterized and efficiently cleaved clade B Env JRFL, binds to bNAbs efficiently but poorly to non-NAbs (Figure 1) and is also efficiently cleaved. In certain aspects JRCSF is antigenically superior to JRFL. We have also determined that the clade A Env A5 is also efficiently cleaved and binds selectively to bNAbs but marginally to non-NAbs (Figure 2). We are currently developing soluble versions of these Envs. We intend to test these Envs in small animals in DNA prime, protein boost format to determine their ability to elicit bNAbs.

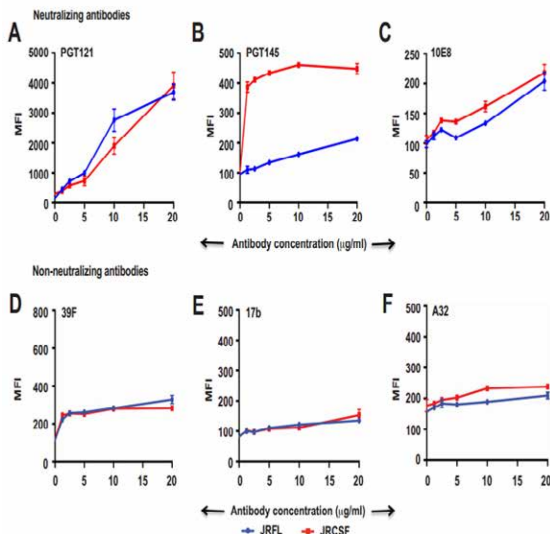

Figure-1: FACS-based cell surface antibody binding assay with clade B ENVs, JRFL and JRCSF and increasing amounts of different neutralizing and non-neutralizing antibodies as shown. MFI: Mean Fluorescence Intensity.

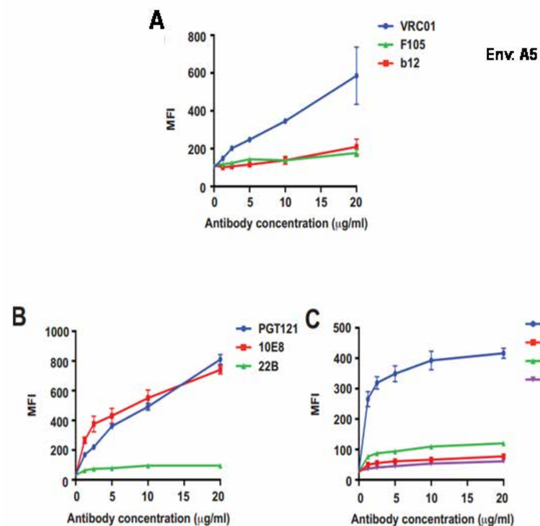

Figure-2: FACS-based cell surface antibody binding assay with the clade A ENV, A5 and increasing amounts of neutralizing (VRC01, PGT121, 10E8, PG9) and non-neutralizing antibodies (F105, 22B, b6, 17b) as shown.

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Stabilization of 4-2.J41 ENV in a native-like trimeric structure for immunogen design

To produce an envelope-based immunogen for HIV-1, a soluble, native-like trimeric form of the envelope, mimicking the immunogen on the cell surface of the virus needs to be designed. In this part of work, to make stable trimeric native trimers, we have identified and swapped a stabilizing domain from a stable envelope to an Indian clade C viral envelope, which is otherwise, less stable.

The primary target of antibodies to block HIV-1 entry to the cell is to block the envelope protein on the viral surface. Designing of a soluble form of the envelope protein that mimics the natural envelope on the viral surface is our current strategy for immunogen design. However, the HIV Env protein is inherently metastable in nature and has a tendency to disintegrate into gp120 and gp41 subunits. Enormous sequence diversity, high mutability rate to escape immune responses and metastable nature of the Env protein makes it difficult and challenging to design a suitable immnogen that can elicit broadly neutralizing antibodies in the immunized individuals. To develop a HIV vaccine candidate, various strategies has been adopted to design and engineer a soluble form trimeric, native-like Env to mimic the naturally occurring Env protein on the viral surface as an immunogen.

The BG505.SOSIP.664, a clade A virus based engineered envelope is so far the best HIV envelope that is amenable to detailed structural studies at atomic resolution. The BG505.SOSIP.664 maintains a very stable near native like trimeric conformation and binds very efficiently to bNAbs. However, the efficiently cleaved Indian clade C envelope, 4-2.J41, does not show its stability when we adopted the identical strategy for making stable trimeric soluble form of the Env. Taking cues from the structure of BG505, we modified the adopted strategy by using a unique “domain swap” design approach to stabilize the Env protein of a clade C strain, 4.2-J41, in a native-like, trimeric, cleaved form by incorporating stable regions from BG505.SOSIP.664. By adopting above approach we designed a clade C based soluble, trimeric, and native like envelope protein with desired immunogenic profile. We have further shown that the above trimeric protein purified through Galanthus lectin column followed by size-exclusion chromatography could bind to a panel of conformation-dependent bNAbs but was poorly recognized by non-neutralizing antibodies. The purified protein was also analyzed by electron microscopy (EM) to confirm the presence of trimeric population in collaboration with The Scripps Research Institute, USA. Currently, we are in the process to recover well-ordered, homogeneous trimers as heterogeneity in the preparation was confirmed through EM.

In another approach we are trying to tether the 4-2.J41.SOSIP.664 envelope into a native like closed conformation. The trimeric envelope consists of three gp120 protomer and three gp41 protomer. The protomers are loosely attached with each other and also with gp41. This gives room for dynamics between closed and open conformation and also shedding of gp120 from the gp41 base. We hypothesised to stabilize the closed conformation by stitching the interface of the protomers to stabilize the closed conformation by cystein tethering. We have identified regions based on the BG505 structure to put cystein residues on some strategic locations of each protomers of gp120 and also between gp120/gp41 interfaces. Such cysteine when form disulphide bond in correct folded conformation will help in stabilizing a native like trimeric closed conformation that may have the required immunogenic

properties for vaccine design.

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Role of C-terminal domain in restoration of wild type conformation for 4.2.J41 Env with deleted cytoplasmic tail at protein level

Research in our lab has shown that when whole cytoplasmic tail (CT) of 4-2.J41 Env is deleted, the Env binds to both neutralizing and non-neutralizing antibodies at a higher extent; unlike JRFL del-CT which binds to non-neutralizing antibodies to lesser extent. This project aims for the validation of rationale and to check for the effect of CT residues of 4-2.J41 in maintaining or restoring the conformation of envelops lacking CT regions similar to wild type Env at the protein level and to develop a soluble immunogen with extended immunogenic epitope region; MPER and Kennedy epitope. We employed different biochemical assays for our designed constructs (length of C-terminal) and identified optimal length of residues sufficient to maintain a stabilized, cleaved conformation. Some structural guided mutations were also introduced to further stabilize the conformation. Preliminary experimental results indicate that designed constructs were soluble and binds with most of the neutralizing antibodies tested so far. The proteins were purified through selective binding with lectin, Galanthus nevalis followed by size exclusion chromatography. The purified proteins were characterized with the help of electron microscopy (EM), which suggests the existence of trimeric population in purified sample. Furthermore, a positive antibody affinity selection with antibody, PGT145 has been employed to further enrich the native trimeric Env. EM characterization for positively selected protein is underway and the results will guide us to even further enrich the trimeric polpulations of the proteins. Once we achieve the goal, the proteins will be characterized following several well established biochemical and biophysical parameters.

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Identification and characterization of HIV envelop surface exposed areas to be used as immunogen for successful vaccine development

Targeting regions around CD4 binding site will give us an alternative approach to probably masking, inhibiting or blocking CD4 binding to gp120, which has been so far not successful by targeting CD4 binding site. Through different structural guided and bioinformatics analysis, we identified 3 surface exposed regions around CD4 binding sites on HIV-1 Env protein and targeted them for scaffold designing. Using several in-house analysis programs, in silico grafting experiments as well as on the basis different biochemical parameters, 3 scaffolds for the “region 1” were selected for further studies. However, in order to characterize the immunogenicity and proper surface exposure of the cloned epitope-scaffold protein before immunization studies we feel the immense need of polyclonal antibodies sera against targeted envelop-peptide and we employed immunization of envelop peptide. Immunized sera against peptide epitope gave high ELISA titer, which is the indicator of binding antibodies. However the sera from animals immunized with linear peptide failed to neutralize HIV-1 lab adapted or tire I/tire II viruses. Furthermore, the sera failed to bind to the Env expressed on cell surface suggesting that either the region is not exposed in native Env or the antibodies present in sera are directed to linear epitopes and so they cannot recognize the conformational

epitope present in Env. Our experimental finding strongly validated our concept of designing scaffold in order to over express the epitopes and its use for immunization studies. Therefore, we overexpressed and purified our epitope harboring scaffold proteins through bacterial expression system up to 95% of homogeneity and initiated immunization studies. The prime-boost strategy has been taken with the same region of HIV-1 Env using different scaffold proteins. Based on the results of present animal immunization studies, further characterization will be employed to validate scaffold based immunization towards vaccine development.

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Stabilization and characterization of soluble immunogen of Clade C Env 4-2.J41 in a near native trimeric conformation

The elicitation of bNAb remains the primary and most challenging goal in HIV-1 vaccine development. DNA priming and protein boost with JR-FL SOSIP.R6 gp140 has been shown to elicit Ab that neutralize HIV-1JR-FL and certain other representative primary isolates in pseudovirus and/or whole-virus assays. Along with JR-FL SOSIP; soluble, cleaved BG505 SOSIP.664 Env trimer which is stabilized and antigenically near-native in conformation provides a solid platform for the structure based immunogen design. Here, we are using our recently identified clade C Env, 4-2.J41, as a target for soluble immunogen designing. It was reported that 4-2.J41 Env expressed on cell surface as efficiently cleaved and binds to cleavage-dependent antibody, PGT151 and several Env-specific conformation dependent neutralizing antibodies.

It was previously shown that the HIV-1 Env with SOS and I to P mutations maintains a native trimeric conformation. However, full length SOSIP conformation of 4-2.J41 shows minimal or negligible binding with cleavage-dependent antibody, PGT151 at cell surface. At soluble protein level, initial data obtain with SOS and IP mutation 4-2.J41 Env also confirms the above finding. This observation suggests the presence of alternate conformation for 4-2.J41 Env when we adopted the same strategy as mentioned above. Hence, we compared the sequences of all functional Env obtained from the plasma of the same patient from which we got 4-2.J41. Sequence analysis confirms that 4-2-J series of clones have some specific sequences, which are the cause for destabilization of the trimeric Env and when the sequences in J41 Env was mutated following the other well characterized stable native trimeric SOSIP form of Env, it could bind to all possibly tested neutralizing antibodies and minimally to non-neutralizing antibodies. Antibody affinity purification is underway.

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A comparative immunogenicity study in rabbits of cleavage competent Indian clade-C Env (4-2.J41) or with clade-B JR-FL Env or in combination of these two by using DNA priming followed by soluble trimeric protein boost immunization format

In this project, we would like to evaluate the immunogenicity of recently identified cleavage competent Indian clade-C envelope (4-2.J41) alone and in combination with JR-FL Env for priming in the form of plasmid DNA followed by relevant protein boost. The sera from animals primed with three times DNA have been examined for the induction of nonnative trimeric Env binding

antibodies. High antibody titers against JRFL Env in sera were detected by ELISA in plate coated with JRFL gp140-Foldon (FT) trimeric proteins. On the contrary, except the sera from control group and the animals immunized with plasmid DNA expressing JRFL Env alone sera from animals immunized with plasmid DNA expressing either 4.2.J41 Env or in combination with JRFL Envs showed high binding antibody titer to trimeric 4-2.J41 Env (4-2.J41-FT Env) protein as measure by ELISA in plate coated with 4-2.J41 gp140-Foldon (FT) trimeric proteins. However, the animals immunized with plasmid DNA expressing JRFL-FT DNA elicited equivalent antibody binding titer to both JRFL-FT and 4-2.J41-FT trimeric proteins.

The neutralizing antibody response is being tested and sera from different groups have shown neutralizing antibody response against tier-1 and a few tier-2 pseudoviruses. Sera of rabbits from clade-C 4.2.J41 Env group showed neutralizing response to the virus pseudotyped with Env isolated from Indian primary isolates. The complete linear epitope mapping of rabbit immune sera revealed strong binding to variable domains of gp120. Importantly, none of the rabbit sera were able to inhibit VRCO1 binding nor reacted to gp41 directed peptides. Indian clade-C 4.2.J41 Env also induced a strong autologous Tier-2 NAb response in rabbits. The immune sera also efficiently competed with PGT like antibodies, in binding to gp120. Thus, naturally cleaved 4-2.J41 Env represent a promising Indian clade-C Env for developing HIV-1 vaccines aimed at inducing bNAbs.

To stabilize the cleaved native-like conformation of cytoplasmic deleted Indian clade-C 4-2.J41 Env at cell surface level

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One of the immune evasion strategies of HIV-1 Env is low number of Env spikes on virion. The cytoplasmic tail of gp-41 carries trafficking signals and conserved motifs that allow internalization of Env from the plasma membrane for degradation or recycling and also alters its conformation. This project aims for rational modifications of trafficking signals and conserved motifs in the cytoplasmic tails of HIV-1 Indian clade C (4-2.J41) Env that can positively regulate Env cleaved native-like conformation, which will further help us we to derive either DNA or virion-based immunogens with greatly enhanced levels of Env trimers at cell surface.

Preliminary studies in our lab have shown when whole cytoplasmic tail (CT) of 4.2.J41 Env is deleted, the Env binds to both neutralizing and non-neutralizing antibodies (higher extent); unlike JRFL del-CT which binds to neutralizing antibodies and less moderately to non-neutralizing antibodies on cell surface level. On further analysis, we found that the cleavage is not affected by deletion of cytoplasmic tail on 4.2.J41 Env. To map the CT, we made several Env constructs with deletions of CT of various length and motifs. We found that the presence of a conserved hydrophilic epitope restores the Env conformation as that of wild type Env on cell surface DNA level. Further characterization and evaluation of the mechanism is going on to check the subcellular localization. The results will enable us to design novel soluble and membrane bound immunogens for HIV-1 Indian clade C Env by exploiting the findings.

Development of vaccine/gene delivery vectors

Though human adenovirus-based vectors have been widely used as gene/vaccine delivery vectors, some of the clinical trials pointed to certain safety and efficacy issues associated with these vectors. Since animal adenoviruses are not known to cause disease in humans and that humans are generally reported to be naïve to neutralizing immunity against animal adenoviruses, we are isolating novel animal adenoviruses from domestic animals and birds, and characterizing them for their suitability as delivery vectors. Additionally, we are also studying the membrane vesicles derived from mycobacteria with a view to ultimately express and deliver proteins of interest as immunogens. Furthermore, a plasmid-based vehicle for delivering therapeutic DNAzymes is also under development.

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Characterization of animal adenoviruses isolated from field animals for their suitability as vaccine/gene delivery vectors

We have isolated several field isolates from apparently healthy bovine, equine, porcine species and poultry bird and have chosen one isolate each representing fowl (FAV), bovine (BAV) and equine (EAV) adenoviruses for further characterization. Complete genome information for the EAV isolate (EAV/H9NS), a serotype 1 EAV, and its pathogenic potential in a mouse model have been established, and currently we are working towards generation of an infectious clone of EAV/H9NS. With respect to the FAV isolate (FAV/B1-7), through bioinformatic analyses, we established this as a member of FAV species C, precisely of FAV serotype 4. As this isolate was obtained from an apparently healthy poultry bird and also that the specific pathogen-free poultry birds infected with the purified preparation of this isolate did not show any visible signs of the disease, we assumed this as a non-pathogenic isolate for analyses purpose. With the availability of sequence information for more numbers of FAV4 isolates from different parts of the world, we compared the genomes of four pathogenic and two non-pathogenic FAV4 isolates with that of FAV/B1-7. Through this analysis, we identified regions that may have roles in dictating the pathogenicity of FAV4 isolates as well as a region that was unique to FAV/B1-7 compared to other known FAV4 isolates, which showed a phylogenetic relationship with that of FAV serotype 10 – another member of FAV species C. Through this, for the first time, we established occurrence of multiple intra- as well as inter-serotype recombination events in the FAV/B1-7 genome. Currently, we are in the process of constructing an infectious clone of FAV/B1-7. Previously, we tested serum samples collected from poultry birds and humans with/without exposure to live, infected poultry birds for the presence of anti-FAV/B1-7 neutralizing immunity as a pre-requisite for vector development. We have now established that several human cell types were susceptible to FAV/B1-7 infection and some of them allowed moderate levels of viral genome replication.

With respect to the BAV isolate (BAV/F14), sequencing of a small region in the Hexon gene followed by bioinformatic analysis suggested it as a possible member of BAV serotype 8. Currently, any information available for a BAV8 isolate is very limited and importantly the vectoral suitability/ability of a BAV8 isolate has not been investigated, and therefore this becomes an important and obvious candidate for further investigation as well as for development as

a novel vector. In this direction, we are in the process of establishing complete genome information and pathogenicity of BAV/F14 in a mouse model. Besides, we are testing large pool of serum samples collected from bovines and human beings who are regularly exposed to bovine species in the field for the presence of anti-BAV/F14 neutralizing immunity. We are also analyzing the BAV/F14-infected cell lysates of human and animal origin to establish the extent of viral genome replication in different cell types and also to establish whether infection of any of these cell types is productive.

Development of therapeutically viable delivery systems for DNAzyme-based antiviral therapeutics against flaviviruses

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DNAzymes (Dz) are single-stranded oligonucleotides with enzymatic activity capable of cleaving the target single-stranded RNA with high specificity. Previously, we have shown that a DNAzyme 3DzG was capable of protecting 100% of the virus-infected mice from JEV-induced encephalitis when injected directly into the brain. We are now using this technology to design Dzs that could inhibit Dengue virus replication. Additionally, we are aiming to develop biocompatible in vivo delivery system(s) for Dz-based therapeutics taking the advantage of the cDNA synthesizing ability from an RNA template of retroviral reverse transcriptase. We have recently expressed the M-MuLV RT gene (kindly provided by Dr. Stephen P Goff) in a bacterial system and testing the functional activity of the purified enzyme. Subsequently, both the M-MuLV RT gene and the Dz-coding cassette will be cloned into a single mammalian expression plasmid to express the DZ and test the functional activity of the expressed Dz against the target in a mammalian system.

Mycobacterial membrane-derived vesicles: Role in pathogenesis and exploration as a novel subunit vaccine vehicle against tuberculosis

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BCG fails to protect adolescents and adults. Neither boosting BCG nor using BCG as booster works. Hence, subunit vaccines are currently being explored as boosters. Those either in clinical trials, or the ones which failed in trails, all contain 1-4 purified antigenic candidates put together with an adjuvant. Experts predict an ideal subunit vaccine must contain multiple antigens targeting different stages of Mycobacterium tuberculosis (Mtb) pathogenesis. As an alternate to liposomal-derived boosters, here we explore if membrane vesicles of mycobacteria could serve similar purpose with the objectives to purify Mtb-derived outer membrane vesicles (OMVs), and determine their contents and delineate their role in Mtb-mediated pathogenesis.

Most bacteria generate membrane/outer-membrane vesicles (MVs/OMVs). Pathogenic bacteria exploit them for their virulence. MVs are nanoscale (approx. 10-300 nm) proteoliposomes produced naturally and thus constitute a unique system in which the antigens and the delivery vehicle per se are naturally derived from the pathogen. Additionally, MVs circumvent safety limitations of attenuated/killed organisms administered as vaccines. Finally, MVs can be engineered to include several naturally un-incorporated antigens. The prediction is, since the pathogen per se delivers the vaccine antigens of interest into recombinant OMVs, they retain native conformations to immune-stimulate better.

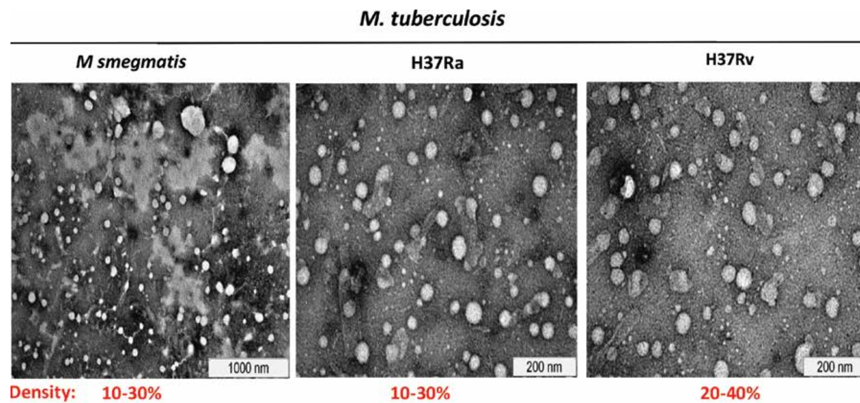

Figure-3: Size of Mycobacteria MVs [200 nm to 250 nm]

Towards achieve this long-term goal, we intend to generate recombinant MVs (rMVs) from Mycobacterium smegmatis (Msmeg), a nonpathogenic mycobacterium species. This warrants that we first identify MVs proteome. Towards this, we had standardized conditions to enrich MVs from large volumes of culture filtrates of Msmeg grown in vitro in minimal media. Using our standardized conditions and Mass Spectrometry, we recently identified 203Msmeg proteins in MVs. These proteins fall into several categories. Mycobacteria MVs too range from 20 to 250 nm in size (Fig. 3)

Interestingly, we and others find approximately 30% of the identified MVs proteome constituted with annotated nucleic acid-binding proteins (NABPs). Given the significant proportion, we tested if the vesicle pellet contains nucleic acids too. Interestingly, we can isolate both DNA and RNA from Msmeg MVs pellet. Access of the isolated nucleic acids (NAs) to digestion with both DNase I and RNase indicate that they are external to MVs. Significantly, NAs could be isolated at all stages of growth. Similar results were obtained with Mtb MVs. Experiments are currently underway to determine if the detected NAs directly interact with MVs or they merely are contaminants. Either way, these NAs and hitch-hiking NABPs can contaminate the true identity of MVs proteome. Eliminating such NAs-associated NABPs and other NABPs- interacting proteins will help identify the true protein candidates in MVs. We are currently in this process.

In the past, with several well established bioinformatics tools, we mined the available data on mycobacterial MVs proteins for possible consensus secretion signal that might drive the identified proteome into MVs. Since we now detect NAs and associating NABPs, it is possible that, until these are eliminated from the total pool, we might not lay hands on a possible consensus signal. It is also highly likely that despite above analyses, a possible consensus sequence might not emerge. If so, we will clone full length and truncations of salient proteins that emerge from MVs, fuse them either with 3X FLAG and/or a reporter to explore if a secretion signal could be identified. Towards this we are in the process of constructing appropriate Gateway vectors.

Biology of medically important viruses and the viral infections

A large number of viral infections persist in India that appear sporadically or have become endemic. Many of these viral infections present themselves as frequent epidemics in various parts of the country. We are interested in studying the viral infections that are associated with poor hygienic conditions such as those spreading though mosquito bites or feco-orally though contaminated drinking water. Thus, we are studying mosquito-borne viruses such as Dengue and Japanese encephalitis, and feco-orally transmitted Hepatitis E virus. Our studies are focused on the biology of the pathogen as well as the infection.

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Identification of correlates of severe dengue disease

Studies in dengue patients from tropical and sub-tropical regions of the world including South America, South- and South-East Asia indicate that hyper-responsive immune system and elevated levels of inflammatory cytokines risk factors. However, a cohort study to comprehensively investigate the kinetics of immune response in dengue patients and identifying the correlation between clinical parameters, plasma factors, viral load and the innate immune response from India has been lacking. We established a pediatric dengue cohort in New Delhi and report the characterization of disease parameters and the correlation between the viral load, thrombocytopenia, plasma cytokine profile and severe disease in pediatric patients.

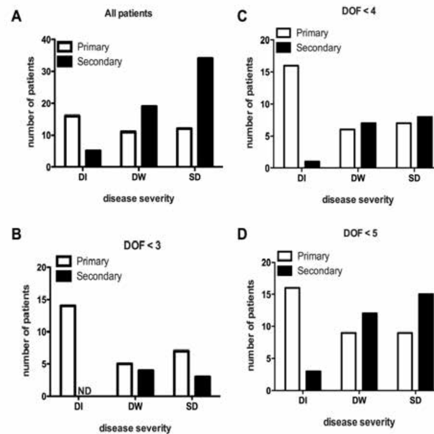

Figure-4: Severe dengue disease occurs in both primary and secondary infections. (A) Patients with primary and secondary dengue infections (N=97) were stratified based on severity at the time of admission. DI – Dengue infection, DW- Dengue with Warning signs and SD – Severe Dengue. Patients with primary and secondary dengue infections with day of fever < 3 (B), <4 (C) or <5 (D) were stratified based on severity at the time of admission (N=36, 45 and 64 respectively).

Here we present for the first time a comprehensive analysis of viral and immunological factors and its correlation with disease severity in pediatric dengue patients in New Delhi. About 40% of our study patients had primary infections and 31% of these patients had severe disease as against 65% of those with secondary infection. Although the proportion of severe dengue (SD) cases were higher in secondary infections (65.5%), about one-third of primary infections (31%) also caused severe disease (Fig 4A). To further exclude recruitment bias in our observations, we determined the proportion of dengue illness (DI), dengue with warning signs (DW) and SD cases from primary and secondary infections in patients with DOF<3, DOF<4 and DOF<5 (Figs 4B-3D).

79% of patients with DOF<3 had primary infection and this gradually decreased to 64% and 53% in DOF< 4 and DOF<5 groups respectively. Overall, 38% of SD cases from DOF<5 group were primary infections suggesting that both antibody-dependent and antibody-independent modes of infection may cause severe dengue in pediatric patients. We determined dengue viral load in whole blood by real-time PCR. Dengue viremia was highest in patients with earlier DOF

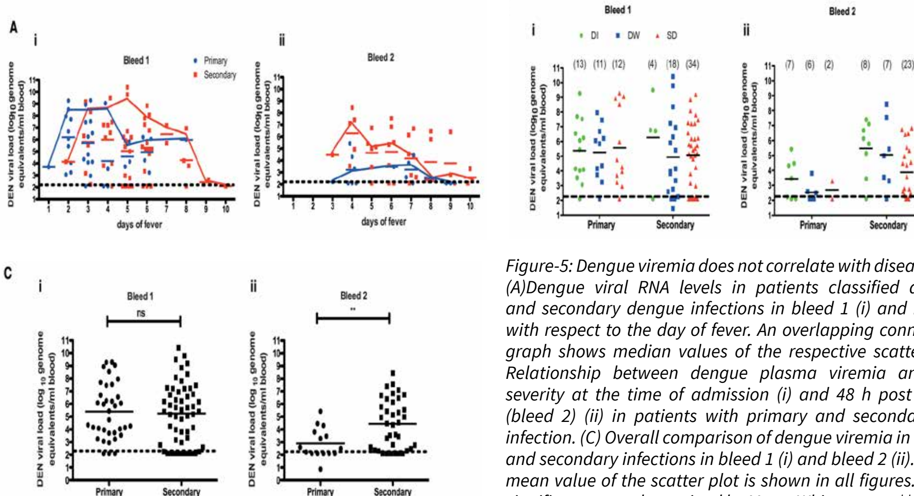

*Figure-5: Dengue viremia does not correlate with disease severity. (A)Dengue viral RNA levels in patients classified as primary and secondary dengue infections in bleed 1 (i) and bleed 2 (ii) with respect to the day of fever. An overlapping connecting line graph shows median values of the respective scatter plot. (B) Relationship between dengue plasma viremia and disease severity at the time of admission (i) and 48 h post admission (bleed 2) (ii) in patients with primary and secondary dengue infection. (C) Overall comparison of dengue viremia in all primary and secondary infections in bleed 1 (i) and bleed 2 (ii). Geometric mean value of the scatter plot is shown in all figures. Statistical significance was determined by Mann-Whitney test. ** P = 0.0074.*

and showed a declining trend 6 days post-fever (Fig 5A i). Viremia was about ten-fold lower in repeat bleeds sampled 48 h after first bleed (Fig 5A ii). We found that both primary and secondary infections had comparable viremia in the first bleed (Figs 5A i) and 5C i) but patients with secondary infections had significantly higher viremia as compared to primary infections in the second bleed (Figs 5A ii and 5C ii). We found that dengue viremia was indistinguishable between patients with DI, DW or SD either in primary or in secondary infection but all the secondary infection cases in the repeat bleed had a significantly higher viremia as compared to primary infections despite showing clinical improvement from SD (Figs 5B i and ii, and 5C ii). These data suggests that DENV infects by both ADE-dependent and ADE-independent mechanisms, however, ADE may contribute to prolonged viremia observed in secondary infections.

Most of the previous studies have reported the association of some of the secreted factors with severe disease and its manifestations in secondary infections. We performed a multiplex magnetic bead based assay to measure 14 analytes in the plasma of dengue patients. Boruta algorithm has been shown to identify important features that are statistically significant in large data sets that may otherwise be masked in univariate analysis. Using this approach, we identified some of the significant features as markers of severe dengue and also in the patients who showed clinical improvement from SD

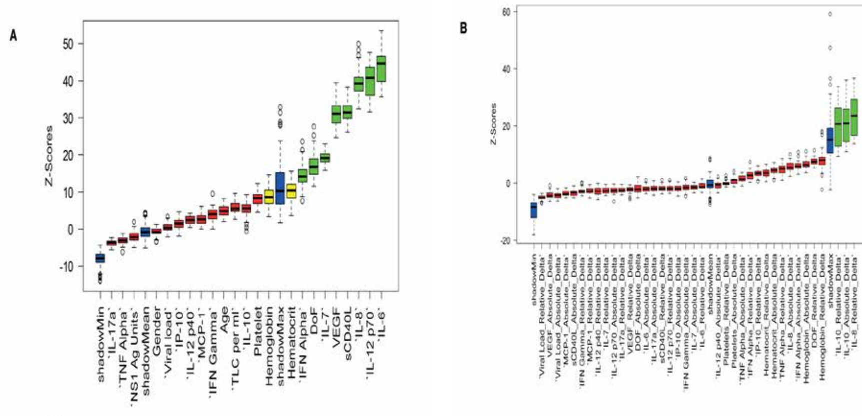

Figure-6: Multivariate selection of features of dengue severity and recovery. (A) The boxplots represent the distribution of variable importances after adjusting for the interactions among the variables. The minimal set of selected features (green) are the ones whose medians lie above the maximal possible median importance in the shadow data. Eight parameters out of all available clinical and biochemical parameters were selected as important for prediction of the severity classes (SD, DW and DI). (B)Identifying markers of recovery from SD using the Boruta algorithm.All parameters from SD patients whose disease status improved clinically from first to second bleed were considered for analysis.Three delta parameters out of all the delta (absolute and relative changes across the first and second bleed, see text) were significantly important for prediction of recovery from SD and were consistent with findings in the univariate analysis.

(Fig 6A and 6B). Most of these factors were found to be important by univariate analysis thus verifying the validity and significance of in silico data.

Further characterization of kinetics of some of the secreted factors that associate with severe disease may prove to be key to understand the effect of immune imbalance in SD. As reduced interferon levels seem to correlate with severe disease in both primary and secondary infections, therapies designed to transiently restore interferon levels to a threshold level required for recovery may prove to be useful in addition to antivirals and vaccine development.

Role of tyrosine kinases in dengue virus and Japanese encephalitis virus life-cycle

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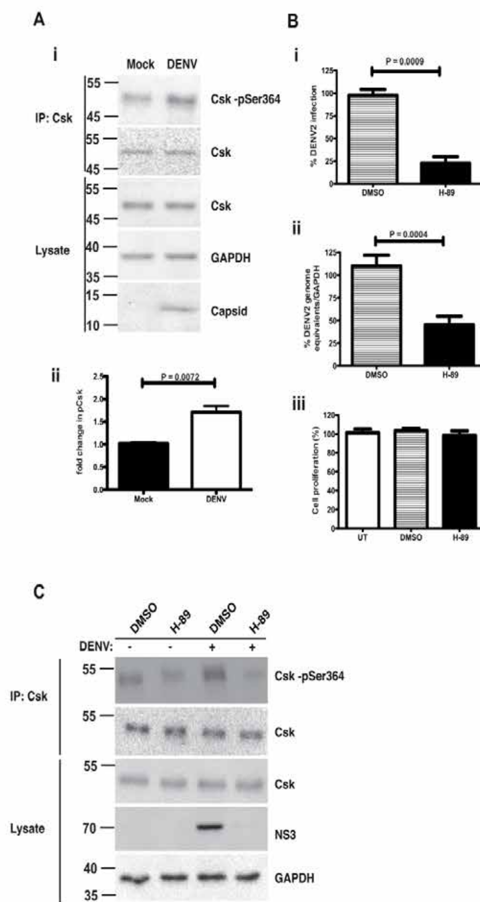
In addition to antivirals targeting viral proteins directly, identifying host factors required for the virus life cycle provides additional targets for drug development and is an alternate plausible approach to counteract viral infections. Tyrosine kinases (TK) participate in intracellular signaling by binding to other proteins in



response to both extrinsic and intrinsic signals. The structure and function of many of the TKs are well conserved across different species, therefore, many viruses have evolved to utilize the function of host TKs at various stages of infections thus providing an opportunity to use host TKs as antiviral targets. The objective of this study was to identify TKs that are necessary for infection of DENV in Huh-7 cells.

We had earlier identified and shown that c-terminal src kinase (Csk) plays a role in DENV and Japanese encephalitis virus replication. CSK has been shown to be phosphorylated on Ser-364 by protein kinase A (PKA). Therefore, we next determined the phosphorylation status of Csk in DENV infection. Csk was immunoprecipitated from infected cell lysates at 24 h post-infection (pi) and the extent of Csk phosphorylation was analysed by immunoblotting with phospho-Csk antibody which recognizes the phosphorylation of Serine 364 residue in Csk. We found a 70% increase in phospho-Csk levels at 24 h pi indicating that DENV infection enhanced phosphorylation of Csk (Fig. 7Ai and ii). We next assessed the effect of inhibiting PKA on DENV infection. Cells were infected with DENV and treated with 5 μM of H-89, a PKA inhibitor from 1 h pi. Viral titers and viral RNA levels were estimated as described earlier. Inhibition of PKA led to significant reduction in viral titers in the supernatant (Fig. 7B i) and this effect was due to reduced viral replication as assessed by reduction in the viral RNA levels (Fig. 7B ii). Inhibition of PKA by H-89 did not affect cell proliferation (Figure 7B iii). To further demonstrate that the effect of PKA inhibition on DENV replication is due to its effect on Csk phosphorylation, cells infected with DENV were treated with DMSO or H-89 at 1 h pi. Csk phosphorylation (pSer-364) was assessed at 24 h pi by immunoprecipitation of Csk from

Figure-7: Csk hyperphosphorylation and protein kinase A activity in DENV infection. (A) (i) Huh-7 cells were infected with an MOI of 3 pfu/cell of DENV2. Cell lysates were prepared at 24 h pi and Csk was immunoprecipitated. Phosphorylation of Csk was detected by antibody specific to Csk-pSer364 by western blot. Total amount of Csk immunoprecipitated was analysed by blotting with Csk antibody. GAPDH in total lysates indicates the equal quantity of protein used for immunoprecipitation. DENV infection was verified by western blotting to detect capsid. ii) Quantitation of Csk-pSer364 western blots is shown. P value was calculated by non-parametric, Mann-Whitney test. Size of pre-stained molecular weight marker band is indicated. (B) (i) Huh-7 cells were infected with DENV2 and cells were incubated in medium containing 5 mM of PKA inhibitor, H-89, or DMSO (vehicle control). Viral titers in the supernatant was measured by plaque assays at 24 h pi. (ii) RT-PCR analysis to measure the DENV RNA in total RNA isolated at 24 h pi from Huh-7 cells infected with DENV2 and treated with DMSO or H-89 post-infection. The data are representative of at least three experiments performed with two or more replicates and indicate mean with SEM. P value was calculated by non-parametric, Mann-Whitney test. (iii) Huh-7 cells were incubated in medium containing 5 mM of PKA inhibitor, H-89, or DMSO (vehicle control) and cell proliferation assay was performed at 24 h post-transfection. UT-Untreated. Error bars represent mean with SEM. (C) Huh-7 cells were mock-infected or infected with DENV2 and treated with DMSO or 5 mM of H-89 from 1 h pi. Csk was immunoprecipitated at 24 h pi and phospho-Csk was detected by western blot analysis with antibody specific to CSK-pSer364. The amount of total Csk pulled down in immunoprecipitation is shown. Total Csk and DENV-NS3 in the cell lysates is shown. GAPDH levels are shown as loading control. Size of pre-stained molecular weight marker band is indicated.

cell lysates. PKA inhibition by H-89 led to reduced phosphorylation of Csk in both mock- and DENV-infected cells. As expected, DENV infection was blocked in cells treated with H-89 as evidenced by reduced expression of dengue non-structural protein 5 (Fig. 7C). These results suggest that phosphorylation of Csk by PKA is potentiated in DENV infection and PKA activity may play an essential role in DENV replication.

Our study has identified a role for Csk and PKA signaling in mosquito-borne flavivirus replication and provides a new perspective into the role of SFK signaling in flavivirus infection. Further efforts to characterize the exact role of Csk in DENV replication may provide novel targets to develop antivirals for dengue disease.

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Investigating the effect of viral infections on zinc homeostasis as a cause of permeability barrier disruption in polarized epithelial and endothelial cells

Cellular zinc levels are known to play a crucial role in the maintenance of permeability barrier. Whether viral infections modulate zinc homeostasis to facilitate viral replication in the host and if this modulation is linked to disruption in the barrier functions of epithelial and endothelial cells observed in viral infections has not been investigated. Here we investigate modulation of zinc transporters in virus infections using dengue virus, respiratory syncytial virus and rotavirus as models of infection.

One of the primary objectives of the study is to determine if viral infections differentially affect permeability barrier functions in epithelial cells. We used Caco-2 cells (epithelial colonic adenocarcinoma cells) as a model system to assess the barrier functions by infecting with dengue virus (DENV-positive-sense, ssRNA virus) and rotavirus, (RV-a dsRNA virus). Effect of infection on permeability functions was assessed by measuring trans-epithelial electrical resistance (TEER) of the monolayer grown on transwell filters at indicated time points post-infection (Fig. 8). Dengue infection caused about 20% reduction in the TEER values by 12 h post-infection but this difference was insignificant and the barrier function was not disrupted further even at 48 h post-infection. However, RV infection led to a rapid drop in TEER values after 18 h post-infection (Fig. 8) suggesting that rotavirus affects barrier functions in Caco-2 cells but DENV has no effect. We next explored the zinc status in infected cells. Caco-2 cells were infected as above and labile zinc levels in the cells was measured by staining with Fluozin-3 and zinc staining was assessed by flow cytometry. As expected, DENV infected cells did not show any change in zinc levels but surprisingly, RV infected cells showed a 50% increase in free zinc indicating that zinc homeostasis is altered specifically during RV infection (Fig.9).

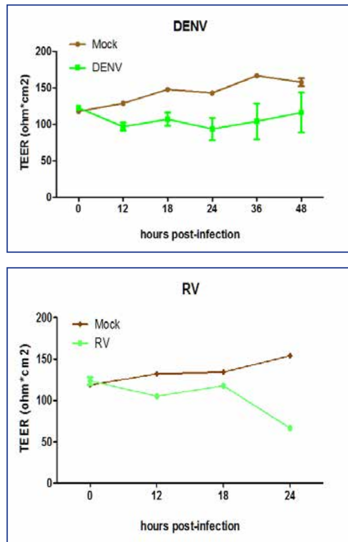

Figure-8: Effect of DENV and RV infection on permeability barrier integrity. (A) Caco-2 cells were infected with 10 MOI of DENV2 or 0.5 MOI of rotavirus. Trans-epithelial electrical resistance was measured at indicated time post-infection. Plot represents Mean with SEM

Our future efforts will focus on identifying the specific components of zinc homeostasis that are affected by RV infection. We will also probe the effect of altering zinc homeostasis either before and after infection on viral proliferation.

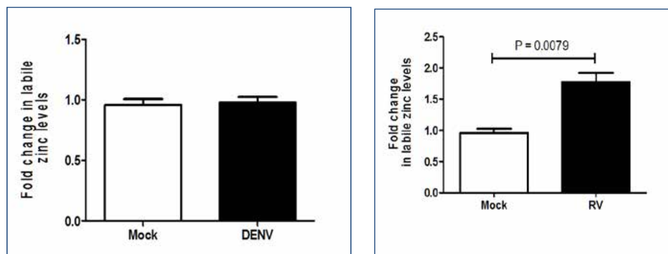

Figure-9: Zinc levels in DENV and RV infection. (A) Caco-2 cells were infected with 5 MOI of DENV2 or 0.5 MOI of rotavirus and intracellular labile zinc levels were measured by fluozin staining at 24 or 16 hours post-infection respectively. Error bars represent Mean with SEM. P value was estimated by Mann-Whitney test.

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Functional significance of neuron/microglia-derived exosomes in Japanese encephalitis virus-induced inflammation and virus dissemination

Acute encephalites syndrome (AES) is a clinical manifestation that is triggered by infection with a wide range of viruses including Japanese encephalitis virus (JEV) or other infectious and noninfectious causes. JEV-infected AES patients suffer severe neurological sequel than AES having non-JEV etiology. It is therefore of utmost importance to differentiate between the various forms of encephalitis with similar clinical manifestations. Studying exosome content from JEV-infected CSF as well as from brain resident cells in culture condition will provide novel information and will help us to identify the components that can lead to new discovery in controlling disease severity.

Exosomes represent a specific subtype of secreted membrane nanovesicles that are approximately 30–100 nm in size and are formed inside the cell in endosomal compartments called multi-vesicular bodies. Bio-fluids like plasma/serum, urine, breast milk, and cerebrospinal fluid (CSF) are potential source for exosomes. These secreted vesicles are known to play an important role in normal physiological processes and may be exploited as a pool for detection of novel biomarkers.

The exosomes contain a lipid bilayer which is known to encapsulate proteins, miRNAs and mRNAs and protects them from degradation. Moreover, miRNAs can be transferred by an exosome secretion route and further exert gene silencing in the recipient cells. Brain function depends on coordinated interactions between the neurons and the glial cells. Recent evidence indicates that these cells release exosomes which play important role in the neuronal-glia communication. Changes of miRNAs in exosome have been reported in diagnosis of different diseases and provided as potential biomarkers for brain neoplasms, degenerative diseases, autism, and schizophrenia. Further, virus may use the exosomal machinery of the host for viral dissemination and may induce pathogenesis. In this study, we hypothesized that CSF-based miRNAs could be useful for understanding JEV-specific pathogenesis in AES cases. Here, we examined the expression pattern of 87 miRNAs in the CSF of AES patients with or without evidence of JEV infection and identified miRNAs that were specifically present in CSF of JEV specific AES. Using a quantitative real-time PCR-based miRNA array, we examined the level of 87 miRNAs expressed in human exosomes isolated from CSF. Subsequently, correlation between cytokine level and miRNAs expression in CSF samples was examined. In this study, we identified and validated the upregulated expression of three miRNAs, miR-21-5p, miR-150-5p, and miR-342-3p that were specifically found in CSF of AES patients infected with JEV. Expression of miR-21-5p, miR-150-5p, and miR-342-3p was also elevated in JEV-infected mice brain. However, the expression pattern of these miRNAs differed in neuronal cells, microglial cells, and the exosome derived from JEV-infected cell culture supernatant. Interestingly, neuronal cells infected with vaccine strain (SA-14-14) did not lead to any upregulation of these three miRNAs. Further, miR-150-5p expression was found to be negatively correlated ($r = -0.5279$, $p = 0.016$) with TNFα level. Pathway analysis of putative target genes of these miRNAs indicated involvement of TGF-β, NGF, axon guidance, and MAPK signaling pathways in JEV patients.

Simultaneously in our in vitro studies, we observed that JEV infection leads to increased release of exosomes into the cell culture supernatant. We also observed the presence of JEV RNA in exosomal fractions. It was interesting to note that microglial cells, which are less infective to JEV infection, package

more viral RNA into the released Exosomes. The packaging of viral RNA within the exosomes suggests a means by which virus may cross blood brain barrier and deliver the content in the neighboring cells. Further work is in progress to understand the role of exosomes on neuroinflammation and virus dissemination.

Use of designer microRNAs against 3'UTR of JEV to inhibit viral replication

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Despite numerous efforts to develop antiviral therapies and vaccine countermeasures, currently there is no antiviral drug or effective vaccine for use against JEV infection. Short interfering RNAs (siRNAs) have shown immense potential to be used as an antiviral therapeutic for various human viral diseases. However, siRNA or shRNA based antiviral studies have several drawbacks including triggering IFN response or saturating RNAi machinery proteins, leading to enhanced toxicity. Artificial designed microRNAs (miRNAs) may help to overcome these problems and are prove to be most effective inhibitors to block genes functions. Our objective here was to design and develop such artificial miRNAs against JEV 3'UTR and test them if they are able to inhibit viral replication in infected in vitro or in the mouse model of JEV infection in vivo.

Artificial miRNAs are small RNA molecules expressed under the backbone of endogeneous cellular miRNAs. These miRNAs are expressed though RNA polymerase II and are supposed to be less cytotoxic than siRNAs due to their relatively low concentration inside the cells. We have designed artificial miRNAs against JEV 3'UTR across different strains, cloned them in a pcDNA6.2GW/EmGFP expression vector. The engineered pre-miRNA sequence structure is based on the murine miR-155 sequence. The 5' and 3' flanking regions derived from the miR-155 transcript were inserted in the vector to preserve as much as possible of the miR-155 structure. The stem-loop structure and a 2 nucleotide internal loop is optimized to result in higher knockdown rate than the 5 nucleotide / 3 nucleotide internal loop found in native miR-155 molecule. In a co-transfection study using artificially cloned miRNA vector and JEV 3'UTR tagged with Renila Luciferase gene in HEK293 cells, we found four out of five miRNA constructs could effectively bind JEV 3'UTR and had desired biological activity. Two of the miRNAs were further tested in JEV-infected neuronal cell culture model. We observed a one log reduction in viral titer in plaque assay compared to control (Fig. 10) demonstrating the potential of these miRNAs in controlling JEV replication. Efficacy of these miRNAs will now be tested in the mouse model of JEV infection.

Transcriptome analysis and identification of novel biomarkers of disease progression in dengue patients focusing on noncoding RNAs

Dengue fever (DF) is now recognized as one of the most important mosquito-borne human infections of the 21st century. The virus is known to promote vascular permeability, cerebral edema leading to Dengue hemorrhagic fever [DHF] or Dengue shock syndrome (DSS). Why DHF/DSS develops only in some patients infected with DENV is poorly understood, but such development appears to be dependent on interaction between viral factors, host genetics and the

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immunologic background of the host. A robust gene expression study comparing patients with DF with those with DHF may provide an opportunity to identify markers associated with immunity, and disease pathogenesis and severity.

There are no effective vaccines available against Dengue and no biomarkers available which can help us to predict disease outcome. To date, dengue research has primarily focused on the deregulation of protein coding genes. Most of the data generated on whole blood or PBMC isolated from different dengue patients through microarray, thereby missing long non-coding RNAs (lncRNAs), which have been shown to play critical role in modulating immune response and viral replication. lncRNAs are designated as the transcripts longer than 200 nt without protein-coding capacity. Similar to mRNA, lncRNAs are spliced products of RNA polymerase II or III, 5′-capped, and with or without 3′-polyadenylation. Recently, increasing evidences have confirmed the crucial roles of lncRNAs in host antiviral response. The broad spectrum of activities and versatile regulatory mechanisms of lncRNAs suggest that lncRNAs are key regulators of host immunity during viral infection. lncRNA is the functional end-product, and the level of lncRNA expression correlates directly with the level of the active molecule. Thus, the use of lncRNAs in diagnostics has intrinsic advantages over the use of protein-coding RNAs. In addition, lncRNAs show greater tissue specificity compared to miRNAs and protein-coding mRNAs, making them attractive in the search for novel diagnostic and prognostic disease biomarkers. Therefore our focus in this study is to identify the early transcriptional signature in the peripheral blood mononuclear cells (PBMCs) in a large number of clinically and virologically well characterized patients with mild and severs dengue infection and establishes their correlation with disease progression. In this study, we will (1) identify known lncRNAs and novel lncRNAs from RNA-Seq data of dengue patients and control; (2) predict the possible functions of the differentially expressed lncRNAs by checking their co-expression patterns with protein-coding RNAs; (3) identify the pathways and biological processes that the candidate lncRNAs are associated with, and (4) identify the lncRNAs to predict clinical outcome (platelet count as one parameter). We have categorized our samples in the following groups. (a) Diseased control (n=8); (b) Group-1, Platelet count ≥50k (n=9); (c) Group-2, Platelet count ≥20K-<50k (n=14); (d) Group-3, Platelet count <20k (n=8). Overall, we have sequenced the RNAs from 39 samples of different groups using the NGS. All the computational analysis is now in progress.

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Antiviral signaling during Japanese encephalitis virus infection of neuronal cells

Activating Transcription Factor 3 (ATF3) belongs to the ATF/cAMP Responsive Element-Binding (CREB) family of TFs and is known to be induced during inflammation and genotoxic stress. ATF3 was shown to be induced by lipopolysaccharides (LPS) and regulate TLR4 signalling via epigenetic regulation. Furthermore, it was shown that ATF3 interacts with HDAC1 thereby causing histone deacetylation and repression of the Il6 and Il12b promoter. Besides being a negative regulator of inflammatory responses, ATF3 has been shown to positively regulate various cellular pathways suggesting that it can act either as an activator or repressor of transcription. Apart from the various TLR ligands, ATF3 has also been shown to be induced by High-Density Lipoprotein (HDL), thus providing mechanistic insights into anti-inflammatory nature of HDL. ATF3 has also been shown to play an important role in inhibition of other cellular responses including the inhibition of allergen-induced airway inflammation in the mouse model of human asthma. These studies thus suggest that ATF3 can be induced by diverse pathways and act as a negative regulator of inflammation. It is well established that viral infections lead to the induction of antiviral and inflammatory responses. However, the role of ATF3 in the context of viral infections, particularly in the regulation of antiviral genes, is not clear.

Our studies have identified a robust induction of ATF3 during JEV infection of mammalian cells. Based on the analysis of published ChIP-seq and micro array data, combined with in-silico promoter analysis, we predicted ATF3 to regulate a cohort of antiviral genes. This was validated in ATF3-depleted mouse neuronal cells (Neuro2a) which showed a significant up-regulation of antiviral genes following JEV infection. Using the

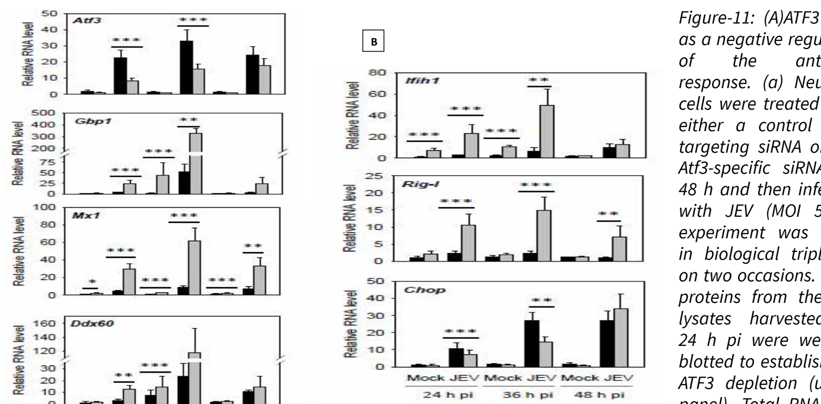

Figure-11: (A)ATF3 acts as a negative regulator of the antiviral response. (a) Neuro2a cells were treated with either a control non-targeting siRNA or the Atf3-specific siRNA for 48 h and then infected with JEV (MOI 5).The experiment was done in biological triplicate on two occasions. Total proteins from the cell lysates harvested at 24 h pi were western blotted to establish the ATF3 depletion (upper panel). Total RNA was isolated from the cells at 24 h pi and qRT PCR performed for various ISGs or members of UPR pathway. Mean values were used to create the heat map demonstrating the gene-expression profiles using Gene-E software (lower panel). (B) Neuro2a cells were treated with either a control non-targeting siRNA or the Atf3-specific siRNA for 48 h and then infected with JEV (MOI 5). The experiment was done in biological triplicate on 2 occasions. Relative expression of various genes at different times pi was studied by qRT PCR for and the mean is plotted. Transcript level in the control siRNA-treated cells is shown by the black bar while that in the Atf3 siRNA-treated cells is shown by the grey bar. The Gapdh levels were used for normalization

chromatin-immunoprecipitation experiment, ATF3 was shown to bind Stat1 and Irf9 promoters and negatively control their expression in Neuro2a and mouse embryo fibroblast (MEF) cells. STAT1 and IRF9 are known to regulate type 1 interferon (IFN) responses. Accordingly, Atf3(-/-) MEF cells showed an abundance of Ifna4 and Ifnb1 transcripts and secreted larger amounts of IFNb1. Consequently, Atf3(-/-)MEF cells showed a significantly suppressed JEV replication compared to wild-type cells. Importantly, in Neuro2a cells where type 1 IFN was not synthesized, ATF3 could modulate the antiviral response through the ISGF3 complex via the regulation of Stat1 and Irf9, and/or by the direct binding to the antiviral gene promoter, for example, Isg15. The enhanced antiviral response in ATF3-depleted Neuro2a cells resulted in suppressed JEV replication. These data indicate that ATF3 functions as a negative regulator of antiviral signaling in mammalian cells and can function in the absence of type I IFNs in JEV-infected mouse neuronal cells by via the regulation of Stat1, Irf9 and Isg15, thereby demonstrating a novel mechanism.(Fig.11A-11B)

Identification of the Japanese Encephalitis Virus Attachment and Receptor System

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Japanese Encephalitis (JE) is the leading form of viral encephalitis in South-East Asia and India. Around 30,000-50,000 cases of JE and up to 15,000 deaths are reported annually. Binding of a virus to its specific receptor is a key event that initiates infection. Identification of the virus receptor can aid in the development of anti-virals that can block virus infection at the first step. The main objective of this study is to identify and characterize the cellular receptor(s) for JEV.

Previously we had validated the use of the JEV-Envelope Protein Domain III (ED3) as an exploratory system to identify the JEV receptor. This domain mediates viral attachment to the host cells, and carries epitopes that elicit a neutralization response. Biochemical Studies identified GRP78 (glucose-regulated protein of 78 kDa) as a JEV-ED3 binding protein. GRP78 is traditionally regarded as a major ER chaperone facilitating protein folding and assembly, protein quality control and regulating ER stress. GRP78 also serves as a co-receptor for Dengue virus serotype 2 and Coxsackie virus. Antibodies directed against GRP78 blocked JEV infection in cell lines and in mouse primary cortical neurons highlighting its role as a virus receptor. The interaction between JEV-Envelope and GRP78 were also validated by biochemical studies.

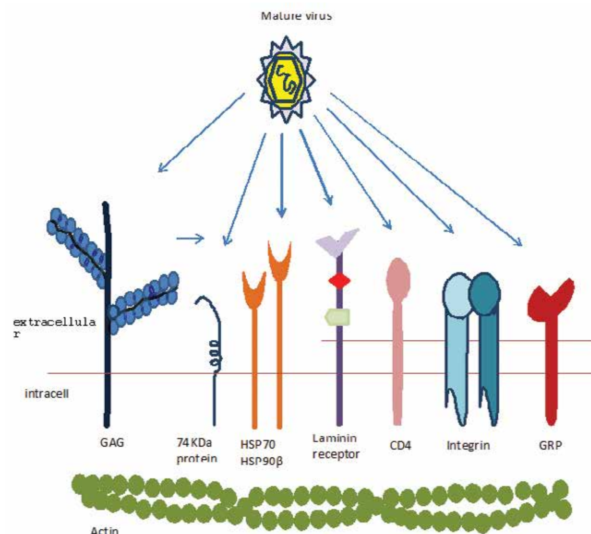

Figure-12: JEV attachment and receptor system. JEV attachment on cells is mediated by charge based interactions with HSPGs that serve to concentrate the virus in closer vicinity of the specific receptor. Several proteins have been implicated as putative receptor molecules some of which are represented – Mr 74,000 protein, HSP70, HSP90β, Laminin receptor, CD4, and integrin α5β3. Our studies indicate a crucial role for GRP78 as an entry receptor.

We have also employed the Subtilase AB toxin derived from Shigatoxigenic E. coli strains that can cleave GRP78 into 44-kDa and 28-kDa fragments, corresponding to the N-and C terminal regions of the protein. As a control the Subtilase AB toxin mutant that lacks this catalytic activity was used. Following treatment with subtilase toxin JEV replication and production of infectious virus particles was decreased several fold indicating that GRP 78 also plays an important role in virus life-cycle at the post-entry step. Our recent studies have established the role of GRP78 in both virus entry and as a chaperone for virus replication and egress.(Fig.12)

We have extended our studies to analyse the role of two additional crucial cell surface molecules in the JEV infection process- Integrins and Epidermal Growth Factor Receptor (EGFR). Our studies indicate that the intergrin $\alpha5\beta3$ does not play a role in JEV infection of neuronal cells. Our preliminary studies indicate that EGFR is likely to play a crucial role in JEV infection either as a receptor or as a signaling molecule.

Cellular Entry Mechanisms of Japanese Encephalitis Virus

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Several endocytic pathways operate at the eukaryotic plasma membrane, which can be exploited by pathogens to gain entry into a permissive cell and establish infection. The route of virus entry can differ between cell types. In addition to utilizing the already-operational endocytic pathways, viruses can induce pathways conducive to entry by receptor binding and signaling events. For flaviviruses, the receptor mediated endocytic pathway has been shown to be preferred internalization route, as low pH of the sorting endosome facilitates viral uncoating and fusion. We are interested in defining the endocytic pathways utilized by JEV for entry into cells in terms of key molecular players.

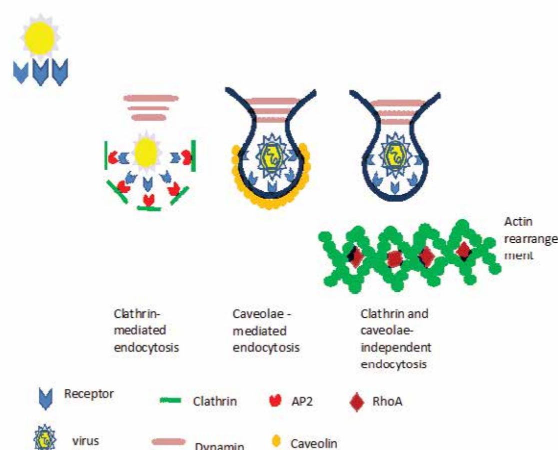

Figure-13: Endocytic pathways for JEV. JEV can get internalized through distinct endocytic routes which include clathrin-mediated endocytosis, caveolae-mediated endocytosis or clathrin-independent route (for neuronal cells). The large GTPase dynamin is essential for all the described entry routes. The clathrin independent pathway requires the small GTPase RhoA and actin rearrangements. The endocytic pathway followed by the virus is likely to be cell type dependent. [From Nain et al., 2016]

Endocytic pathways display extensive cross-talk with respect to molecular players and cargo sorting and recent studies have demonstrated that a high degree of plasticity exists in eukaryotic cells. Studies in our lab have shown that JEV entry in neuronal cells occurs via a clathrin independent endocytic mechanism. By using fluorescently labeled virus particles, a combination of pharmacological inhibitors, RNA interference (RNAi), and dominant-negative (DN) mutants of regulatory proteins involved in endocytosis we established that JEV infects fibroblasts in a clathrin-dependent manner, but it deploys a clathrin-independent mechanism to infect neuronal cells. The clathrin-independent pathway was shown to require the scission molecule- dynamin and plasma membrane cholesterol. Virus binding to neuronal cells leads to rapid actin rearrangements and an intact and dynamic actin cytoskeleton, and the small GTPase RhoA plays an important role in viral entry. (Fig. 13) We have extended these studies to identify host membrane trafficking genes involved in JEV life cycle- entry, replication and infectious virus particle production, by RNA interference screen (of 144 membrane trafficking genes) in human neuronal and epithelial cells. The RNAi screen was standardized in HeLa (epithelial) cells and IMR-32 (neuronal) cells. A total of 144 genes have been tested for their role in JEV life-cycle in epithelial and neuronal cells. Several hits have been obtained, implicating a crucial role of these genes in the virus life-cycle, which are being validated in our ongoing research.

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Interactions between Japanese encephalitis virus and host cellular pathways: implications for pathogenesis

Host-pathogen interaction is a complex interplay that results in triumph of the pathogen (and establishment of disease), or of the host (and clearance of the pathogen). In an attempt to skew the balance towards the host we are studying several aspects of JEV-host interaction with a focus on cellular pathways such as autophagy and its role in determining innate immune activation. We are also studying immune evasion, virus persistence and identification of pattern recognition receptors (PRRs) for JEV in dendritic cells.

The first objective of our study is to characterize how the host autophagy cellular mechanism responds to infection by JEV and its role in pathogenesis. Autophagy is a lysosomal degradative pathway that has diverse physiological functions and plays crucial roles in several viral infections. Autophagy can function as a mechanism of host defense by clearing the pathogen or its proteins through degradation or enhancement of type I interferons. On the other hand microbes can abrogate and/or exploit the autophagy process to enhance their replication. Recent studies in our laboratory have shown that JEV replication was significantly enhanced in autophagy deficient cells resulting in higher viral titers. Autophagy was functional during early stages of infection however it becomes dysfunctional as infection progressed resulting in defective endosomal acidification (Fig.14) and accumulation of misfolded proteins. Autophagy deficient cells were highly susceptible to virus induced cell death. We have extended our studies to establish the role of autophagy in the pathogenesis of JEV. We have examined the contribution of infection induced host responses- oxidative stress and ER stress in the induction of autophagy. Currently we are characterizing the role of autophagy in interferon production during JEV infection. Modulation of interferon production by autophagy is likely to play an important role in the pathogenesis of JEV and disease outcome. Inhibition of autophagosome maturation by JEV can potentially have two possible pathological outcomes. Firstly, deficiency of autophagy leads to the accumulation of disordered proteins that are the underlying causes of neurodegeneration. It could be possible that dysfunctional autophagy contributes to the neurological symptoms of JEV infection. Secondly, lysosomal dysfunction can lead to release of lysosomal proteases in the cytosol of infected cells causing caspase activation and initiation of apoptosis.

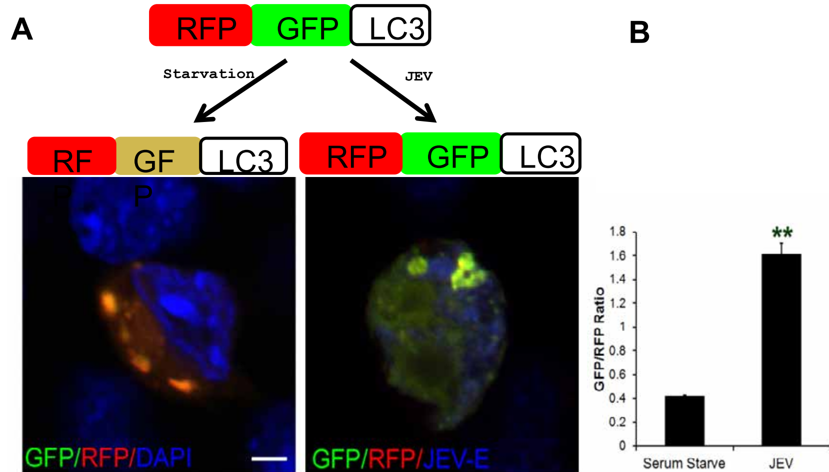

Figure-14: JEV infected cells show defective endosomal acidification. (A) Neuro2a cells were transfected with RFP-GFP-LC3 plasmid and were serum-starved (left panel) or JEV infected (right panel). GFP fluorescence is quenched in serum-starvation indicating that lysosomes are acidic, while in JEV infected cells GFP fluorescence is bright indicative of defective acidification. (B) Quantitative representation of expt. conducted as in (A). Ratio of GFP/RFP fluorescence is higher in JEV infected cells compared to serum-starved condition.

Our second key objective is to study JEV replication and activation of innate immune responses in monocyte derived dendritic cells. Dendritic cells have proven to be important for the transport to lymphoid organs of viruses related to JEV, such as Dengue and West-Nile virus. While dendritic cells play a pivotal role in the initiation of the host defence against invading JEV, a recent study has correlated the elevated replication rate of JEV in dendritic cells with the high mortality of mice in an experimental model of JEV infection. Preliminary data from our collaborators indicate that JEV has the capacity to induce the maturation of monocyte-derived dendritic cells, together with the expression of PD-L1, suggesting that the virus skews the immune response by conferring tolerogenic properties to antigen presenting cells, thus escaping from the host immune system. We are now elucidating at the molecular level the functional consequences of the interaction of JEV with dendritic cells.

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Mechanism of thrombocytopenia during Dengue virus infection

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Infection by Dengue virus (DENV) is responsible for causing Dengue fever which in its severe form is associated with hemorrhage, spontaneous bleeding in the lungs and visceral spaces, in addition to thrombocytopenia or an acute drop in the level of blood platelets. Platelets are small anucleate cells, produced from large mother cells called megakaryocytes, play a major part in blood homeostasis by plugging disruptions in vascular endothelium through formation of thrombus or clots. In addition to DENV infection by a number of other viruses, including Simian Immunodeficiency virus (SIV) and Human Immunodeficiency virus (HIV) are known to induce thrombocytopenia. The etiology of thrombocytopenia by many of these viruses indicate a combination of impeded biogenesis and accelerated decay of platelets, resulting in the observed rapid decrease in steady state level of platelets. Reports from multiple groups indicate high-affinity non-covalent association of DENV-specific Immunoglobulins with platelets, which has been indicated to augment platelet decay through a mechanism, the details of which are not yet clear. On the other hand, a mechanism involving virus infection of bone marrow cells has been suggested to attenuate generation of platelets by megakaryocytes. We are exploring details of both mechanisms using a variety of in vitro approaches using primary cells as well as unique hematopoietic cell lines.

In final stages of megakaryopoiesis, a hematopoietic progenitor capable of producing either erythrocyte or megakaryocyte (termed Megakaryocyte-Erythrocyte progenitor or MEP), differentiates into a megakaryocyte through elaborate steps of differentiation. K562 is a human cell line derived from bone marrow, which behaves like an MEP and can be pharmacologically

induced to undergo changes that mimic in vivo megakaryopoiesis. We have standardized an in vitro model of megakaryopoiesis based on K562 cell line and developed the indices for qualitative and quantitative assessment of differentiation steps. In order to assess any impedance to megakaryopoiesis by virus infection, we are comparing megakaryopoiesis in cells of this line with or without infection. Preliminary studies indicate interference in one of the crucial steps in megakaryopoiesis, by DENV infection. Currently, our efforts are directed to understanding the molecular basis of this impedance. In the future we plan to conduct similar studies using a primary hematopoietic stem cell based model system of megakaryopoiesis.

Investigation of the mechanisms dictating poor replication efficiency of genotype-1 Hepatitis E virus

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HEV is one of the most common causes of acute and sporadic viral hepatitis. It is a positive strand RNA virus, transmitted through the feco-oral route. Even after 30 years of discovery of the virus, little information exists regarding viral life cycle and replication machinery. HEV is divided into seven genotypes. Genotype-3 and 4 viruses infect humans and a few animals (such as pigs, deer, mongeese) and have been reported from industrialized countries. Genotype-3 and 4 viruses have been successfully propagated in the laboratory in mammalian cell culture. However, genotype-1 virus, which is known to infect human and is a major public health concern in south Asian countries, replicates poorly in mammalian cell culture and no other efficient model exists to investigate its life cycle. We are attempting to identify a permissive cellular condition that would allow efficient viral replication in human hepatoma cells. Despite sharing similarity in genome organization and encoding similar proteins, genotype-3 hepatitis E virus (HEV) replicates more efficiently in cell culture than genotype-1 HEV. We are interested in identifying the possible mechanisms underlying the poor replication efficiency of genotype-1 HEV.

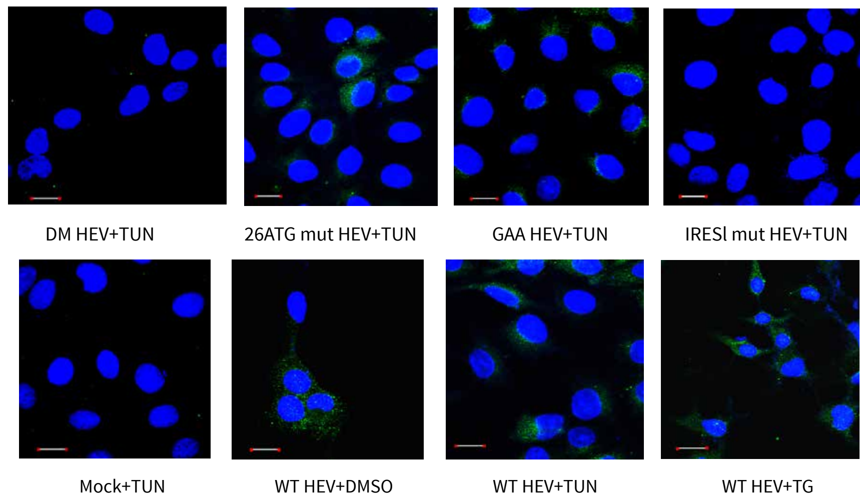

Figure-15: Tunicamycin and thapsigargin induce ORF4 expression. Immuno fluorescence of ORF4 in Huh-7 cells transfected with indicated in vitro synthesized RNA. Scale: 20μm. Shown are merged images of nuclei (blue) and ORF4 (green). "→": positive staining, "→" : unstained.

Our studies revealed that endoplasmic reticulum stress inducing agents promote genotype-1 HEV replication by initiating cap-independent, internal translation mediated synthesis of a novel viral factor, which we named ORF4 (Figure 15).

Further investigations revealed that ORF4 is expressed only in genotype-1 and it acts by interacting with multiple viral and host proteins and cooperates with host eEF1α1 (eukaryotic elongation factor 1 isoform 1) to enhance the activity of viral RNA dependent RNA polymerase. (Fig.16) Moreover, a proteasome resistant ORF4 mutant significantly enhanced viral replication. Thus, our study identifies an optimal condition required for efficient replication of genotype-1 HEV and dissects out the molecular mechanism governing that. These data will be instrumental in developing an efficient model of the virus. Ongoing studies aim at further characterizing the properties of ORF4.

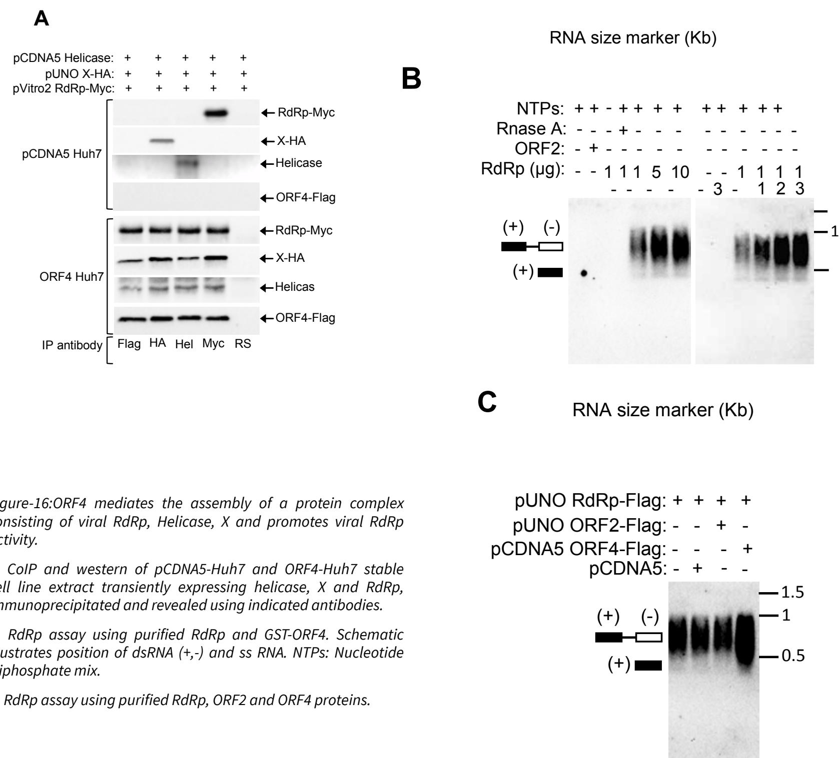

Figure-16:ORF4 mediates the assembly of a protein complex consisting of viral RdRp, Helicase, X and promotes viral RdRp activity.

A. CoIP and western of pCDNA5-Huh7 and ORF4-Huh7 stable cell line extract transiently expressing helicase, X and RdRp, immunoprecipitated and revealed using indicated antibodies.

B. RdRp assay using purified RdRp and GST-ORF4. Schematic illustrates position of dsRNA (+,-) and ss RNA. NTPs: Nucleotide triphosphate mix.

C. RdRp assay using purified RdRp, ORF2 and ORF4 proteins.

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Understanding the functions of uncharacterized proteins of Hepatitis E virus

ORF1 of HEV contains domains of unknown functions such as X domain, V domain and Y domain. Recent reports suggest some of these domains to be important for viral replication. However, no clear conclusion exists regarding that. We are interested in defining the function of these domains during the life cycle of HEV. This information will advance our understanding of the virus biology and may be useful in identifying suitable targets for developing therapeutic strategies.

Towards understanding the function of the HEV X domain, we characterized its interaction partners among other HEV encoded proteins. We observed that X-domain directly interacts with the viral methyltransferase and the ORF3 proteins. ORF3 association with the X-domain was mediated through two independent motifs, located within its N-terminal 35aa (amino acids) and C-terminal 63-123aa. Methyltransferase interaction domain was mapped to N-terminal 30-90aa. The X-domain interacted with both ORF3 and methyltransferase through its C-terminal region, involving 66th,67th isoleucine and 101st,102nd leucine, conserved across HEV genotypes. Furthermore, ORF3 and methyltransferase competed with each other for

associating with the X-domain. While working on the interaction between X domain and methyltransferase of HEV, we have also identified interaction between a human macro domain protein and a methyltransferase, which suggests that such interaction could have important functional significance not only in the life of HEV but also in other organisms. Functional significance of these associations is being investigated.

Expression and purification of recombinant Hepatitis E virus-like particles in Pichia pastoris

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Pichia pastoris has been successfully employed as a host to express recombinant proteins for therapeutic use. In contrast to the bacterial expression system, Pichia pastoris allows glycosylation of the expressed protein, which might be a superior option for some natively glycosylated proteins. Since HEV capsid protein is known to harbor functional glycosylation sites, we are interested in expressing Hepatitis E virus-like particles (VLPs) in Pichia pastoris, which might be useful as a better diagnostic and/or prophylactic agent.

We have expressed 112-608 aa region of the genotype-1 HEV ORF2 protein with a N-terminal α-factor secretory signal and C-terminal His-tag in the Pichia pastoris. The protein was purified by Ni-NTA chromatography, followed by PEG precipitation. Using different glycosidases, we have confirmed glycosylation status of this protein. Using density gradient centrifugation and electron microscopy, we have confirmed VLP nature of the protein. (Fig.17) It has also been successfully tested against a number of HEV patient serum in ELISA. We are employing a similar strategy to generate chimeric VLPs, which may be a better diagnostic reagent and/or provide better immune response against HEV.

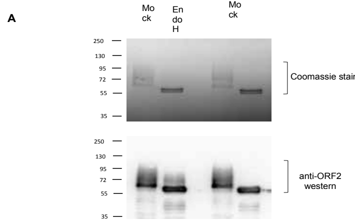

Figure-17: Glycosylation and VLP assembly by Pichia expressed ORF2 protein

Dr. Milan and his team

Interference of innate immunity response by hepatitis E virus

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Viral RNAs harbor molecular signals known as pathogen associated molecular patterns (PAMPs) that are detected by innate immunity receptors to activate a suite of cellular defense responses. Viruses interfere with the host responses either by blocking recognition of molecular patterns or by cleaving adaptor proteins. Understanding the mechanisms by which viruses evade the antiviral host response could lead to the identification of drug targets or in the development of attenuated vaccines. The objective of this study is to characterize the role of HEV proteins in interfering with innate immunity response.

Dr. Ranjit Kumar C.T.

The type I interferon signaling is integral to the innate immune system's ability to create an antiviral state. We investigated the role of HEV proteins in interfering with innate immune responses. We observed that innate immune receptor RIG-I was activated by HEV RNA. The role of HEV proteins in interfering with RIG-I signaling was investigated and we observed that the papain-like cysteine protease (PCP) blocked RIG-I mediated interferon production. We also found that the conserved residues of the protease catalytic site are not involved in the inhibition of RIG-I signaling. Instead, PCP physically interacted with one of the adaptor proteins required for activation of RIG-I response. The N-terminal region of PCP was found to be important for the interaction with the adaptor protein. Further characterization of the modulation of innate immune response by PCP is underway.

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Characterization of hepatitis E virus RNA-dependent RNA polymerase and its associated proteins in the replicase complex

The viral replicase complex, which is responsible for the replication of the viral RNA, is formed by the association of RdRp with other viral non-structural proteins and some of the host proteins. In spite of the critical role of RdRp in the viral life cycle, the replication of HEV is poorly understood. Understanding viral replication will be crucial for the development of more effective antivirals. The objective of this study is to gain comprehensive understanding of the HEV replication process through biochemical characterization of the RdRp and the replicase complex.

Using pull-down assays we have identified host proteins that interact with HEV RdRp. Further characterizations of these proteins to understand their role in HEV replication are in progress. Unlike RdRps from hepatitis C virus and poliovirus, there is very little information available on the mechanism of action of HEV RdRp. Therefore, we have purified HEV RdRp from bacterial and mammalian cells and are in the process of developing a non-radioactive assay. Understanding the viral replicase complex and detailed insights into the functioning of RdRp will provide important information on the replication of HEV and help in developing new therapeutic interventions.

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Screening of small molecule compounds to identify inhibitor(s) of hepatitis C virus (genotype 3a) RNA-dependent RNA polymerase.

Hepatitis C virus (HCV) is a serious health issue for the entire world in general and for India in particular. The newly developed direct acting antivirals (DAA) have shown significant improvement in HCV treatment. However, high drug prices are preventing treatment options for majority of the patients in low- and middle-income countries. Also, HCV genotype 3, the most prevalent form of HCV in India, continues to show inferior response to DAAs. The objective of the study is to develop antivirals against RNA-dependent RNA polymerase (RdRp) of HCV.

RdRp of HCV is the catalytic subunit of the replicase complex responsible for the replication of viral RNA and therefore an important drug target. We developed a cell-based assay for quantitating the activity of HCV genotype 3a

RdRp and used it to screen a small molecule compound library. We screened about 3500 small molecule compounds with the aim of identifying potential HCV inhibitors. We found that 6 compounds specifically inhibited HCV genotype 3a RdRp. Further, we obtained HCV genotype 3a replicon with a fused firefly reporter from Dr. Charlie Rice, USA and tested the 6 identified inhibitors of RdRp on the HCV replicon. We observed that one compound showed concentration dependent inhibition of HCV replication without any cytotoxic effects. Quantitative real-time PCR analyses further confirmed inhibition of viral RNA replication (Fig.18A). Furthermore, structure-activity analysis of the compound was performed using commercially available compounds that harbored moieties similar to our lead compound. Interestingly none of the derivatives of the lead compound could replicate the inhibition observed suggesting that the entire molecule is necessary for antiviral activity. There are 6 major genotypes of HCV and hence it is important to develop an antiviral that could inhibit multiple genotypes. Therefore, we developed cell-based assays for RdRps from all six major genotypes of HCV. The lead compound showed inhibition of RdRps from all the six genotypes thus exhibiting a pangenotypic anti-HCV activity. Biochemical analyses with recombinant HCV RdRp suggested that the compound might not be a direct-acting inhibitor of HCV RdRp (Fig. 18B). This implies that the compound may have to be metabolized inside the cells to convert it into its active form. It is also possible that the compound is interacting with a host factor necessary for viral replication. Importantly, this compound also inhibited both HEV and dengue virus replication (Fig. 18A)suggesting that it could be a broad-spectrum antiviral.

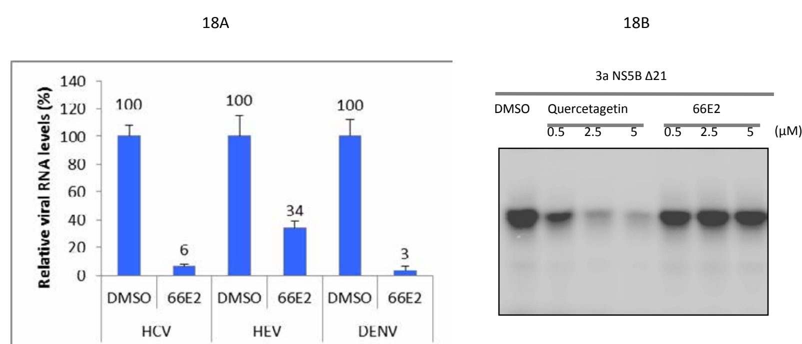
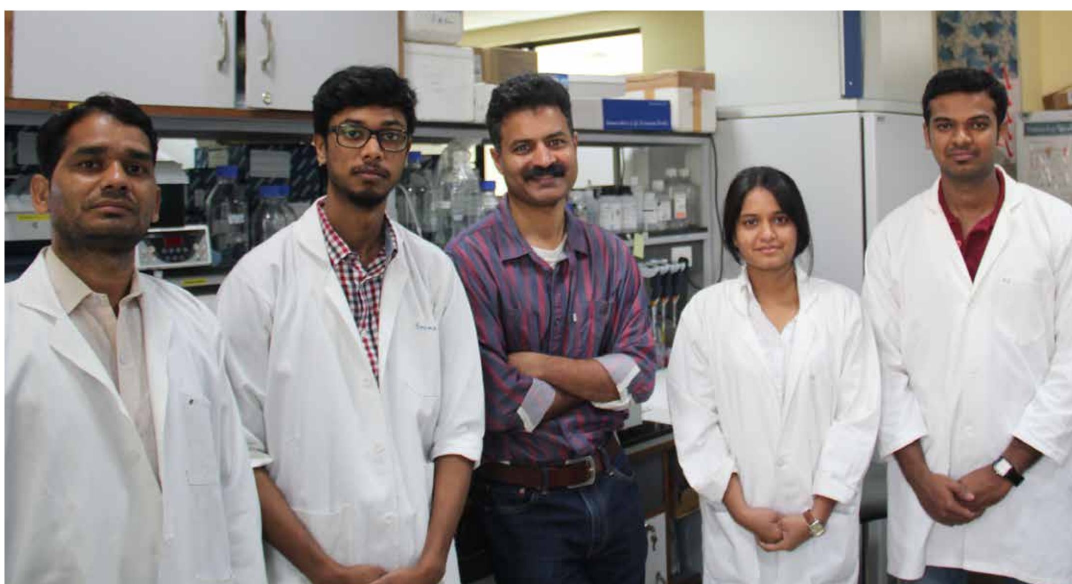


Figure-18A. Quantitative real-time PCR analyses to determine viral RNA levels after 48 h of treatment with DMSO or our lead compound 66E2.

18B. RNA synthesis assay with purified HCV genotype 3a RdRp (3a NS5BΔ21). Concentrations of 66E2 and Quercetagetin (a known inhibitor of HCV RdRp) used are given on the top.



Dr. Ranjith and his team

Biology of Mycobacterium tuberculosis

India has the highest burden of tuberculosis (TB). The World Health Organization (WHO) statistics for 2013 gives an estimated TB incidence of 2.1 million cases for India out of a global incidence of 9 million. It is estimated that about 40% of the Indian population is infected with TB bacteria, the vast majority of whom have latent rather than active TB. We are studying the biology of Mycobacterium tuberculosis (Mtb), the TB causative agent with a view to identify novel genes/proteins/pathways that could be potential drug targets or vaccine candidates.

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Understanding the role of polyphosphate kinases and polyphosphatases in physiology of Mycobacterium tuberculosis

Inorganic polyphosphate (PolyP), a linear polymer of inorganic phosphate linked by phosphoanhydride bond is upregulated in various bacterial pathogens upon exposure to various stress conditions. PolyP is ubiquitously present in all domains of life (archaea, bacteria and eukarya) and plays an important role in various cellular physiological functions. In bacterial pathogens, polyphosphate kinase -1 (PPK-1) catalyzes the reversible transfer of the terminal phosphate group of ATP to form long chain polyphosphates and the exopolyphosphatase (PPX) enzyme cleaves the phosphoanhydride bonds of PolyP to generate inorganic phosphate. Here we aimed to validate these PolyP metabolic enzymes as in vivo drug targets for Mtb.

Mtb genome harbors enzymes involved in both PolyP synthesis (PPK-1, Rv2984) and its utilization (PPK-2, Rv3232c and PPX, Rv0496 and Rv1026). We have developed a luciferase based assay system to quantify in vitro PolyP levels in mycobacteria. We observed that mycobacteria accumulates PolyP at later stage of growth, upon exposure to stress conditions such as oxidative, nitrosative, nutritional, low oxygen and drugs such as rifampicin (Rif), levofloxacin (Levo), Isoniazid (Inh) and Gentamycin (Gm). This accumulation of PolyP in Mtb is dependent on intracellular levels of guanosine pentaphosphate. Using temperature sensitive mycobacteriophages we have constructed both ppk-1 and ppk-2 mutant strains of Mtb. Using these bacterial strains, we show that PolyP deficiency is associated with increased susceptibility of Mtb to front-line TB drugs. We have also biochemically characterized PPK-1 and PPK-2 enzymes from Mtb and show that both these enzymes can utilize PolyP and ADP in a dose dependent manner to synthesize ATP. We have also demonstrated that any dysregulation of PolyP levels impairs the ability of Mtb to cause disease in guinea pigs. The animals infected with these mutant strains have significantly less bacterial loads and gross pathology in comparison parental strain infected guinea pigs.

We have also biochemically charaterized Rv0496 and Rv1026 as exopolyphosphatases using Malachite Green reagent. We show that both these enzymes can utilize both short chain and long chain polyphosphates as substrates. The polyphosphatase double mutant had a survival defect upon exposure to various in vitro stress conditions. The ppx mutant of Mtb was also observed to be highly attenuated for growth in both lungs and spleens of guinea pigs. In concordance significantly less tissue damage was observed in lung sections from ppx infected mutant strains. These findings suggest that PolyP accumulation occurs in Mtb upon exposure to various stresses and PolyP

levels dysregulation impairs the growth of Mtb in guinea pigs. Experiments are in progress to identify the pathways that are regulated by Mtb PolyP levels. We have also adapated this luciferase based assay system to 96-well format for small molecule screening. The most active non-cytotoxic compound would eventually be evaluated for its ability to inhibit growth of Mtb using the mouse model of tuberculosis. We have also initiated collaboration to determine X-Ray crystal structure for PPK-1 enzyme.

Integrative approaches to understand function, stability and structure of bacterial toxin-antitoxin systems

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Isogenic populations of antibiotic-sensitive bacteria can have rare cells that transiently become drug tolerant 'persisters' but an understanding of the emergence of persistence remains elusive. Transcriptional profiling of isolated persisters has suggested a possible role of the toxin-antitoxin (TA) systems in persistence. In bacteria, TA systems are found in both plasmids and chromosomes, but in many cases, their functional role is unclear, especially for genomic TA systems. TA systems are found on both chromosomes and plasmids of virtually all bacteria and can represent as much as 2.6% of total coding sequence. TA systems belong to 5 different types and often show the phenomenon of conditional cooperativity wherein toxin and antitoxin form complexes of different stoichiometric ratios, to repress transcription from its cognate promoter. In most cases their role is unclear. We are therefore aiming to (i) elucidate structure for some of these TA systems, (ii) study regulation among these TA systems, and (iii) understand role of these TA systems in drug tolerance and virulence.

In Mtb, TA systems are primarily of Type II and belong to either VapBC, MazEF, RelBE, ParDE , or HigBA families with the first two having the most members. RelE, MazF, YafQ, HigB, HicA and MqsR toxins cleave mRNA in either ribosome independent or dependent manner. CcdB and ParE toxins inhibit DNA replication by regulating DNA gyrase activity. The VapC family of toxins from enteric bacteria also inhibit protein translation by cleaving initiator tRNA. Depending on the dosage and inducible system, overexpression of these toxins leads to either bactericidal or bacteriostatic effects. We had earlier biochemically characterized MazF toxins from Mtb. We showed that these toxins are differentially regulated and Mtb devoid of three functional MazF toxins is imapired for growth in guinea pigs. Subsequently, we have also characterized VapC toxins of Mtb using anhydrotetracycline inducible based systems. We observed that despite the presence of PIN domain only few of these VapC toxins are functionally active. Using temperature sensitive mycobacteriophages we have also generated mutant strains for few of these active VapC toxins. Our preiliminary study suggests that VapBC TA systems individually contribute to stress adaptation and virulence of Mtb. RNA-seq experiments using these overexpression strains suggest that these TA systems are also regulated via cross-talk. These findings suggest that TA systems are differentially regulated under different stress conditions and upregulation of these toxins subsequently leads to upregulation of "stress responsive genes" that enables the bacteria to adapt under different conditions and in guinea pigs.

The combination of genetic tools and deep sequencing methodologies has led to identification of essential and conditionally required genes for many prokaryotes. Future experiments include utilizing these sequencing tools to delineate the role of these TA systems in Mtb physiology, persistence and

virulence. We are currently in the process to generate a library of strains overexpressing individual toxins under Atc inducible integrative constructs. The pooled library would be subsequently exposed to stress conditions such as nutritional, nitrosative, oxidative or low oxygen conditions for two different time points. Since TA systems have been described as terminal effectors of bacterial persistence, we would also determine the contribution of these toxins to Mtb drug tolerance. To investigate this, the induced library strains would be exposed to drugs with different mechanism of action (rifampicin, isoniazid, levofloxacin and gentamycin) at 1x and 10x at two different time points. We would also use these pooled libraries to understand the contribution of these toxins in intracellular survival using macrophages and guinea pigs. To understand the role of toxins in Mtb physiology and intracellular survival, at designated time points genomic DNA of pooled library would be isolated and subjected to plasmid junction sequencing as per standard protocols.

Identification of novel scaffolds and drug targets to combat tuberculosis

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The situation of TB has worsened due to BCG failure to combat adult TB and emergence of various drug resistant strains of Mtb. Therefore there is an urgent need to identify new targets and chemical scaffolds that (i) inhibit growth of drug-resistant bacteria, (ii) target drug-tolerant and latent bacteria, (iii) are compatible with current TB and anti-retroviral treatments, and (iv) have the potential to reduce the duration of current TB therapy.

We have developed a 96-well assay system to identify scaffolds that inhibit the growth of Mtb. Using this methodology we have screened various diamine derivatives, isoniazid-amidoether derivatives and Bile acid amphiphiles for anti-tubercular activities. These analogs were non-cytotoxic and showed anti-tubercular activity around 5-10 mM. These compounds were also tested in animal models and were comparable in their activity to that of isoniazid in murine model of tuberculosis. As a follow up of these studies second generation derivatives for these scaffolds have been prepared and activity assays are in progress. We are also evaluating activity of various natural products and thiozolidones derivatives against susceptible and resistant bacteria.

Additionally, we have screened nearly 10,000 compounds in our whole cell based assays. Based on these screening efforts, we have identified scaffolds that are more potent against slow growing mycobacteria in comparison to fast growing mycobacteria and E. coli. These compounds are also active against drug resistant bacteria, which suggests that these scaffolds have a different mechanism of action and target a metabolic pathway that is specific for slow growing mycobacteria. The exact MIC99 values for some of these compounds is <1 mM, with the most active compound has a MIC99 value of 300 nM. Most of the compounds (~30) were observed to be non-cytotoxic in THP-1 cells even at 100 mM concentration (highest concentration tested in the study). Based on these readings, therapeutic index (MIC99/TC50) was calculated for all these 40 compounds and compounds with Ti value >25 were evaluated for their ability to inhibit intracellular mycobacterial growth using BCG infected macrophages. We have performed mechanistic studies for few of these molecules. We show that 2 of these molecules are activated in F420 dependent manner but the nitroreductase required for their activation still needs to be identified. We have designed various analogs which have improved the activity of the parent compound by 8.0 fold. Future experiments include animal experiments of the parent compound and its derivatives either alone or in combination

with current TB regimen. We are also planning experiments to understand mechanism for the remaining active scaffolds and also perform screening for new libraries of compounds.

Analysis of Mtb genome sequence together with a series of transposon mutagenesis studies and whole genome sequencing approach has led to identification of various metabolic pathways, which are essential for Mtb growth in vitro and therefore are potential targets for anti-tubercular therapy. Numerous studies have validated enzymes involved in cell wall biosynthesis, DNA replication, central carbon metabolism, amino acid biosynthesis, stringent response pathways, two component systems, sigma factors and co-factor biosynthesis as drug targets. Since mammals are unable to synthesize most of these amino acids we are trying to validate various amino acid biosynthetic enzymes as drug targets. Using CRISPRi approach we have established essentiality for few of these enzymes for Mtb growth in vitro. Previously, we have validated Phosphoserine phosphatase (PSP), a key essential metabolic enzyme involved in conversion of O-phospho-L-serine to L-serine as a drug target. Using HTS approach we had identified chlorobiocin and rosaniline as inhibitors specific for Mtb PSP enzyme. We are screening more libraries against Phosphoserine Phosphatase. Additionally, we have also biochemically characterized enzymes involved in L-Threonine and L-Aspartate biosynthesis. Experiments are in progress to screen small molecule libraries against these targets. The identified sacffolds would then be evaluated for their ability to inhibit Mtb growth in macrophages and mice.

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CRISPRi-based genetic screen to identify therapeutic targets of TB-causing human pathogen Mycobacterium tuberculosis

Our fight against tuberculosis stands on the shoulders of about a century old BCG vaccine and a half century-old regimen of drugs that have largely failed to control the spread of disease. Additionally, emergence of multi-, extremely-, and total- drug resistant (MDR, XDR & TDR, respectively) strains of Mtb urgently demands new targets for development of better and stronger line of preventive and treatment measures. This study involves rapid screening of Mtb genes by CRISPRi approach to identify those required for maintaining in vitro growth that can be later explored as potential therapeutic targets of Mtb.

Mtb genome comprises of ~4000 open reading frames, of which ~25% are uncharacterized and annotated as 'hypothetical' or 'conserved hypothetical'. Importantly, ~5% of the uncharacterized hypothetical genes are predicted essential for mycobacterial growth in the synthetic culture media by transposon site hybridization analysis. Genetic incompetence and lack of an efficient tool for manipulating mycobacterial genome have severely impeded the functional characterization of these unknown genes. To address these limitations, we have recently developed an approach based on CRISPR interference (CRISPRi) for feasibly disrupting the expression of ORFs in Mtb. Using CRISPRi approach the lab is involved in characterizing the functions of many essential genes of Mtb such as those regulating DNA replication, cellular proteostasis and protein translocation. The common work-flow involves disruption of predicted 'essential gene' followed by analysis of its effect on bacterial growth in synthetic culture medium and in macrophages. Based on growth analysis we proceed with those targets that are essential for maintaining optimal growth. Subsequently, we perform deeper analysis of each individual target gene to better understand their function by involving whole-genome transcriptomic and proteomic studies. A thorough analysis of each of these target genes will not only help firmly establishing them as potential drug/vaccine target but will also be critical for developing an in vitro assay to screen small molecule inhibitors. Moreover, characterization of some of these genes will unfold new metabolic pathways that mycobacteria employ for long-term survival under adverse conditions in the host which will enable us designing drugs against latent population of Mtb that has engrossed one-third of world's population. With the successful implementation of CRISPRi approach, ours has become the first laboratory in India which has thus far created >70 knockdown strains of Mtb. At present we are at early stages of characterizing the functions of some of the essential genes by using their respective knockdown strains.

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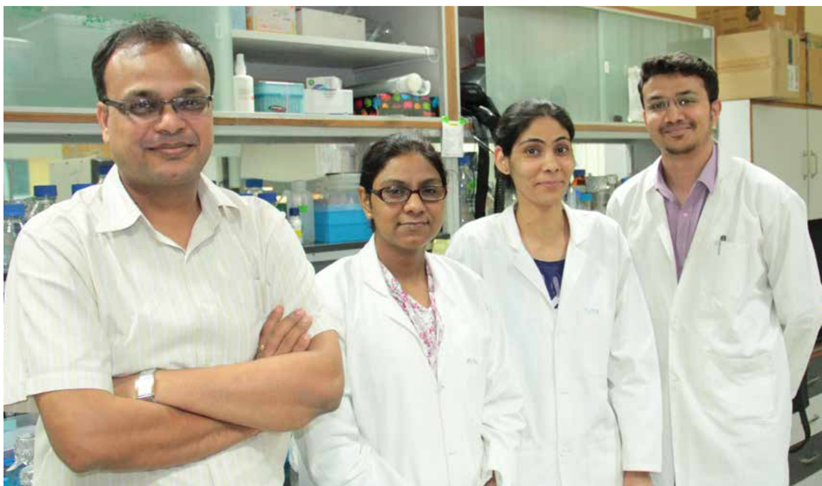
To characterize the role of putative preprotein translocase in Mycobacterium tuberculosis

Mtb maintains its virulence by the help of several proteins embedded in both the cell membrane and outer wall. Cell surface proteins regulate important biological processes such as transport of materials, interaction with host cells and subsequent elicitation of host immune response upon infection etc. In this project we aim at (1) studying the mechanism of protein translocation in Mtb by an essential protein Rv3921c, which exhibits poor homology with a preprotein translocase YidC, (2) establishing its role in intracellular survival of mycobacteria, and (3) developing an assay system to screen inhibitor library against Rv3921c.

So far we observed that Rv3921c is a constitutively expressed protein, which is localized to cell envelope of mycobacteria. Importantly, overexpression of this protein in Mycobacterium smegmatis results in depletion of at least two proteins in the cell membrane, ribonucleotide reductase and a peptidase of M48 superfamily. By conditional depletion of Rv3921c we showed that it is essential for in vitro growth of Mtb. In order to understand the physiological consequences of Rv3921c depletion, we analyzed whole genome transcription profiles of the control and the Rv3921c-depleted strains of Mtb by microarray. Our results indicated that suppression of Rv3921c significantly alters the expression of >350 genes that are majorly involved in intermediary metabolism and respiration (24%), cell wall and cell processes (16%) and lipid metabolism (13%). Overall the microarray results specify a plausible role of Rv3921c in regulation of respiration in mycobacteria. Next, we investigated the effect of Rv3921c depletion on global expression of proteins. Quantitative proteomics by isobaric tag for relative and absolute quantitation (iTRAQ) was performed with the whole cell extracts (WCEs) of Rv3921c-depleted and control strains of Mtb $H_{37}Rv$ after 4 days of depletion. It was observed that Rv3921c depletion modulates expression of ~170 proteins by >1.3-fold, majority of which belong to intermediary metabolism and respiration category. Many ATP synthases (AtpA, AtpD, AtpF, AtpG and AtpH) and electron transport proteins (QcrA, Rv0247c, Rv0248c, Rv0688 and LldD2) are accumulated, while a number of ribosomal proteins (RplD, RplF, RplI, RplJ, RplL, RplM, RplO, RplQ, RplT, RplU, RplX, RpmA, RpmC, RpmF, RpmG2, RpsA, RpsB, RpsD, RpsG, RpsI, RpsJ, RpsM, RpsN1, RpsP, RpsQ, RpsR1, RpsS), RNA polymerase subunits (RpoC and RpoZ), transcription regulators (SigA, Rho, Crp, DosR, EspR, GreA, MtrA, Rv0144 and Rv3295) and stress response regulators (AhpC, GroEL1, GroEL2, GroES, Rv2581c and VapB47) are downregulated in Rv3921c-depleted cells relative to control. In line with these observations, we also report that Rv3921c-depleted cells exhibit reduced ATP levels, altered NADH/NAD+ ratio towards a reducing state and reduced membrane potential. Subsequent experiments established that Rv3921c interacts with few respiratory proteins such as Rv0247c, Rv0248c and QcrA which are dependent on Rv3921c for their translocation. Finally we report that Rv3921c is required for bacterial growth not only in culture medium but also in macrophages.



Dr. Nisheeth and his team

Overall, our results concluded that Rv3921c behaves like YidC preprotein translocase which controls the respiratory metabolism in mycobacterium. These results also propose YidC as a drug target due to its essential requirement for in vitro and intracellular growth of Mtb and its absence in humans. Currently, we are in process of identifying more envelope proteins that are exclusively dependent on Rv3921c for their translocation. Once these proteins are identified, we will use some of them in designing an assay system for screening inhibitor library.

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A systems approach to analyze changes in global phosphorylation status of proteins in macrophages infected with Mycobacterium tuberculosis complex bacteria and their repercussions on mycobacterial virulence

Upon infection of macrophages both pathogenic as well as non-pathogenic mycobacteria exert multiple physiological changes primarily by causing alterations in expression and posttranslational modifications (PTMs) of various host proteins. Phosphorylation is one such PTM event, which universally happens in host cells under different extracellular stimuli including infections. Here we aim at capturing maximum number of host proteins that undergo differential phosphorylation after infection with virulent and avirulent strains of mycobacteria by LC-MS/MS. The results will not only unravel novel host pathways controlling mycobacterial infection, but can also be implemented in designing new biomarkers for tuberculosis.

In order to understand the phosphorylation pattern of host proteins upon infection, we began with infecting PMA-treated THP-1 cells with avirulent M. bovis BCG using 1:10 MOI for different time points. Immunoblotting of lysates with anti-phospho antibodies depicted a distinct phosphorylation pattern in the infected cells at day 3 onwards, post-infection. Subsequently, infection at different MOIs for 3 days demonstrated that 1:10 MOI is the best infection dose to obtain the consistent data. Once we optimized conditions with BCG, we proceeded with virulent strain, Mtb $H_{37}Rv$ by following the same procedures as described above. Our results demonstrated that similar to BCG, Mtb can also impact the host phosphorylation status at day 3 onwards, when infected with 1:10 MOI. Subsequent dose dependent analysis revealed that unlike BCG, Mtb can cause changes in host phosphoproteome profile at much less MOI, which is 1:2. Interestingly, at higher MOI such as 1:10, there was significant cell lysis and complete dephosphorylation of host proteins. Based on our initial data, we decided to continue with 1:10 MOI of BCG and 1:2 MOI of Mtb for 3 days of infection to analyze changes in host phosphorylation status. Our results from the work so far clearly indicate that THP-1 macrophages infected with BCG or Mtb $H_{37}Rv$ for 3 days exhibit massive dephosphorylation of proteins. We now plan to conduct the infection experiments using these conditions followed by enrichment of host phosphoproteins and analysis by mass spectrometry. Simultaneously we will also attempt standardizing conditions for suppression of host proteins by siRNA- or CRISPR-Cas9 approach.

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Deciphering Mycobacterium tuberculosis artillery

Despite BCG availability, annually, 10 million fresh TB cases emerge. To fulfill WHO TB strategy 2035, experts predict an alternate superior vaccine to existing BCG a must. In recent past, several recombinant BCG versions developed have failed in trials. This has urged researchers to rethink the whole TB vaccine design. Experts suggest “for vaccination, perhaps we MUST select the antigen repertoire, rather than relying on those the pathogen LIKES US TO SEE”. Therefore, there is urgent need to identify the “right vaccine antigens by discovering pathogen’s arsenal. Hence, the objectives here are to (1) identify Mtb’s protein stockpile that gains access into macrophages, and (2) determine the corresponding host cellular targets to the identified protein arsenal.

Dr. Krishnamohan Atmakuri

To identify Mtb's protein effectors that access macrophages, we designed a novel genetic approach that exploits Cre-recombinase from bacteriophage P1 as a reporter. Exploiting Gateway technology, we first tagged each ORF with NLS-Cre. Then we moved them into mCherry-expressing virulent Mtb to infect recombinant macrophages carrying loxP-nptII-loxP-gfp genetic element under a ubiquitous promoter. When such a Cre-fused Mtb protein accesses host environment, the NLS promotes its entry into the nucleus. The hitch-hiking Cre then promotes recombination of loxP sites to bring promoterless gfp (the reporter for this screen) in close proximity to the ubiquitous promoter for continued expression. Macrophages that receive an Mtb-fused protein thus turn green. (Fig 18)

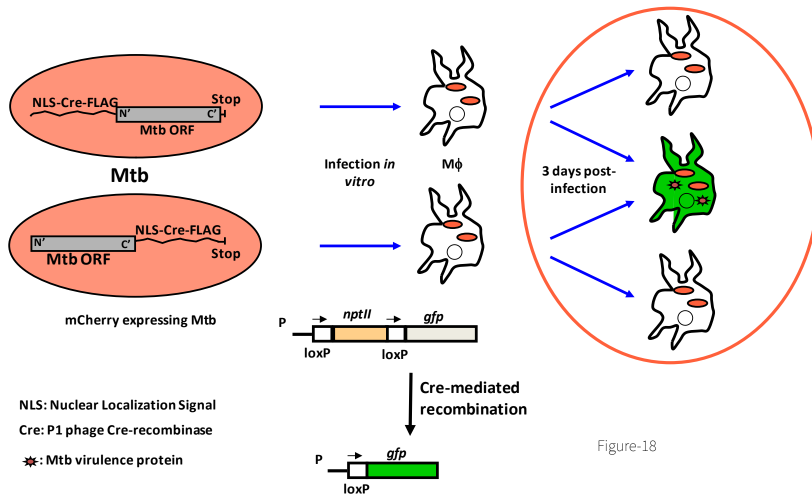

Figure-18

We had earlier procured the Mtb ORFeome entry clone library from BEI Resources, USA. We had earlier designed and constructed a complex Gateway destination vector that help fuse Mtb ORFs in frame at their N-termini with NLS-Cre. We have thus far moved around 1200 Mtb genes into the N-NLS-Cre vector and transformed approx. 600 constructs into Mtb. Once the appropriate transgenic mice are ready, their bone marrow will be extracted monocytes differentiated and then set for infection with modified Mtb library.

Functional characterization of mycobacterial aminopeptidase

PRINCIPAL INVESTIGATOR
Krishnamohan Atmakuri

INVESTIGATORS
Nishant Sharma
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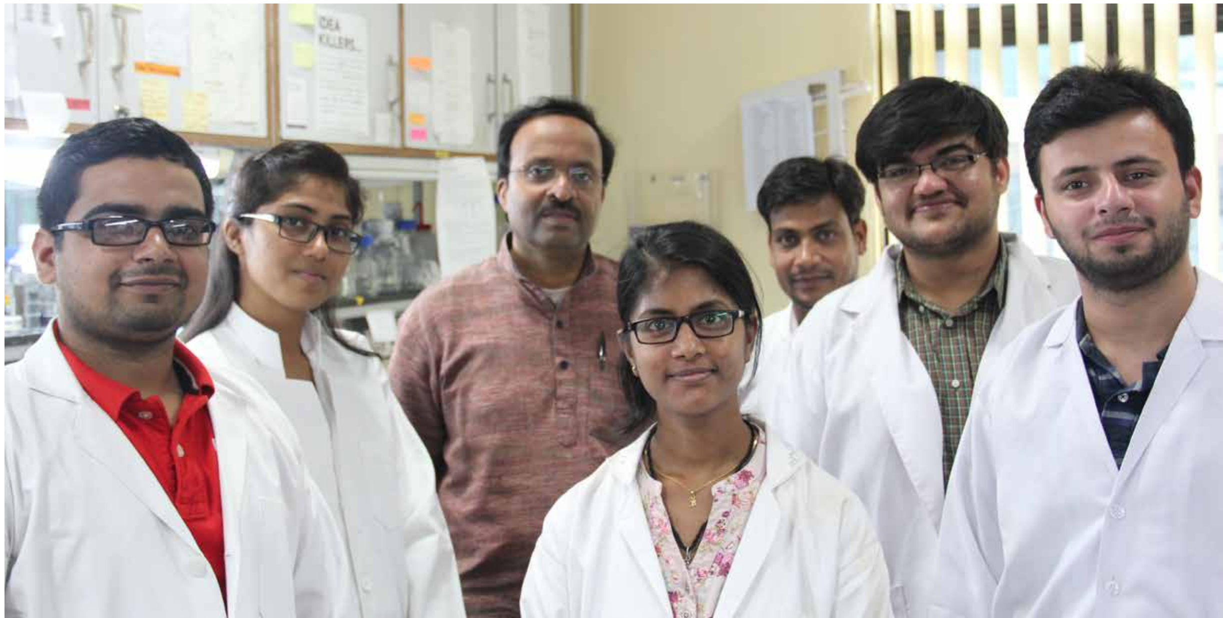
COLLABORATOR
Anmol Chandele
(Emory University, USA)

From a candidate screen described above we identified one Mtb aminopeptidase (AP) accessing macrophages. We and others detect this AP in the spent media of Mtb in vitro from cultures. Interestingly, of the Mtb APs, this alone has a N-terminal peptidase and a C-terminal human AP-like domains. We set out to understand the functional role of this protein in both Msmeg and Mtb.

Interestingly, bioinformatics analyses indicate that, while this protein's N-terminal peptidase domain is highly conserved across pathogenic and non-pathogenic mycobacteria, its C-terminal domain is well conserved across pathogenic mycobacteria alone. Intriguingly, the C-terminal domain resembles two well conserved host aminopeptidases. As expected, our homology-based structural prediction for Mtb's aminopeptidase again identified the same host aminopeptidases. Superimposition of either host

APs crystal structures (from PDB) to the predicted structure obtained for pathogen's AP indicated RMSD values less than 2.9 angstrom indicating more than significant structural homology between host and pathogen APs. Its secretion into CF (reported by other groups and also identified by us); its delivery into macrophages (our study); and its role in vivo but not in vitro (reported by others through transposon mutagenesis studies) in Mtb, all indicate towards a possible role in pathogenesis for the C-terminal domain.

To decipher its role and to characterize its host targets/substrates, we designed and constructed, Tet-inducible, native and N-terminally 3X FLAG-tagged - full length, - truncated N- and C-terminal domains, and - active site mutants of both Msmeg (non-pathogenic) and Mtb (pathogenic) APs. We also generated a marker-free full length Knockout (KO) for this AP in Mtb. Interestingly, though we could easily generate a KO in Mtb, it has thus far been impossible to generate the KO in Msmeg, indicating its possible role in vitro. We also cloned and expressed the Mtb's full length protein in E. coli, gel purified it, and generated polyclonal antibodies specific to this protein. Because of high homology of the N-terminal peptide domains of Mtb and Msmeg, the generated polyclonal recognizes Msmeg homolog. We are currently in the process of generating KO of the homologous AP in both Msmeg and attenuated Mtb strain, H37Ra. Lastly, while we could easily overexpress and detect accumulation of virulent (Mtb) and non-pathogenic (Msmeg) AP versions in both pathogenic (H37Rv) and attenuated (H37Ra) wild-type strains, we failed to over express similar versions in Msmeg. We noticed that any overexpression of AP in Msmeg was inherently strictly regulated by chopping off excess from its C-terminal end by a peptidase (possibly carboxypeptidase). This again indicates that AP from Msmeg and Mtb might play different roles. We are currently in the process of identifying the cognate substrates of APs in the pathogen and host. We are also in the middle of localization experiments to find clues to their possible function.



Dr. Krish and his team

PRINCIPAL INVESTIGATOR
Amit Kumar Pandey

INVESTIGATORS
Sakshi Talwar
Manitosh Pandey
Indu Bisht
Pooja Shrawat

Dr. Amit Kumar Pandey

Carbon metabolism in Mycobacterium tuberculosis and its implications on mycobacterial pathogenesis

"Tubercle or Granuloma" the hallmark of tuberculosis defines the truce in the war between the host and the causative pathogen Mtb. The core of the structure is packed with infected phagocytic cells. These infected cells release chemokine that actively recruits mononuclear cells from adjacent blood vessels forming the secondary layer. This layer also has large numbers of lipid-laden macrophages appropriately called as "foamy macrophages". Finally all these layers are delineated for the rest by a boundary of fibrous connective tissue layer. The lipid rich core of the structure harboring Mtb is shown to be very hypoxic and ideally favors the growth of slow growing non-replicating persisters. Mtb is known to survive for decades under this extreme environment. How Mtb is able to sustain under this very nutrient limiting condition is indeed very intriguing. Although, Mtb is thought to survive on lipids inside the host macrophages, the exact intra-cellular diet of Mtb is not very clear. Various research findings suggest sugars along with lipids, derived from the host could be a major source of carbon for Mtb during the intracellular logarithmic growth phase. After the onset of the host mediated adaptive immunity, Mtb infection moves into the persistent stage. Various studies have demonstrated that cholesterol is required for the maintenance of the persistent stage of Mtb infection. We hypothesize that this carbon switch is very critical for Mtb to slow down its replication and metabolic rate thereby activating a more latent form of infection. A better understanding of the type of intracellular carbon source available and the carbon source specific genetic signature would widen knowledge of the disease process. This would extend the range of potential genes that can be targeted for better therapeutics. The proposal involves generation and characterization of Mtb strains lacking genes critical for cholesterol utilization. Information from the project on regulatory genes and the motifs of the related regulatory proteins would be very helpful in unraveling the complex regulatory network. The ultimate goal will be to generate an interactome map of the regulatory pathways of cholesterol utilization in Mtb.

We did a preliminary study on genes that are differentially regulated under cholesterol specific media. In this microarray-based study Mtb was grown in media containing cholesterol and glycerol as the sole carbon source. A preliminary analysis of the transcription signature thus obtained indicated a re-wiring that explains the reduced growth rate specific to cholesterol sensing and metabolism. Interestingly, a majority of the differentially regulated Mtb genes were associated with cell growth and metabolism. These observations very strongly associate cholesterol metabolism to Mtb replication and growth.

Based on our transcriptome data and a previous study that identified genes essential for cholesterol utilization, we identified a set of 40 genes that could possibly be critical for carbon specific regulation of Mtb physiology and metabolism.We have so far generated clean deletion knockout strains specific to 15 of the above genes. Molecular and functional characterization of each of these genes is in progress. Majority of the genes included in this study belong to genes that either directly or indirectly regulate the Mtb transcriptome under specific growth and stress condition. The list includes genes from the toxin-antitoxin system, transcription factors, two-component systems, adenylate cyclases and gene of unknown function. We intend to extend this study and incorporate other physiologically relevant carbon source available to Mtb inside the host viz., fatty acids, amino acids, low and high-density lipoproteins.

We have also initiated studies analysing differential protein profiling of Mtb grown under cholesterol vs glycerol media. We hypothesize that a detail proteome analysis would unravel pathways and network critical for generation of slow growing persisters that help Mtb to hide against a very robust host surveillance mechanism.

Integrative genomics of host-pathogen interaction to identify new drug targets against persistent Mycobacterium tuberculosis

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INVESTIGATORS
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Amit Singhal
(ASTAR, Singapore)

Due to the emergence of multi drug resistance (MDR) and extremely drug resistance (XDR) strains of Mtb, and a sudden increase in the incidence of human immune deficiency virus (HIV) and diabetes in the population, TB, a seemingly treatable disease is turning out to be a major public health hazard. One of the most challenging aspects of TB treatment is the presence of a slow growing, non-replicating, metabolically inactive "persister" population of bacilli inside host cells that requires extremely long treatment regimen. Clinical and experimental evidences shows that the capacity of Mtb to enter a dormant state leading to latent infection is the key to the survival of Mtb in its host and to induce chronic infection, thus delaying the efficacy of current therapy. Inhibiting dormancy or altering the metabolic state of dormant Mtb could increase the effectiveness of antibiotics and shorten treatment duration.

We hypothesize that the differentially regulated critical metabolic pathways triggered by the intracellular nutrient availability and requirements contribute significantly towards the generation of Mtb persisters. We have 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. This indicates that Mtb actively modulates the host biosynthetic machinery for the generation of nutrients required for its own survival. Utilizing genetic and high dimensional informatic approach we will identify differentially regulated metabolic pathways both in Mtb and its host, which could lead to better understanding of host-pathogen symbiosis and thus Mtb pathogenesis, and designing of novel intervention strategies targeting persisters.

Cholesterol utilization pathway genes as therapeutics target

PRINCIPAL INVESTIGATOR
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Current TB treatment regimen involves multiple drugs for a prolong period. The duration could be from three months to two years depending on the type of infection. Prolong treatment leads to non-compliance and emergence of newer drug resistance strains. Shortening the therapy would go a long way in alleviating this problem. It is widely perceived that the major culprits are the so-called non-replicating and metabolically inactive "persister" population. The importance of cholesterol metabolism during the persistence stage of Mtb infection and its potential role in generation of persisters is very intriguing. In light of the above facts and hypothesis the focus of the current proposal is to screen for chemical inhibitors that specifically target these pathways. The long-range goal would be identify novel anti-TB drugs that specifically targets "persisters". These novel compounds in combination with the standard frontline anti-tubercular drugs would significantly enhance the success

rate in tuberculosis therapy. We are in the process of screening a library of almost 2000 compounds for scaffolds that specifically inhibit growth of Mtb in media containing cholesterol as a sole carbon source. We have successfully standardized an resazurin based whole cell growth inhibition protocol in a 96-well plate format. We have identified several compounds that demonstrated carbon source specific growth inhibitory activity. The compounds demonstrating a very low MIC and cellular toxicity would further be tested for antimycobacterial activity in an in vitro cell culture based model. Our lab is also exploring the potential of targeting cholesterol catabolic pathway genes as novel therapeutic target against Mtb. We have generated several Mtb strains deficient in genes critical for cholesterol catabolism and are in the process of characterizing these strains. The long term goal is to identify critical cholesterol catabolic pathway genes as novel target for developing a live-attenuated vaccine against tuberculosis.

PRINCIPAL INVESTIGATOR
Amit Kumar Pandey

INVESTIGATOR
Manitosh Pandey

Genetic essentiality study of Mycobacterium tuberculosis under various growth and stress conditions

Advancement in new cost-effective high throughput sequencing techniques has led to the identification of complete genome of various pathogens. The volume of the data generated, failed in its objective of further understanding of microbial pathogenicity. Genetic essentiality study of a pathogen is one such technique where in a gene is functionally characterized and associated with a phenotype. We are standardizing protocol to study genetic essentiality of Mtb under various growth and stress condition.

To achieve the goal, the use of mariner based mycobacteriophage system for generating high-density transposon mutant library is planned. The library will passage through different growth and stress conditions and the genetic essentiality would be determined by comparing input and output libraries. Since it is demonstrated that cholesterol is required only during the late stages of Mtb infection, the hypothesis, that a genetic essentiality screen for the gene required for bacterial growth in cholesterol would be more relevant physiologically, if done under hypoxic conditions, is proposed. A better understanding on cholesterol metabolism at the molecular level under physiologically relevant conditions would definitely help in designing of effective therapeutic solutions for TB. We have successfully generated mariner based high density transposon mutant libraries using both Mtb H37Rv and M. bovis BCG strains.

Dr. Amit and his team

Peer-reviewed Publications

1. Kumari B, Jain P, Das S, Ghosal S, Hazra B, Trivedi AC, Basu A, Chakrabarti J, WW S, Banerjee A (2016) Dynamic changes in global microRNAome and transcriptome reveal complex miRNA-mRNA regulated host response to Japanese Encephalitis Virus in microglial cells. Scientific Reports 6: 20263.
2. Goswami S, Banerjee A, Kumari B, Bandopadhyay B, Bhattacharya N, Basu N, Vrati S, Banerjee A (2016) Differential expression and significance of circulating microRNAs in cerebrospinal fluid of acute encephalitis patients infected with Japanese Encephalitis Virus. Molecular Neurobiology DOI: 10.1007/s12035-016-9764-y.
3. Mason RD, Welles HC, Adams C, Chakrabarti BK, Gorman J, Zhou T, Nguyen R, O'Dell S, Lusvarghi S, Bewley CA, Li H, Shaw GM, Sheng Z, Shapiro L, Wyatt R, Kwong PD, Mascola JR, Roederer M (2016) Targeted Isolation of Antibodies Directed against Major Sites of SIV Env Vulnerability. PLoS Pathogens 12: e1005674.
4. Nain M, Abdin MZ, Kalia M, Vrati S. (2016) Japanese encephalitis virus invasion of cell: allies and alleys. Rev Med Virol 26: 129.
5. Patil S, Kumar R, Deshpande S, Samal S, Shrivastava T, Boliar S, Bansal M, Chaudhary N K, Srikrishnan A K, Murugavel K G, Solomon S, Simek M, Koff W C, Goyal R, Chakrabarti B K, Bhattacharya J (2016) Conformational Epitope-Specific Broadly Neutralizing Plasma Antibodies Obtained from an HIV-1 Clade C Infected Elite Neutralizer Mediate Autologous Virus Escape through Novel Mutations in V1 Loop. Journal of Virology 90: 3446.
6. Kumar R, Agrawal T, Khan NA, Nakayama Y, Medigeshi GR (2016) Identification and characterization of the role of c-terminal Src kinase in dengue virus replication. Scientific Reports 6: 30490.
7. Kakumani PK, Medigeshi GR, Kaur I, Malhotra P, Mukherjee SK, Bhatnagar RK (2016) Role of human GRP75 in miRNA mediated regulation of dengue virus replication. Gene 586.
8. Deshpande S, Patil S, Kumar R, Shrivastava T, K Aylur, Srikrishnan, Murugavel K G, Koff W C, Chakrabarti B K, Bhattacharya J (2016) Association of mutations in V3/C3 domain with enhanced sensitivity of HIV-1 clade C primary envelopes to autologous broadly neutralizing plasma antibodies. Retrovirology 13: 41.42
9. Singla M, Kar M, Sethi T, Kabra SK, Lodha R, Chandele A, Medigeshi GR (2016) Immune response to Dengue virus infection in pediatric patients in New Delhi, India- Association of viremia, inflammatory mediators and monocytes with disease severity. PLoS Neglected Tropical Diseases 10: e0004497.
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11. Thakur P, Gantasala N P, Choudhary E, Singh N, Zainul Abdin M, Agarwal N (2016) The preprotein translocase YidC controls respiratory metabolism in Mycobacterium tuberculosis. Scientific Reports 6: 24998.
12. Choudhary E, Lunge A, Agarwal N (2016) Strategies of gene inactivation in mycobacteria: achievements and challenges. Tuberculosis 98: 132.

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Patents

Patent Application type:	Provisional U.S. Patent Application [62/189,418]
Title:	HIV-1 Clade C Envelope Glycoproteins
Date of Filing:	July 07, 2015
Inventors:	Jayanta Bhattacharya, Suprit Deshpande, Shilpa Patil, Rajesh Kumar, Bimal K. Chakrabarti

Patent Application type:	PCT application [WO2016065252]
Title:	Native Trimeric Env Immunogen Design
Date of Filing:	October 23, 2015
Inventors:	Chakrabarti Bimal K, Boliar Saikat, Das Supratik, Shrivastava Tripti, King Charles Richter, Bhattacharya Jayanta, Samal Sweety

Patent Application type:	Provisional Indian Patent Application [201611004727]
Title:	Isolated Nucleotide Sequence Useful for the Prevention of HIV-1 Infection
Date of Filing:	February 10, 2016
Inventors:	Jayanta Bhattacharya, Suprit Deshpande, Shilpa Patil, Rajesh Kumar, K.G. Murugavel

Patent Application type:	Complete Korean Patent Application [10-2016-0035673]
Title:	7-substituted 2-nitro 6,7- dihydroimidazo [2,1-b][1,3] oxazine derivatives of their optical isomers, pharmaceutical composition containing the same as an active ingredient
Date of Filing:	March 25, 2016
Inventors:	Lee iII Young, Park Chan Yong, Jung Myoung Geun, Kaung Hyun Hye, Ramandeep Singh, Saqib Kidwai

Seminars and Conferences

Dr. Jayanta Bhattacharya

Title of the Talk: Dissecting Molecular Specificity of a Broadly Cross Neutralizing HIV-1 Clade C Plasma Antibody obtained from an Indian Donor.

Place and Date: IAVI Design & Development Laboratory, Brooklyn, New York, USA [May 01, 2016]

Title of the Talk: Innovative approaches for vaccine design - HIV experience.

Name of the Meeting: 6th IMAPAC World Vaccine Summit

Place and Date: Hyderabad [March 10, 2016]

Title of the Talk: Neutralizing antibodies as gateway HIV-1 entry inhibitors and their translational implications.

Name of the Meeting: Bio-Epoch 2015

Place and Date: School of Biotechnology, Jawaharlal Nehru University, New Delhi [April 10, 2015]

Title of the Talk: Vulnerabilities associated with immune evasion and escape of HIV-1

Place and Date: South Asian University, New Delhi [September 10, 2015]

Dr. M. B. Appaiahgari

Title of the Paper: Transplacental rotavirus IgG interferes with immune response to live oral rotavirus vaccine OrV-116E in Indian infants

Name of the Meeting: 4th Molecular Virology Meeting

Place and Date: Rajiv Gandhi Centre for Biotechnology, Trivandrum, Kerala, [April 16-17, 2015]

Dr. G. R. Medigeshi

Title of the Paper: Identification of viral and immunological correlates of disease severity and recovery in pediatric dengue patients.

Name of the Meeting: 17th International Congress on Infectious Diseases

Place and Date: Hyderabad, India [March 2-5, 2016]

Title of the Paper: Change in plasma cytokine levels with treatment in childhood tuberculosis – association with outcome.

Name of the Meeting:	27th National Conference of Respiratory chapter of Indian Academy of Pediatrics (RESPICON 2015)
Place and Date:	Mumbai [Dec 11 - 13, 2015]

Title of the Paper:	Effect of Acute Respiratory Infection on Infant Pulmonary Function Test at 18 months of age: A prospective Cohort Study.
Name of the Meeting:	27th National Conference of Respiratory chapter of Indian Academy of Pediatrics (RESPICON 2015)
Place and Date:	Mumbai [Dec 11 - 13, 2015]

Title of the Paper:	Development of Th1/Th2 balance and correlation of serum IgE with respiratory infections during infancy.
Name of the Meeting:	1st Asian Paediatric Pulmonology Society Annual Scientific Congress cum 18th Hong Kong Society of Paediatric Respirology and Allergy Annual Scientific Meeting.
Place and Date:	Hong Kong and Macao [October 03 - 05, 2015]

Title of the Paper:	Etiology of Acute Respiratory Infections in Infancy: A Prospective Birth Cohort study.
Name of the Meeting:	1st Asian Paediatric Pulmonology Society Annual Scientific Congress cum 18th Hong Kong Society of Paediatric Respirology and Allergy Annual Scientific Meeting
Place and Date:	Hong Kong and Macao, [October 03 - 05, 2015]

Title of the Paper:	Etiology of Acute Respiratory Infections in Infancy: A Prospective Birth Cohort study.
Name of the meeting:	17th International Congress on Infectious Diseases
Place and Date:	Hyderabad, India [March 02 - 05, 2016]

Title of the Paper:	Interplay between dengue nonstructural proteins and the human RNA silencing machinery.
Name of the meeting:	The long and the short of non-coding RNAs (Aegean Conferences Inc.)
Place and Date:	Chania, Crete, Greece [June 14-19, 2015]

Dr. Arup Banerjee

Title of the Talk:	Dynamic changes in MicroRNAome in JEV infected human microglial cells: possible implication in pathogenesis
Name of the Meeting:	4th Molecular Virology Meeting
Place and Date:	RGCB, Thiruvananthapuram [April 16-17, 2015]

Dr. Manjula Kalia

Title of the Talk:	Interactions between Cellular Autophagy Machinery & Flaviviruses”.
Name of the Meeting:	4th Molecular Virology Meeting
Place and Date:	Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram [April 16-17, 2015]
Title of the Talk:	Interactions between cellular autophagy and RNA viruses”
Name of the Meeting:	8th RNA group meeting at CSIR-Centre for Cellular and Molecular Biology
Place and Date:	Hyderabad [January 8-10, 2016]
Title of the Poster:	Role of autophagy in Japanese encephalitis virus infection
Name of the Meeting:	Gordon Research Conference: Autophagy in Stress, Development & Disease
Place and Date:	Ventura CA, United States [March 20-25, 2016]

Dr. Ramandeep Singh

Title of the Paper:	Toxin-antitoxin systems in Mycobacterium tuberculosis: Role in drug tolerance and virulence.
Name of the Meeting:	Association of Microbiologists of India – 2015
Place and Date:	Jawahar Lal Nehru University, [Dec 2015]
Title of the Paper:	PolyP metabolism in mycobacteria: Role of PPK-1 and PPK-2 in Stationary Phase survival and virulence of M. tuberculosis.
Name of the Meeting:	Ramalingaswamy Conclave – 2015
Place and Date:	Regional Centre for Biotechnology, Faridabad [Dec 2015]

Dr. Nisheeth Agarwal

Title of the Paper:	Gene silencing in Mycobacterium tuberculosis by CRISPR/Cas9
Name of the Meeting:	Genome Biology and Big Data Bioinformatics
Place and Date:	University of Delhi South Campus, New Delhi [29-03-2016]
Title of the Paper:	Gene silencing in Mycobacterium tuberculosis by CRISPR/Cas9
Name of the Meeting:	Seminars in Molecular Medicine & Biotechnology
Place and Date:	All India Institute of Medical Sciences, New Delhi, India [Date: 08-03-2016]

Extramural Grants

Funding agency:	Department of Biotechnology, India
Title of the Grant:	Identification of correlates of disease severity in pediatric Dengue patients in New Delhi.
Duration:	May 2012 to Oct 2015
Amount:	Rs. 1,23,84,972

Funding agency:	Welcome Trust-DBT India Alliance
Title of the Grant:	Investigating the effect of viral infections on zinc homeostasis as a cause of permeability barrier disruption in polarized epithelial and endothelial cell.
Duration:	Oct 2014 to Sep 2019
Amount:	Rs. 3,63,18,986

Funding agency:	Department of Biotechnology, Government of India
Title of the Grant:	Role of microRNAs in establishment of Japanese Encephalitis Virus (JEV) infection and disease progression
Duration:	Feb, 2013 to Feb 2016
Amount:	Rs. 55.6 Lakhs

Funding agency:	Department of Biotechnology, Government of India
Title of the Grant:	Transcriptome analysis for identification of novel biomarker for disease progression in Dengue patients
Duration:	Aug, 2014 to July, 2017
Amount:	Rs. 140 Lakhs

Funding agency:	Indo-French Centre for the Promotion of Advanced Research
Title of the Grant:	Host-Virus Interactions and Antibody Therapy for Japanese encephalitis.
Duration:	March, 2015 to February, 2018
Amount:	Rs. 68.14 lakhs

Funding agency:	Department of Biotechnology, Government of India
Title of the Grant:	Characterization of hepatitis E virus RNA-dependent RNA polymerase and its associated proteins in the replicase complex
Duration:	From 2013 to 2016
Amount:	Rs. 8170100

Funding agency:	Department of Biotechnology, Government of India
Title of the Grant:	Programme support on integrative approaches to understand structure, function and stability of bacterial toxin-antitoxin systems
Duration:	From November 2015 to November 2018
Amount:	Rs. 78,46,000

Funding agency:	Department of Science and Technology, Government of India
Title of the Grant:	Understanding the role of citrate lyases in physiology and persistence of M. tuberculosis
Duration:	From November 2015 to November 2018,
Amount:	Rs. 46,00,040

Funding agency:	Department of Biotechnology, Government of India
Title of the Grant:	Mycobacterial membrane-derived vesicles: Role in pathogenesis and exploration as novel subunit vaccine vehicles against Tuberculosis
Duration:	From 2012 to 2017
Amount:	Rs 85.6 lakhs

Funding agency:	Department of Biotechnology, Government of India
Title of the Grant:	Deciphering Mycobacterium tuberculosis artillery
Duration:	From 2012 to 2017
Amount:	Rs 64.6 lakhs

Funding agency:	Indo-Singapore grant jointly funded by Dept. of Science and Technology (DST), India and Agency for Science, Technology and Research (ASTAR)
Title of the Grant:	Integrative genomics of host-pathogen interaction to identify new drug targets against persistent Mycobacterium tuberculosis
Duration:	From 2016 to 2019
Amount:	Rs 50 lakhs

Funding agency:	Department of Biotechnology
Title of the grant:	The animal facility for research on infectious diseases
Duration:	2014-2019
Amount:	Rs. 17,14,30,400

Honors and Awards

Dr. Ramandeep Singh

Ramalingawami Fellowship (2010- 2015)

National Bioscience Award (2014)

Dr. Krishnamohan Atmakuri

Ramalingaswami Fellowship (2012-2017)

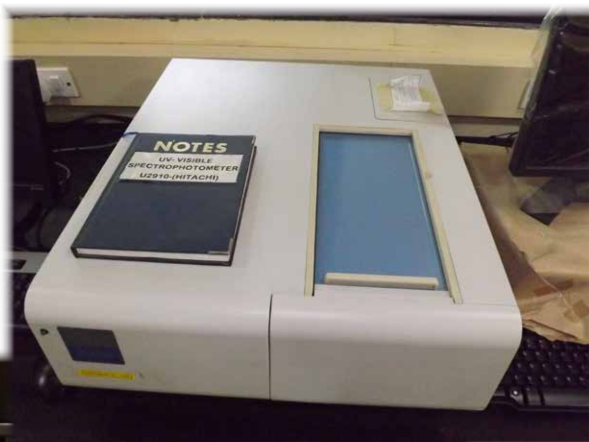
Dr. Amit Kumar Pandey

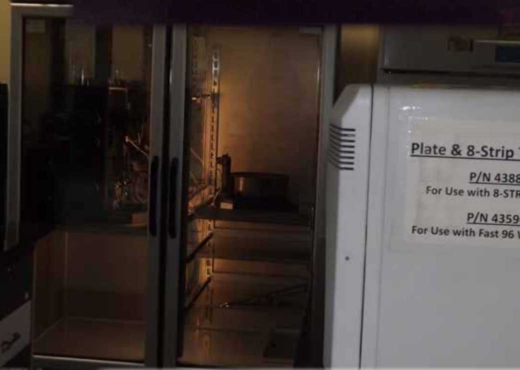
Ramalingaswami Fellowship (2013-2018)

Dr. Guruprasad Medigeshi

Welcome Trust-DBT India Alliance Intermediate Fellowship (2014-2019)

Hing-end Instrument at THSTI





Pediatric Biology Centre (PBC)

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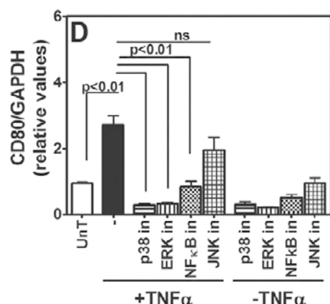

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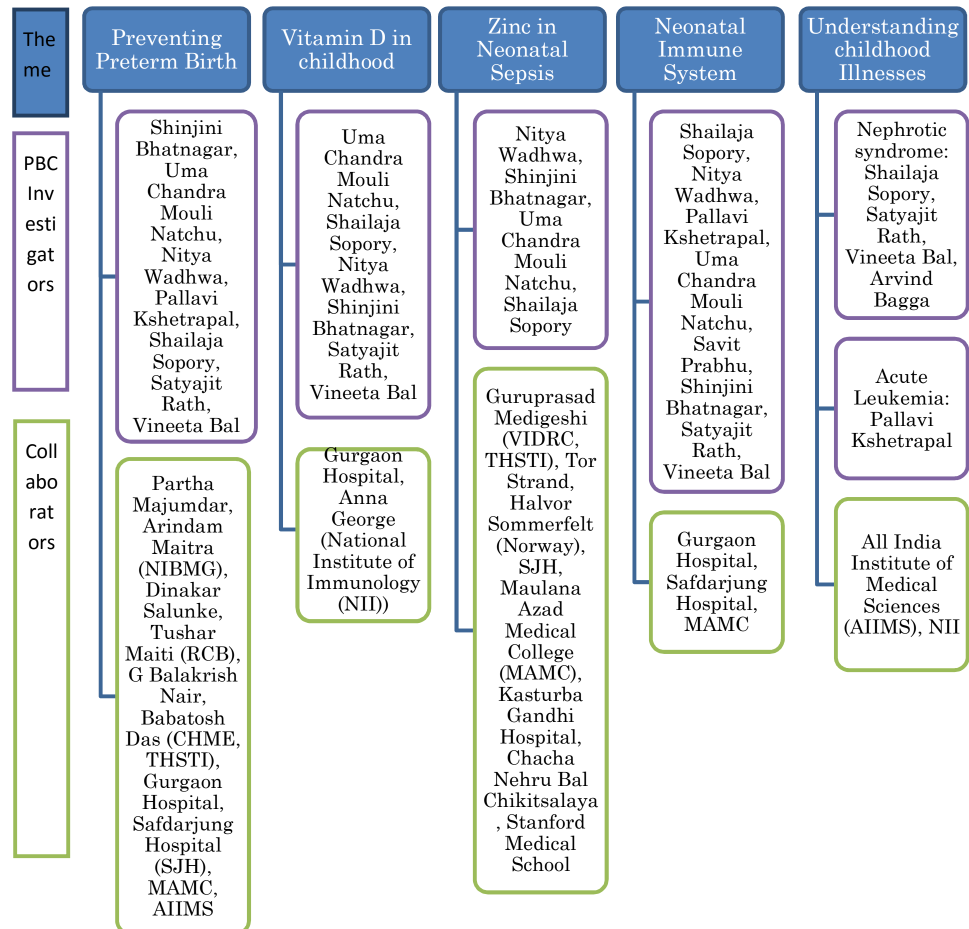
An Overview

Dr. Shinjini Bhatnagar

Research and development at the Pediatric Biology Centre (PBC) focuses on important issues of health and disease in mothers and children. PBC uses an approach where clinical researchers and biologists work together to target such issues in well planned human studies. Scientists at PBC work in three main areas: (i) Create an understanding of health and disease in mothers and children (ii) develop and test interventions against important childhood illnesses and (iii) design solutions for maternal and child health (in partnership with Centre for Biodesign and Diagnostics). Currently, research on understanding illnesses and in developing interventions, with collaborations with researchers at THSTI, multiple hospitals and international collaborations, is being conducted in five thematic areas (Figure 1).

Figure 1. Thematic areas, investigators and collaborators at PBC



Preventing Preterm Birth

Inter Institutional Program for Maternal, Neonatal and Infant Sciences-A translational approach to studying preterm birth

Long-term goals of the program:

1. Stratify women early in pregnancy into various levels of risk of Preterm Birth (PTB).
2. Identify simple and better prediction tools that will recognize the optimal time of prediction and clinical intervention.
3. Develop additional strategies to identify the presence of unusual/novel microbes that could serve as biomarkers.
4. Identify focused remedies targeting one or more mechanistic pathways (e.g. infection, inflammation, hormonal), and apply currently available interventions (tocolytic agents) based on better understanding of biological mechanisms.

Immediate goals

The immediate goal is to establish a hospital-based cohort of pregnant women at our partner district hospital at Gurgaon, Haryana. Women will be enrolled at < 20 weeks of period of gestation, and followed up until delivery to identify:

1. Modifiable clinical and epidemiological determinants for better sub-classification of PTB; to facilitate better understanding of mechanisms within well-defined sub-categories.
2. Maternal genotypes and epigenomic factors to predict and aid in disease stratification.
3. Proteomic alterations at different stages of pregnancy to predict and monitor PTB.
4. Composition and diversity of the vaginal microbiome during different stages of pregnancy and its association with PTB.

Details of the study:

The primary enrolment site and the referral site (Safdarjung Hospital) were setup for the study. The hospital based prospective cohort was initiated at the Gurgaon Civil Hospital in May 2015. This cohort is enrolling women early in their pregnancy and serially following them through their pregnancy until childbirth till 42 days (6 weeks) post-partum. Pregnant womenat less than 20 weeks gestation as determined by a 'dating' ultrasound and who are willing to come to GHG for follow up antenatal visits and participate in the study are eligible if they give their written informed consent. Those women who have an extra-uterine, molar or heterotopic pregnancy are excluded.

Information related to socio-demographic details, medical history, and history of past and present pregnancy is obtained and recorded in structured and pretested case recording forms (CRF). This includes information on established socio-demographic, environmental and obstetric risk factors for preterm birth and/ IUGR.

Collection of biological samples (maternal blood, stool, urine, high vaginal fluid, saliva) is done in pre-packed collection kits, which are labeled with a unique identification code (UIC) for each participant to ensure confidentiality of participants. This unique identification code is scanned at each step in the flow of biological samples from collection to immediate processing to temporary storage to transportation and till final storage. Scanning of UICs

at each step will inform us about the actual time taken for flow of samples from one point to other, thus will help us to monitor adherence to the protocol while handling these samples and facilitate the quality assurance process of laboratory parameters.

The enrolled participants are followed up at pre-defined periods during the course of the pregnancy. They are asked to come at 18-20 weeks, 26-28 weeks, 30-32 weeks, at the time of labor and delivery and then at 42 days post-partum. At each time-point clinical data is collected along with the anthropometry and a clinical examination in structured and pre-tested follow-up forms. Biospecimens (maternal blood, urine, saliva, high vaginal fluid and additionally cord blood, cord tissue and placental tissue at delivery) are collected at 18-20 weeks, 26-28 weeks, delivery and 42 days post-partum following the same rigor as described for the enrolment samples.

Progress till date:

We have screened 5114 pregnant women at the antenatal clinic of Gurgaon Civil Hospital. Of these, 991 eligible women have been enrolled. Clinical outcomes from 240 of these enrolled participants have been collected todate.

Creation of a Biorepository and Imaging Data Bank for Accelerating Evidence Generation to Facilitate Children to Thrive

PRINCIPAL INVESTIGATOR
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Broad objectives of the study: The primary objective is to develop and maintain a bio repository for long term storage of bio specimens viz. blood (maternal, cord, neonatal), saliva (maternal, neonatal), feces (maternal), urine and high vaginal swabs (maternal), placental punches, paternal saliva some of which are already being collected in the ongoing pregnancy cohortdescribed above. This will build infrastructure and processesfor collection, storage and retrieval of biospecimens for our research endeavors. Raw and processed images from serial ultrasound scans performed, as part of the pregnancy cohort, will be stored as an e-bank.

Relevance of the project for research in the country: Such a resource is likely to reduce time and cost required for future research in this area. The biospecimens and imaging data stored can be analyzed in conjunction with clinical data as and when new insights emerge in the field. The image data will serve as a resource for a number of subsequent analyses for the following:

1. Uterine measurements during pregnancy and predictors of impending birth
2. Normative data for fetal biometry that are India specific and or indicative of specific disease conditions
3. Image processing and analysis that can automate interpretation of ultrasound images.

Studies of such kind, which involve large serial data collection and a multifaceted approach are needed to identify causal factors and mechanisms in normal and abnormal pregnancies.

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Dr. Nitya Wadhwa

Stress outcomes on pregnancy, fetal growth and birth weight: Development of methods to identify mothers at risk of Preterm birth and intrauterine growth restriction resulting from maternal stress

Broad objectives of the study: The objectives of this study are to contribute to the understanding of the causes of adverse pregnancy outcomes particularly preterm birth and intra uterine growth restriction and to find handles to ameliorate these outcomes. In particular, we shall test that sustained stress during pregnancy is a major contributor to adverse pregnancy outcomes. We further posit that the nature of adverse outcomes is correlated with the extent of maternal stress during pregnancy. The key research questions are:

1. Does sustained moderate-level stress during pregnancy lead to intra-uterine growth restriction?
2. Do mothers who deliver preterm (+IUGR) experience sustained high-level stress during pregnancy?
3. What are the biological markers of stress during pregnancy that correlate with enhanced risk of adverse outcomes in mothers and their babies?

Details of the study: The goal of our study is to identify biological markers of stress during pregnancy that correlate with enhanced risk of adverse outcomes in mothers and their babies

This study is rooted in the large multicenter cohort-study on preterm birth to identify the correlates, causes and predictive biomarkers of preterm birth, funded by the Department of Biotechnology, Government of India and implemented by Translational Health Science and Technology Institute (THSTI), Gurgaon Civil Hospital (GCH). Consecutive pregnant women will be considered in the "pregnancy cohort" established at GCH. Stress will be ascertained at two time points, at 18-20 weeks of pregnancy and again at 26-28 weeks. The justification of measuring stress twice separated by 8-10 weeks is to confirm if the woman has been under sustained stress during her pregnancy. Stress will be measured by a validated psychological instrument. Our target is to obtain 250 women in the top 20-th percentile of stress (women under high stress), 250 women in the middle 20-th percentile of stress (women under moderate stress) and 250 women in the bottom 20-th percentile of stress (women with low stress)

In the study cohort, we will measure stress biologically by assaying for telomere length, epigenomic alterations and levels of hair cortisol, plasma epinephrine and other biochemical markers. Staff has been recruited and training is underway.

Vitamin D in child health

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Dr. Uma Chandra Mouli Natchu

Vitamin D supplementation to improve immune responses to Vaccines administered in early infancy – the Nutrivac-D Trial

Broad objectives of the study: We are attempting to evaluate if daily supplementation with vitamin D could modify immune responses (or "take") to vaccines administered in the first few months of life.

Relevance of the project for research in the country: Such evidence of nutritional modification of immune responses could pave the way for simple, effective and sustainable interventions to improve immune responses to childhood vaccines in infancy. Since deficiencies of many micronutrients including vitamin D are common and widespread, the potential to develop nutritional interventions that improve vaccine immunogenicity is immense. In addition, these interventions are likely to produce collateral beneficial effects on infectious morbidity, infant growth, childhood mortality and long-term health in general.

Details of the study: Newborns are enrolled in a randomized controlled trial in Haryana (District of Gurgaon). Children will be randomized to (i) 400 IU of vitamin D or (ii) placebo for a period of 24 weeks and vaccinated according to the Universal Immunization Program. Seroconversion to OPV & Hepatitis B and response to tuberculin skin test will be evaluated as outcomes. This trial allows us to examine the effects of Vitamin D on responses to three vaccines that implicate various components of the immune system (OPV–mucosal immunity, Hepatitis B – humoral immunity, BCG – primarily cell-mediated immunity.Immunophenotyping performed on samples from study subjects is being used to generate hypotheses about the developing human immune system. The trial will examine the effect of vitamin D supplementation on growth and intercurrent infections/ morbidity.

Progress till date: The study has been in progress for over 3 years; during this period over 600 infants have been enrolled in the study and nearly 500 subjects have been followed up for a period of 6 months. Immunophenotyping of samples from the umbilical cord and peripheral blood have yielded data on patterns of immune system development with age in infancy. These results will be assessed based on vitamin D supplementation status when the study is completed. We expect to add to a policy decision on routine vitamin D supplementation in the first 6 months of life.

Dr. Mouli and his team

Zinc in Neonatal Sepsis

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Binod Man Shreshtha and Ram Hari Chapagain
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Zinc as an adjunct for the treatment of clinical severe infection in infants younger than 2 months

Broad objectives of the study: This study will evaluate the efficacy of zinc administered orally as an adjunct to standard therapy to infants aged 3 days to 2 months hospitalized with clinical severe infection in reducing case fatality. It is also proposed to test the efficacy of zinc on reducing time to death until 12 weeks from the day of enrollment. The secondary objectives will be (i) failure of primary treatment, defined as a need to change antibiotics or requirement for life support or death, (ii) time to cessation of clinical signs of clinical severe infection, (iii) time to failure of primary treatment, (iv) time to discharge, and (vi) death or severe illness at any time after discharge from hospital until 12 weeks from day of enrollment. In addition to the above, an evaluation of the incremental cost effectiveness of zinc supplementation will be conducted to inform policy. This study will also measure the efficacy of zinc on survival and function of peripheral blood mononuclear cells and on innate immunity.

Details of the study: This double-blind randomized placebo-controlled parallel group superiority trial is a collaborative study between Pediatric Biology Centre at THSTI, Tribhuvan University, Nepal and Centre for International Health, Bergen, Norway. It has been awarded a Research Grant on Global Health and Vaccination Research (GLOBVAC) by the Research Council of Norway and additional funding from Centre for Intervention Sciences in Maternal & Child Health (CISMAC), Norway.

The trial will be conducted over a period of 3 years and 6 months. This is a multicenter study where recruitments will take place in 7 centres, 4 in New Delhi India: (Maulana Azad Medical College (MAMC) and associated Lok Nayak Hospital, Vardhman Mahavir Medical College & Safdarjung Hospital (VMMC & SJH), Chacha Nehru Bal Chikitsalaya (CNBC) and Kasturba Hospital (KH)), and 3 in Kathmandu, Nepal (Patan Hospital [PH], Kathmandu, Kanti Children's Hospital (KCH), Kathmandu and Institute of Medicine (IOM), Kathmandu). The study will be coordinated by PBC at THSTI.

4140 infants with clinical severe infection will be enrolled, given intervention for 14 days and followed up till discharge and until 12 weeks from the day of enrollment. The trial will measure the efficacy of 10 mg of elemental zinc administered orally as an adjunct to standard therapy to infants aged 3 days to 2 months hospitalized with clinical severe infection identified using an adaptation of the WHO Integrated Management of Childhood Illnesses (IMCI) criteria. The participants will be randomized to receive zinc or placebo in a 1:1 allocation ratio. The intervention (zinc/ placebo dispersible tablets) will be co-administered with the standard therapy which includes intravenous antibiotics and other supportive therapy like intravenous fluids, supplemental oxygen, etc. daily at 12 hourly intervals from the time of enrollment for 14 days. Clinical data will be collected from enrolled infants every 6 hours until discharge from the hospital. After discharge, the infants will be contacted at three time points: at a follow up visit in the hospital (after completion of the 14-day course of intervention, around day 15 of enrollment) and twice on telephone at one and a half month and again at three months (12 weeks) from the day of enrollment.

The Consortium agreement with Research Council of Norway was signed in

February 2015. Since then we have identified the hospital sites and signed partnership agreements with them. We have developed the protocol, informed consent document and case report forms. The protocol was submitted to REC West, Norway in March 2016 and their approval came in June 2016. We have also submitted the protocol to the Institutional Ethics Committees of THSTI, MAMC and associated Lok Nayak Hospital, VMMC and SJH, CNBC and National Health Research Council, Nepal for their review and approval.

Dr. Nitya and her team

Meanwhile, the process of procuring the intervention, getting a clinical trial insurance policy, preparing the site and recruiting research staff and training them is underway.

Neonatal Immune system

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Understanding the distinct developmental and functional properties of the neonatal immune system and their clinical consequences in the neonatal period

Broad objectives of the study: India's high neonatal mortality contributes to nearly two-thirds of its high infant mortality, particularly in low birth-weight (LBW) neonates. Infections contribute to more than 25% of neonatal mortality. Immune system limitations are plausible contributors to this problem, particularly in LBW neonates. The neonatal immune system is known to be quantitatively and qualitatively distinct from the adult immune system, but the differences are not well enough under stood as yet for explaining neonatal susceptibility to infections and fore ventual interventions.

Our earlier study had shown differences in frequencies and number of certain immune cells, picking up from there we will be characterizing the immune subsets further and also looking at functional differences between the different subsets of the B-cell, T cell and monocyte populations between SGA and AGA infants (cord blood) and also compare it with adult blood. At the same time these infants will be followed up for a period of 6 weeks to look at neonatal infection-related morbidities in the term AGA and SGA neonates and correlate it with the function of the immune cells.

Details of the study: The study is being conducted at Safdarjung hospital, all ethical clearances have been obtained, staff recruited and trained for taking informed consent, cord blood collection, immediate processing, temporary storage at site and sample transportation to the research laboratory at THSTI. The laboratory at PBC, THSTI has standardized the process of separation of preparing peripheral blood mononuclear cell (PBMCs) from the fresh whole blood and the process of immunopheno typing by flowcytometry.

The protocols for staining, gating, sorting of different subsets of monocytes (patrolling, classical, and inflammatory), B-cells (naïve, memory, B1B and immature) and T cells (CD4 and CD8) has been standardized in addition to protocols for the stimulation of monocytes and T cells. Assays to study the Notch intra expression have been standardized.

HLA-G 5'URR genotyping in small for gestational age (SGA) neonates compared to appropriate for gestational age (AGA) neonates.

PRINCIPAL INVESTIGATOR
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PARTICIPATING SCIENTIFIC STAFF AND STUDENT
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COLLABORATOR
Sunita Sharma
(Gurgaon Civil Hospital)

Dr. Pallavi Kshetrapal

Broad objectives of the study: Small for gestational age (SGA) babies has been a rising public health problem where these babies are at a higher risk for neonatal mortality, morbidity and have a poor neurological outcome. There is a growing body of evidence suggesting pregnancy complications such as preeclampsia, recurrent pregnancy loss (RPL), Intra Uterine Growth Restriction (IUGR), and premature birth could be associated with aberrant immunologic interactions at the fetal-maternal interface due to differences in the HLA-G expression of the placenta. Human leukocyte Antigen G (HLA-G) is a nonclassical major his to compatibility complex (MHC) class I molecule that exists as membrane bound or soluble isoforms. HLA-G is expressed on the placental trophoblast cells on the feto-maternal interface. Despite evidence for HLA-G playing an important role during pregnancy, the precise relationship between genetic variation in HLA-G and the pregnancy outcome, i.e. birth of SGA/AGA neonates remains unresolved.

The broad objective of our study is to study single nucleotide polymorphisms (SNPs) at the 5` URR region of the gene between the two categories (SGA vs AGA) and understanding how these polymorphisms lead to change in expression patterns of this protein if any. Our study will help to investigate the biological phenomenon of HLA-G production by the fetus. The initial findings will facilitate development of diagnostic tests that can be translated into clinical practice.

Dr. Pallavi and her team

Details of the study: Standardizations of macro-dissections of the human placental tissue and handling cord blood have been carried out for histo-pathology and DNA isolation. Reference sequence has been generated using three appropriate for gestational age neonates (AGA) (using stored samples from a previous study conducted at PBC). The collection of human bio-specimens, namely maternal blood, cord blood and placental tissue has been initiated.

Understanding childhood Illnesses

PRINCIPAL INVESTIGATOR
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COLLABORATOR
Rachna Seth *(AIIMS, Delhi)*

Role of Notch synergies in acute lymphoblastic leukemias

Broad objectives of the study: Childhood acute lymphoblastic leukemia (ALL) is an aggressive type of hematologic malignancy that results from malignant transformation of normal developing B cells. Recent reports about the involvement of Notch 3 and Hes5 in B-ALLs substantiate our objective to investigate the role of Notch and its synergistic partners in these disease conditions. Our study focuses on the role of Notch and its synergies in pediatric B-ALLs.

Project Details: In my earlier work, I carried out a modifier screen utilizing Drosophila genetics and a unique collection of mutations housed at the HMS to search the genome systematically for genes capable of synergizing with a constitutively active Notch receptor to influence prolife ration in vivo. Initial analysis of the modifiers (using Panther and David GO databases) has been encouraging and demonstrates that many of the human homologues of these fly modifiers have be reported and documented to be involved in cancers, like hepatocellularcarcinoma, prostratecancer, breastcarcinoma, colorectal cancers and manymore. Therefore we embarked studies to probe the other candidates to test if they have a role to play in the human B-ALLs.

We began our investigations by carrying out expression analysis of the candidate genes in Jurkat cell line, a T-ALL specific cell line and standardized Real-time PCR conditions for the gene expression profiling of receptors (Notch), ligands of Notch, candidate synergistic factors. We have now analyzed the expression of Notch and a few other candidate modifier genes (LMO2, HLF, NLK, etc.) in pediatric B-ALL samples by qPCR using SYBR green chemistry and normalized the data with GAPDH, a reference gene and compared it with healthy controls.

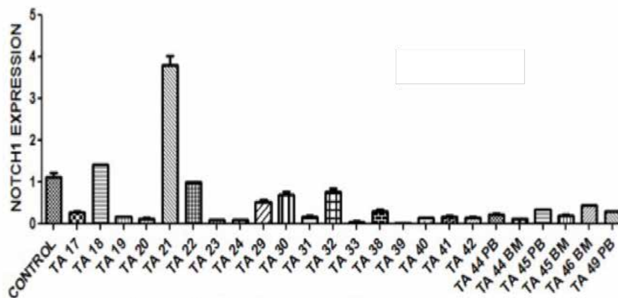

Figure-4

As published in recent studies by Kuanget. al., 2013, that Notch 3 is hyper methylated in the B-all patients. Interestingly in the blood samples collected from pediatric patients from the AIIMS hospital, we found a down regulation of the Notch 1 transcript in 86% of the total 22 samples tested so far. 9 % showed no change and 4% showed up regulation (Fig.2). This is a novel finding, as Notch 3 and Notch 4 have been associated with B cell acute lymphoblastic leukemia, but not Notch 1. We are in the process of analyzing the same in a larger sample size.

Our preliminary results also reveal a trend in the expression pattern of two other candidate genes in the patients when compared to the controls. We are in the process of analyzing these transcript expression patterns with the gene expression of Notch 1 to identify synergies and understand the mechanism leading to such synergies, if any.

Further we would like to understand the mechanism these genes adopt in the progression of the disease using invitro cell culture approaches.

PRINCIPAL INVESTIGATOR
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PARTICIPATING SCIENTIFIC STAFF AND STUDENT
Bhavya Khullar

COLLABORATORS
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Vineeta Bal

Dr. Shailaja Sopory

Molecular Mechanisms of Minimal Change Disease Nephrotic Syndrome: Role of CD80

Broad objectives of the study: Nephrotic syndrome is a kidney disease that is associated with loss of large amount of protein in the urine (proteinuria), low blood protein levels, high cholesterol levels and oedema. Minimal change disease nephrotic syndrome is the most common cause of nephrotic syndrome in children. Pathologically there is no change under the light microscope (hence the name minimal), but under electron microscope visible changes to the podocyte epithelial cells (that maintain the final barrier to protein loss) are seen. CD80 (a T-cell costimulatory molecule) was shown to be upregulated on the podocytes of patients with active disease and CD80 was also excreted in the urine of patients.

With this background the main objective of this study was to understand the mechanism of CD80 mediated proteinuria seen during nephrotic syndrome and look at the causes and consequences of CD80 upregulation in the podocyte at the cellular level.

Details of the study: Using mouse model systems of LPS (Lipopolysaccharide, a TLR4 ligand) mediated proteinuria and bone marrow chimeras between wild type and TLR signaling mutant mice we showed that LPS does not directly act on the podocytes but TLR receptor is required on the bone marrow cells to cause proteinuria and CD80 upregulation. We narrowed down on TNFα as the soluble factor that is secreted by the hematopoietic cells and acts on the podocytes to cause injury and albuminuria.

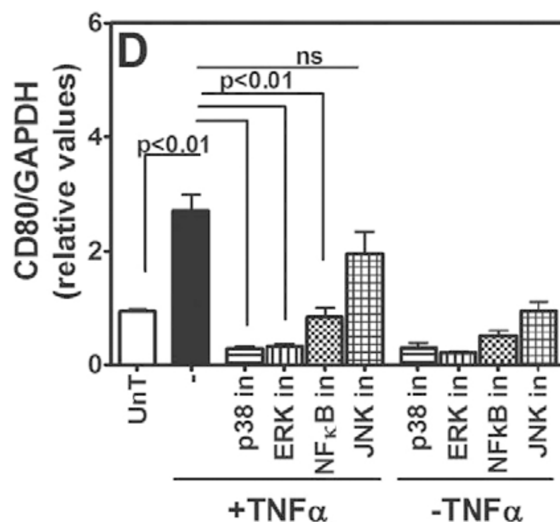
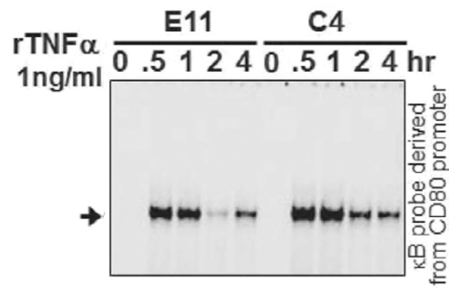


Figure-3: Relative CD80 mRNA expression levels in E11 podocytes with or without treatment with recombinant TNFα, in the presence or absence of inhibitors as shown (n=6, three independent experiments). Data represents mean±s.e.m. in, inhibitor; ND, not detectable; ns, not significant; UnT, untreated.

We next examined potential signaling intermediates by which TNFα might lead to upregulation of renal CD80. Podocyte cells were treated with TNFα for 6 h in the presence or absence of various well-characterized chemical inhibitors of MAPK and NFκB pathways, and subsequently CD80 mRNA levels were examined. Although inhibition of p38 MAP kinase, MEK-Erk as well as NFκBsignalling severely attenuated TNFα-induced CD80 upregulation, JNK inhibitor had only a marginal effect (Figure 3).

Furthermore, using a DNA probe derived from the CD80 promoter in our EMSA, we confirmed that TNFα-induced NFκB dimers are capable of directly binding to the CD80 promoter in podocytes (figure 4). Supershift analyses established the participation of canonical RelA-p50 NFκB dimers in podocytes because these dimers specifically bound to the CD80 promoter sequence (data not shown). The ability of NFκB inhibitors, but not of JNK inhibitors, in attenuating CD80 expression suggests that AP1 is unlikely to mediate TNFα-induced CD80 expression in podocytes and that this function is dependent on NFκB activity.



Our data further indicate that p38-MAPK as well as MEK-Erksignallingcooperate with the NFκBpathway in TNFα-induced CD80 gene expression.

Figure-4: TNFα-mediated TNFαB activation in nuclear extracts of cultured podocytes over time, determined with EMSA analysis using a DNA probe derived from the CD80 promoter encompassing the −2969 to −2945 region with NFαB sites

Dr. Shailaja and her team

PRINCIPAL INVESTIGATOR

Savit B Prabhu

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Dr. Savit B Prabhu

Comparison of human neonatal and adult blood leukocyte subset composition phenotypes

Background: The human peripheral leukocyte subset composition depends on genotype variation and pre-natal and post-natal environmental influence diversity. We quantified this composition inadults and neonates, and compared the median values and dispersal ranges of various subsets in them.

Details: We confirmed higher frequencies of monocytes and regulatory T cells (Tregs), similar frequencies of neutrophils, and lower frequencies of CD8 T cells, NKT cells, B1 B cells and gamma-delta T cells in neonatal umbilical cord blood. Unlike previous reports, we found higher frequencies of eosinophils and B cells, higher CD4:CD8 ratios, lower frequencies of T cells and similar frequencies of CD4 T cells and NK cells in neonates. We characterized monocyte subsets and dendritic cell (DC) subsets in far greater detail than previously reported, using recently described surface markers and gating strategies and observed that neonates had lower frequencies of patrolling monocytes and lower myeloid dendritic cell (mDC):plasmacytoid DC (pDC) ratios. Our data contribute to South Asian reference values for these parameters. We found that dispersal ranges differ between different leukocyte subsets, suggesting differential determination of variation. Further, some subsets were more dispersed in adults than in neonates suggesting influences of postnatal sources of variation, while some show the opposite pattern suggesting influences of developmental process variation. We will follow up these studies with extensive functional profiling of various immune subsets in neonates.

PRINCIPAL INVESTIGATOR
Savit B Prabhu

PARTICIPATING SCIENTIFIC STAFF AND STUDENT
Angika Bhasym

COLLABORATORS
Shailaja Sopory
Nitya Wadhwa
Uma Chandra Mouli Natchu
Shinjini Bhatnagar
Prasenjit Gucchait
Satyajit Rath
Vineeta Bal
Anna George

Functional assays for dendritic cell (DC) subsets in healthy adults, appropriate for gestation neonates and neonates with intra-uterine growth restriction

Broad objectives of the study: The objectives of the study are: (1) to come up with a easy-to-perform, flow-cytometry based assay that can be performed in relatively small sample volumes from frozen PBMCs from adult blood and cord blood. (2) To compare dendritic cell function (of all the subsets of DCs) between 30 healthy adults, cord blood of 30 healthy neonates and 30 small for gestation neonates.

Details of the study: Background of the study (Past): Our group has performed an extensive immunophenotyping of dendritic cells, including the recently described subsets of DCs and found significant differences in healthy adult versus neonates and between healthy neonates and small-for-gestation neonates. This has led to the necessity to perform functional studies of these subsets and in the long run, correlation of functional studies with early neonatal outcome.

Present status of the study: At present, a single-tube flow cytometry based assay has been optimized that can assess dendritic cell subset function in as small as 10 ml of adult blood and cord blood samples. Sample collection will be initiated.

Other activities for translational research preparedness and innovation in maternal & child health

PRINCIPAL INVESTIGATOR
Shinjini Bhatnagar

CO-PRINCIPAL INVESTIGATORS
Dinakar Salunke
(RCB, Faridabad)
Prasun Roy
(NBRC, Gurgaon Civil Hospital)

CO- INVESTIGATORS
Uma Chandra Mouli Natchu
Nitya Wadhwa

Collaboration for translational and clinical research between Translational Health Science and technology Institute, National Brain Research Center, Regional Centre for Biotechnology and General Hospital Gurgaon

Broad objectives of the study:

1. Preparedness for clinical and translational research at GCH
    a) Strengthening clinical care in relevant departments to be able to provide quality services round the clock
    b) Basic training in clinical and research protocols to establish a core group of clinicians who will be able to provide standardized support for research
2. Create a model of ‘grassroots’ level implementable clinical research that can be conducted outside of tertiary academic medical centers.
3. Provide exposure to intermediate level health care centers, their problems and needs for researchers to devise appropriate research programs to provide sustainable solutions in collaboration with clinical departments and this could be an educative prototype approach from a national-level perspective.

Details of activities accomplished

Preparedness for clinical and translational research at GCH: Expansion of the infrastructure at the GCH hospital site set up for clinical research. With an

increase in the number of clinical studies being conducted at GCH, the "Clinical & Translational Research Unit (CTRU)" established by THSTI and RCB for clinical research activities such as collecting clinical data,obtaining written informed consent from eligible participants, taking anthropometric measurements was expanded. A research laboratory (named Molecular Research Unit) has been initiated for collection of research related bio-specimens, their immediate processing and temporary storage at the hospital site and for facilitating transportation of these biospecimens to THSTI labs for long term storage in the biorepository. A separate unit (CTRU extension) has been established within a couple of km from the hospital for setting up a satellite unit of the data management centre and coordination of field activities like home visits.

a). **Strengthening clinical care in relevant departments to be able to provide quality services round the clock**

A medical team comprising of senior and junior residents have been posted in the Pediatric and Gynecology departments at GCH and two laboratory technicians to augment the laboratory services of the hospital have been recruited and posted by THSTI and RCB. This team works under the supervision of the clinical faculty at the hospital and the Principal Medical Officer, GCH.

b). **Basic training in clinical and research protocols to establish a core group of clinicians who will be able to provide standardized support for research**

The research knowledge of the clinical staff of GCH and the THSTI-RCB research teams have been augmented by workshops organized by THSTI on clinical research methodology. All newly recruited research staff undergo GCP (Good Clinical Practice) training.

Create a model of 'grassroots' level implementable clinical research that can be conducted outside of tertiary academic medical centers.

THSTI helped GCH form and register it's Independent Institutional Ethics Committee (registration number: ECR/278/Inst/HR/2013). Several collaborative research studies have been initiated between THSTI, RCB, NBRC and GCH.

Provide exposure to intermediate level health care centers, their problems and needs for researchers to devise appropriate research programs to provide sustainable solutions in collaboration with clinical departments and this could be an educative prototype approach from a national-level perspective.

THSTI organized CMEs every 3 months on various clinical topics relevant for maternal & child health -for the GCH physicians and nurses and for doctors from surrounding districts of Haryana as part of the commitment for contributing to their continuing clinical education.

As part of this partnership program GCH physicians have attended several national level workshops on clinical research. THSTI has organized national and international faculty visits for interactions with the doctors at GCH as part of their capacity development.

Integration achieved between basic and clinical partners

As a result of this program considerable “glue” has been created between the GCH physicians, the clinical scientists at THSTI and NBRC and the biologists at RCB-THSTI and NBRC. Basic scientists from THSTI and RCB and the physicians at GCH are members of the Institutional Ethics Committee (Human Research) at GCH and work together to improve the quality of research being done at the hospital and ensure the safety and rights of the patients participating in the research studies. Similarly some GCH physician staffs are members of the HumanEthicsCommitteeof NBRC.

Several collaborative research studies have been initiated between THSTI-RCB and GCH, and NBRC and GCH and are either ongoing or have been completed. The GCH physicians are co-investigators in these collaborative research studies and have used this opportunity to build their research skills.

PROJECT COORDINATORS

Uma Chandra Mouli Natchu *(PBC)*
Jonathan Pillai *(CBD)*

Dr. Uma Chandra Mouli Natchu

SPARSH – Social innovation program for Products: Affordable and Relevant to Societal Health. Fellowship program for innovation in maternal and child health

Details and Progress: This BIRAC funded grant was awarded in 2014 and began in 2015. Three recruited fellows were trained in human physiology, anatomy, health care systems and basic maternal and child health. They also underwent training in the biodesign process. After a 6-8 month long immersion and needs assessment process, the fellows completed needs filtering with supervision from clinical mentors from AIIMS, Gurgaon Hospital and Maulana Azad Medical College. The top identified needs were a low cost “disposable” incubator for newborns, a simple to use bone marrow biopsy needle (biopsy and aspiration in a single entry) and a bedside hand handle device to detect hypocalcemia. First iteration of concepts and prototyping have been completed and physical prototypes are likely to be completed by September 2016

Peer-reviewed publications

1. Chaudhuri S, Maurya P, Kaur M, Tiwari A, Borth N, Bhatnagar S, Kumar N. (2015) Investigation of CHO Secretome: Potential Way to Improve Recombinant Protein Production from Bioprocess. J Bioprocess Biotech. http://dx.doi.org/10.4172/2155-9821.1000240. (I.F: 1.74)

2. Yoshida S, Martines J, Lawn JE, Wall S, Souza JP, Rudan I, Cousens S; neonatal health research priority setting group, Aaby P, Adam I, Adhikari RK, Ambalavanan N, Arifeen SE, Aryal DR, Asiruddin S, Baqui A, Barros AJ, Benn CS, Bhandari V, Bhatnagar S, et al. (2016) Setting research priorities to improve global newborn health and prevent stillbirths by 2025. J Glob Health; 16 Jun;6(1):010508. (Cited: 04, I.F: 3.208)

3. Sharma C, Sankhyan A, Sharma T, Khan N, Chaudhuri S, Kumar N, Bhatnagar S, Khanna N, Tiwari A. (2015). A repertoire of high-affinity monoclonal antibodies specific to S. typhi: as potential candidate for improved typhoid diagnostic. Immunol Res; 62(3):325-40. (I.F: 3.098)

4. Singh P and Wadhwa N, Lodha R, Sommerfelt H, Aneja S, Natchu UCM, Kabra SK, Bhatnagar S#, Strand TA. 2015. Predictors of time till recovery in infants with probable serious bacterial infections. PlosONE; 10(4):e0124594. I.F: 3.234)

5. Rathore DK, Nair D, Raza S, Saini S, Singh R, Kumar A, Tripathi R, Ramji S, Batra A, Aggarwal KC, Chellani HK, Arya S, Bhatla N, Paul VK, Aggarwal R, Agarwal N, Mehta U, Sopory S, Natchu UCM, Bhatnagar S, Bal V, Rath S, Wadhwa N. (2015) Underweight full-term Indian neonates show differences in umbilical cord blood leukocyte phenotype. PlosONE; Apr 21;10(4):e0123589. (I.F.: 3.730)

6. Bagri NK, Bagri N, Jana M, Gupta AK, Wadhwa N, Lodha R, Kabra SK, Chandran A, Aneja S, Chaturvedi MK, Sodhi J, Fitzwater SP, Chandra J, Rath B, Kainth US, Saini S, Black RE, Santosham M, Bhatnagar S. Efficacy of oral zinc supplementation in radiologically proven pneumonia: Secondary analysis of a Randomized controlled trial. (manuscript submitted in Bull WHO)

7. Prabhu SB, Rathore D, Nair D, Chaudhary A, Raza S, Kanodia P, Sopory S, George A, Rath S, Bal V, Tripathi R, Ramji S, Batra A, Aggarwal KC, Chellani HK Arya S, Agarwal N, Mehta U, Natchu UC, Wadhwa N Bhatnagar S. Comparison of human neonatal and adult blood leukocyte subset composition phenotypes. (manuscript submitted to PlosONE)

8. Yadav P, Khalil S, Mirdha BR, Makharia GK, Bhatnagar S. (2015). Molecular characterization of clinical isolates of Cyclospora cayetanensis from patients with diarrhoea in India. Indian J Med Microbiol; 33(3):351-6. (I.F: 0.94)

9. Bothra M, Bhatnagar S. (2015). Probiotics in pediatrics. Indian J Pediatr; 82(5):399-400. (I.F: 0.867)

10. Diana M. Ho, Pallavi S. K, Spyros ArtavanisTsakonas. (2015). The Notch-mediated hyperplasia circuitry in Drosophila reveals a Src-JNK signaling axis. (e-life. 2015; 4: e05996.)

Chapters in books

1. Vohra P, Bhatnagar S. 2015. When to Suspect Celiac Disease in a Child? Handbook of Celiac Disease: Pub: Kontentworx, a division of KWX Communications Pvt. Ltd.;37-41
2. Bhatnagar S, Dabas A, Shah D. 2015. Persistent Diarrhea. PG Textbok of Pediatrics, Volume 2. Pub: Jaypee Brothers Medical Publisher (P) Ltd.;1404-1408

Patents

Patent Application type:	Indian Provisional (1350/DEL/2015)
Title:	Production of recombinant Cytolethal Distending Toxin B protein and its uses as diagnostic tool thereof.
Applicant:	THSTI
Date of filing:	May 14, 2015
Inventors:	Ashutosh Tiwari, Tarang Sharma, Anurag Sankhyan, Chandresh Sharma, Navin Khanna, Shinjini Bhatnagar

Patent Application type:	Indian Complete (683/DEL/2015)
Title:	Monoclonal Antibodies specific to Salmonella typhiflagellin, and use thereof
Applicant:	THSTI
Date of filing:	March 13, 2016
Inventors:	Ashutosh Tiwari, Tarang Sharma, Anurag Sankhyan, Chandresh Sharma, Navin Khanna, Shinjini Bhatnagar

Product Leads

S. Typhi specific anti-flagellin 4 murine monoclonal antibodies for diagnostic use S.

Typhi specific CdtB antigen for diagnostic use.

(Both products are under the process of technology transfer)

Seminars and Conferences

Prof. Shinjini Bhatnagar

Name of Workshop:	Yakult India Microbiota and Probiotic Science Foundation, “Gut, Its Microbes and Health”
Place and Month:	New Delhi, March 2015
Name of Workshop:	5th Annual Research Symposium of Public Health Foundation of India “Buildingcapacity for Implementation Research”
Place and Month:	New Delhi, March 2015

Name of the Meeting:	Biorepository Synchronization meeting
Place and Month:	London, April 2015
Name of the Meeting:	Global coalition advance preterm birth research annual meeting, “Preterm Birth and Maternal-Newborn R&D: Current Investment”
Place and Month:	Washington, July 2015
Name of the Meeting:	JIPMER Research Day celebration “Prematurity”
Place and Month:	Puducherry, September 2015
Name of the Meeting:	2nd National conference of Indian Society of Pediatric Gastroenterology, Hepatology and Nutrition (ISPGHAN) “Diarrhea and Malnutrition”
Place and Month:	New Delhi, October 2015
Name of the Meeting:	2nd National conference of Indian Society of Pediatric Gastroenterology, Hepatology and Nutrition (ISPGHAN) “Diarrhea and Malnutrition”
Place and Month:	New Delhi, October 2015
Name of the Meeting:	Clinical and Public Health Selection Committee Meeting
Place and Month:	Hyderabad, October 2015
Name of the Meeting:	International symposium on Pneumococcal Conjugate Vaccines in India – a Roadmap to Introduction, “Importance of timely introduction of PCV in India”
Place and Month:	New Delhi, November 2015
Name of the Meeting:	Preterm Birth International Collaborative (PREBIC) Biomarker Working Group, “Diagnostic needs in preterm birth and pPROM: A role for PREBIC Biomarker working group”
Place and Month:	Prague, November 2015
Name of the Meeting:	Provocative ideas on Human Placental Research, “Epidemiologic relevance of placenta based study in the Indian Society”
Place and Month:	THSTI, Faridabad, December 2015
Name of the Meeting:	Meeting of writing committee for celiac guidelines
Place and Month:	Hyderabad, January 2016
Name of the Meeting:	Global Biotechnology Summit
Place and Month:	New Delhi, February 2016

Name of the Meeting:	Grand Challenges: All Children Thriving Community Meeting, “Research from evidence to policy”
Place and Month:	Nairobi, February 2016
Name of the Meeting:	Young Investigator Meeting (YIM), “Research from evidence to policy”
Place and Month:	Manesar, February 2016
Name of the Meeting:	The Grand Challenges All Children Thriving Community
Place and Month:	Nairobi, February 2016
Attendees:	Dr. Shinjini Bhatnagar, Dr. Uma Chandra Mouli Natchu, Dr. ShailajaSopory, Dr. Pallavi Kshetrapal

Dr. Pallavi Kshetrapal organized a two day DBT sponsored international meeting on generating provocative ideas on “Human Placental Research” at Translational Health Science and Technology Institute, Faridabad, India on 1st and 2nd December 2015.

Extramural Grants

Funding Agency:	Grand Challenges India- All India Thriving by BIRAC
Title of the grant:	Stress outcomes on pregnancy, fetal growth and birth weight: Development of methods to identify mothers at risk of preterm birth and intrauterine growth restriction resulting from maternal stress
Duration:	2016-2018
Amount:	Rs 134.22 lakhs
Funding Agency:	Grand Challenges India- All India Thriving by BIRAC
Title of the grant:	The simple absolute neutrophil count as a predictor of linear growth and a measure of mucosal inflammation in Indian infants
Duration:	2016-2018
Amount:	Rs 3,19,72,560
Funding Agency:	Grand Challenges India- All India Thriving by BIRAC
Title of the grant:	Zinc as an adjunct for the treatment of clinical severe infection in infants younger than 2 months
Duration:	2015-2018
Amount:	18.02 million NOK [Research Council of Norway] and 6.5 million NOK [Centre for Intervention Sciences in Maternal & Child Health (CISMAC)]

Funding agency:	Dept. of Biotechnology, Govt. of India
Title of the grant:	Understanding the distinct developmental and functional properties of the neonatal immune system and their clinical consequences in the neonatal period
Duration:	2015-2018
Amount:	Rs 173.41443 lakhs

Funding agency:	Dept. of Biotechnology, Govt. of India
Title of the grant:	Inter Institutional Program for Maternal, Neonatal and Infant Sciences-A translational approach to studying preterm birth
Duration:	2013-2018
Amount:	Rs 48,85,32,796

Funding agency:	Biotechnology Industry Research Assistance Council
Title of the grant:	Social Innovation Immersion Programme Fellowship: The development of immersion based innovation program that targets the neglected tail of health care innovation for maternal and child health in India
Duration:	2015-2016
Amount:	Rs 91,17,000

Funding agency:	Dept. of Biotechnology, Govt. of India
Title of the grant:	Neonatal immune profiles: infections and toxicants
Duration:	2014-2016
Amount:	Rs 88,08,576

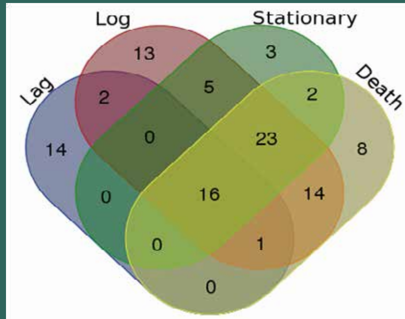
Funding agency:	Dept. of Biotechnology, Govt. of India
Title of the grant:	Clinical and Translational Research Unit Glue Grant Scheme, Department of Biotechnology Collaboration for translational and clinical research between Translational Health Science and Technology Institute, National Brain Research Centre, Regional Centre for Biotechnology and Gurgaon Civil Hospital
Duration:	2011-2016
Amount:	Rs. 5,73,06,000

Honors and Awards

Dr. Savit Prabhu
Recipient of Indo-Australian Career Boosting Gold fellowship (2016)

Centre for Biodesign and Diagnostics (CBD)

CHO Cell Line Engineering for 92



Technology Platform for Simple and Efficient Production of Recombinant Antibodies and Antigens 99

Diagnostic development for different etiologies tropical fevers 100

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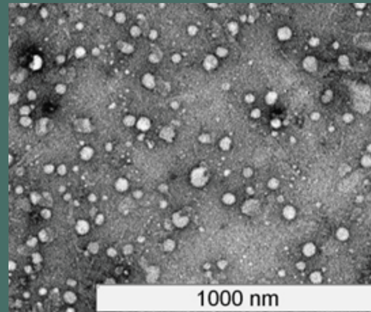

Microfluidic devices for isolation and evaluation of drug resistance of single bacteria 104

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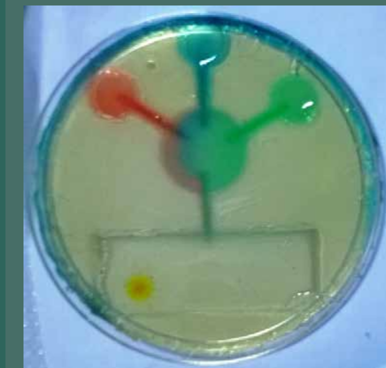
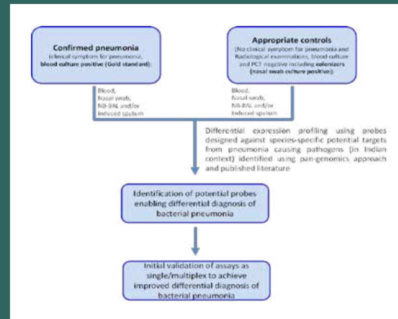
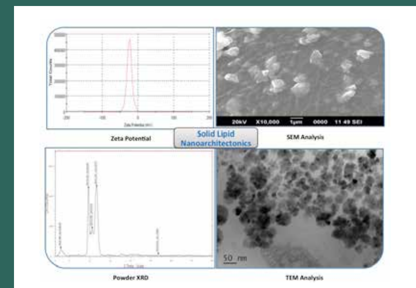


Biomarker Discovery and Diagnostic Development for Efficient Diagnosis of Pneumonia 94



Diagnosis and Treatment of Tuberculosis

- Novel sample processing for the simple and rapid diagnosis of TB, MDR-TB and XDR-TB: Enhanced filtration device 95
- Dynamic Molecular Platform for the rapid detection of Drug Resistant TB 97
- Utility of antigen detection for the accurate and rapid diagnosis of extrapulmonary TB (EPTB 97
- Generation of high affinity DNA aptamers for the detection of Tuberculous Meningitis 98
- Development of aptamer as a therapeutic agent for Tuberculosis 99
- Fixed dose combinations of anti-TB drugs in Solid Lipid Nanoparticles and Nano Lipid Crystals formulations for enhanced protection in gastric pH environment 101
- Lipid Drug Conjugates of hydrophilic drugs targeting pulmonary and extra-pulmonary TB 102



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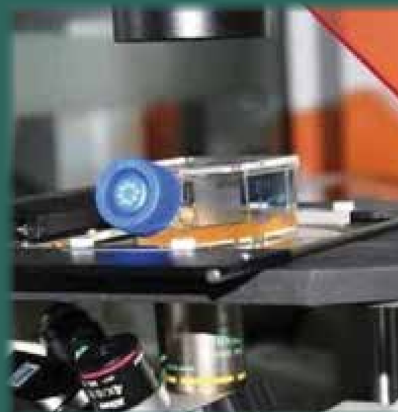

An Overview

Dr. Shinjini Bhatnagar

The Centre for Biodesign and in vitro Diagnostics (CBD) was established in 2011, thereby creating an organization for medtech innovation and establishing best practices for translation within the milieu of a larger public health institute. CBD therefore emerged out of the fledgling efforts to operationalize a unique Health Science Technology program that had never been previously envisaged or implemented in the country until that point.

The Center was established with the ambitious mission of driving medical technology innovation in India for affordable health care utilizing the Biodesign concept for systematic, clinical needs-inspired innovation and commercialization of medical technology. This mission was broadly articulated in the original SFC as follows:

- To develop leaders in biomedical technology innovation in India
- To promote world class quality science and application enabling affordable platform technologies for use in in-vitro diagnosis, implants, devices, imaging and similar disruptive technologies
- To develop transforming molecular and other diagnostics for personalized medicine and public health
- To develop low cost, high quality, indigenous implants and devices
- To promote techno-entrepreneurship

The uniqueness of this Center as compared to its peer programs within THSTI lies in its broad implementation of the Biodesign process, which emphasizes “clinical needs-driven innovation”. CBD is the only program of its kind in India (and possibly at a global level) to formally employ the Biodesign process for selection and execution of “translational research” projects in an academic setting.

In endeavoring to fulfill its mission over the last five years, CBD has been successful in establishing a foundation for multi-disciplinary translational science and technology, tightly integrating biotechnology with engineering and clinical medicine. From small beginnings in 2011, CBD has patiently built up a relatively modest core team of 7 researchers with PI or co-PI status. Nonetheless, the scientific productivity as measured by tangible output such as publications, patents, product leads or prototypes, and extramural funding generated is commendable, as enlisted here:

- Multi-disciplinary team of 7 faculty including biologists, biomedical engineers, clinicians and pharmacologists, 5 post-doctoral Innovation Awardees, 3 Ph.D. Students, and 7 technical support staff
- Infrastructure development including establishment of state-of-the-art facilities for diagnostic development, device prototyping, drug delivery systems formulation and pharmacokinetic evaluation
- Twenty one peer-reviewed publication with a cumulative impact factor

of 100.266

- Six patent applications targeting a market opportunity of US$ 2 billion
- Commercial launch of Celiac disease diagnostic kit, (in collaboration with ICGEB and J Mitra)
- Three products in final stage of development (i. Typhoid rapid diagnostic test, ii. Filtration device for improved smear microscopy for TB, iii. Aptamer based tool for TB diagnosis)
- Three products in pipeline (i. A mammalian engineered cell line for improved product yield and stability, ii. Blood safety for emergency setting and blood bank, ii. Dynamic molecular test for MDR and XDR TB)
- Six presentations in national and international conferences
- Five competitive fellowships (one Indo-US, two Indo-Australian, two DST)
- Six extramural grants worth Rs. 488 Lakhs from DBT, BIRAC, ICMR
- One entrepreneurial venture (Tritek Innovations Pvt. Ltd.)
- 4 international collaborations and 6 national academic partnerships and 3 national industry partnerships.
- One post-graduate level Biodesign course and one Biodesign-inspired Social Innovation Fellowship

CBD is at the forefront of venturing into techno-entrepreneurship in THSTI. Its scientists have initiated knowledge-based entrepreneurial ventures based on innovations generated in CBD labs with a defined goal of commercialization of research outputs for benefit of the society. CBD faculty, with generous support from the THSTI leadership, took the initiative in establishing an Incubation Facility at the new campus at Faridabad. Comprising of ~2000 sq. ft. of mixed wet lab and office space, the facility is capable of hosting up to six startup incubatees and is geared for real-life commercialization of product leads generated in house. In early 2016, CBD faculty also successfully pitched for and secured dedicated funding of INR 1 Crore from the Technology Development Board of the Department of Science and Technology, Govt. of India for seed funding of faculty-led startups.

Apart from PI-led scientific projects, a key success of CBD has been its pioneering role in developing and expanding Biodesign education in a research-oriented academic setting. In this regard, CBD faculty has facilitated formal and informal Biodesign training, both within THSTI as well as with NBA partner institutions. This includes the introduction of a formal course on Biodesign for Ph.D. students, and the successful completion of a pilot fellowship program in Biodesign innovation in the clinical space of maternal and child health (SPARSH-Social Innovation Immersion Program) funded by BIRAC and initiated as a collaborative program by CBD and Pediatric Biology Centre at THSTI.

CBD also recognizes the need to provide an organizational structure, ecosystem and governance process that ensures long-term sustainability and scope for growth for a new cadre of professionals who work at the interface of biology, engineering and medical science. The National Biodesign Alliance (NBA), a pan-India initiative by DBT to facilitate exchange of ideas, expertise, and resources between like-minded partner institutions, was established in order to collectivity address the whole value chain for development and delivery of affordable diagnostics and medical devices in an integrated manner. This unique alliance was anchored at THSTI with CBD operating as the secretariat for the NBA for the past 4 years, and was instrumental in 3 multi-institutional projects, one of which (an affordable glucose monitoring device) has been

commercialized by a start-up Janacare; and another has now translated into a successful tech transfer and secured BIG funding from BIRAC (Aptamer based TB diagnostics).

As a whole, CBD has now matured from a fledgling entity in to a mature program at the forefront of fulfilling the translational mandate of the Institute. Its success is being recognized, both in terms of scientific output by its faculty, as well as in terms of the substantial extra-mural funding it has been able to generate over the last 2 years. In the coming year, CBD is therefore well poised to not only consolidate its position of leadership in the development of diagnostic devices and assays, but also substantially expand its reach in the areas of drug delivery, implants and devices.

CHO cell line engineering for improved product yield and stability

PRINCIPAL INVESTIGATORS

Niraj Kumar
Susmita Chaudhuri

PARTICIPATING SCIENTIFIC STAFF AND STUDENTS

Babu Mathew
Sneha Arora
Misba Majood
Srikant Kumar
Shailesh Kumar

Dr. Niraj Kumar

Demand for recombinant proteins for therapeutic and diagnostics is increasing worldwide and hence improvements in the overall yield of such products from bioprocess are of great interest. Chinese hamster ovary (CHO) cells are the most commonly used cell lines for large scale production of such high-quality (human-like) recombinant protein products. This project is aimed to identify molecular regulators that may improve the yield from bioprocess.

Impact of microvesicles on recombinant protein product

Typically, product is released, along other secretory proteins, by the cell into the culture media. These secreted proteins may have significant impact on cell growth, product quality & quantity during the production culture and the designing of strategies for efficient product purification. It is observed that ~50% of the product gets lost during production process by the cells and/or the components present in the culture (Figure-1). Further investigation revealed that the microvesicles are typically enriched with proteolytic enzymes and significantly contributes towards product degradation during bioprocess.

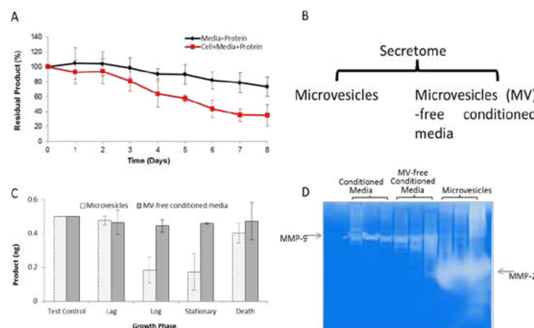

Figure-1: Impact of secretome on stability of recombinant protein product during CHO batch culture. A. Degradation of recombinant protein product during CHO batch culture. (B) Composition of secretome (C) Identification of fractions associated with product degradation. proteolytic enzymes in fractions of secretome and Inhibition of proteolytic enzymes with chemical inhibitors. (D) Identification of Proteolytic enzymes enriched in microvesicles using gelatin zymography.

Exosomal Secretome

Microvesicles may contain proteins that may have role in regulation of cell growth and recombinant protein productivity of CHO cells. Therefore, we aimed to reveal the proteome of microvesicles over batch culture. For this, CHO cells were grown in chemically-defined protein-free culture medium in shake flask and microvesicles were isolated from lag, log, stationary and death-phase of culture and quality tested using Western blotting and Zetasizer and both were as per the expectation (Figure-2).

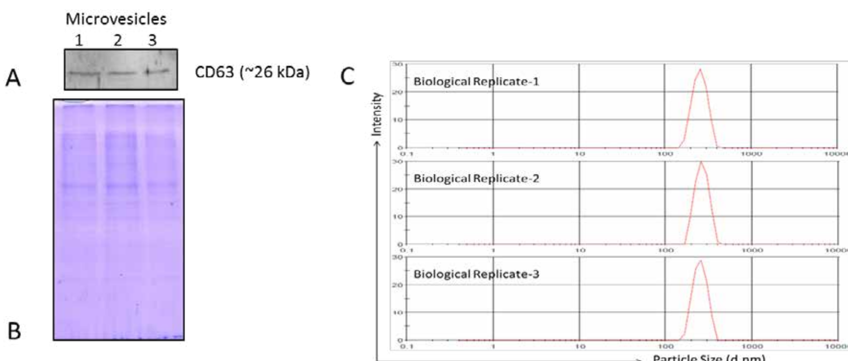

Figure-2: Evaluation of exosomes. (A) Western blotting. (B) Commassie stained gel as loading control. (C) Size estimation of exosomal samples using Zetasizer.

The purified microvesicles were investigated using gel-free proteomics tools (LC-LTQ Orbitrap MS) in order to identify microvesicular proteins that may have impact on cell growth, viability and/or productivity and also better define HCP. A total of 99 proteins were identified over batch (Figure-3). The work is ongoing to further analyze the data.

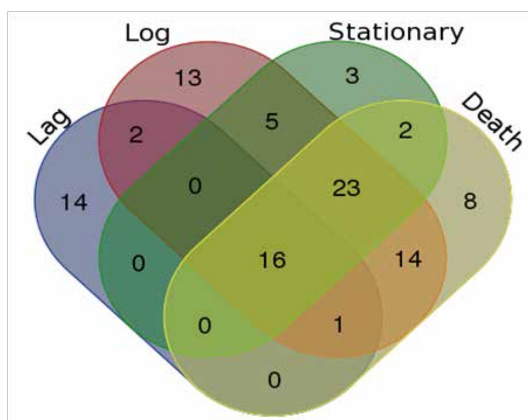

Figure-3: Venn-diagram of microvesicular protein identified over CHO batch culture

Current translational status & future pathway

Work is on-going to file complete specification of the provisional patent for the process development and to design a low-cost filtration device that can online remove microvesicles from the production culture during the batch/fed-batch run and also enable to collect these microvesicles in sterile conditions for bioprocess, medical, research or other purposes.

Dr. Niraj and his team

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Biomarker Discovery and Diagnostic Development for Efficient Diagnosis of Pneumonia

Pneumonia is a major cause of childhood mortality and morbidity, especially in resource-poor countries. It is primarily caused by bacterial and viral acute lower respiratory infection and is the single largest cause of deaths (27.5%) among children in post-neonatal period in India, with an incidence of 0.03-0.52 episodes per child per year.

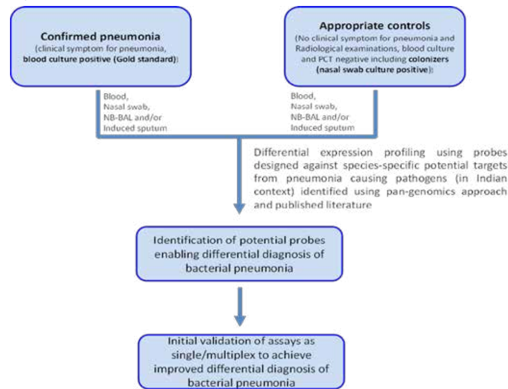
This project is aimed to identify biomarkers that may help in development of specific, sensitive and affordable diagnosis of pneumonia causing bacterial pathogens in Indian context.

Pneumonia is clinically diagnosed based on cough, difficulty in breathing, rapid respiratory rate, chest in-drawing and/or decreased level of consciousness/ danger signs. However, these clinical criteria over-diagnose bacterial pneumonia, as children with viral infections in lower respiratory tract will also be diagnosed as pneumonia. Even the chest radiography do not discriminate between various causes. Of the currently available diagnostics, nucleic acid based methods have shown highest sensitivity (70-90%) and specificity (60-90%). However, they have also have issues, such as –

- These tools use probes against only a few pathogens (1-2 most prominent pathogens), however pneumonia is caused by number of pathogens
- Even if the probes have been used for multiple pathogens, the test has not been clinically validated, contributing to large number of false positive or negative cases, reducing clinical sensitivity and specificity.
- Existing tools do not differentiate between bacterial and/or non-bacterial pneumonia and are not much useful in maximizing targeted antibiotic therapy and hence significantly reduce emergence of drug resistance.
- These tests do not differentiate between colonizing and invading pathogens and also do not provide information about the presence of the any drug-resistant pathogen

Therefore, the team is aimed to identify and validate biomarkers that may differentiate between invasive bacterial and non-invasive non-bacterial pneumonia with information about the presence of any drug-resistance pathogen.

Figure 4



For this, we have designed species-specific probe(s) for all bacterial pathogens commonly known to be associated with childhood pneumonia in India using pan-genomic approach and evaluate their potential in diagnosis as uniplex and multiplex test (Figure 4). Besides, a number of potential targets which may enable efficient differential diagnosis of bacterial pneumonia have already been reported in sporadic studies. We will also evaluate their performance for the purpose in the proposed study. The discovery and early-evaluation of targets will be performed using invasive samples (blood, induced sputum and NB-BAL) as they have less-contamination from the upper-respiratory tract specimens, however the performance of the targets will be established using non-invasive samples (nasopharyngeal swab and sputum) as these are the specimen of choice for our final assay(s).

Current translational status & future pathway

The project has received permission from institutional human ethics committee to start work.

The proposed work will enable development of specific, sensitive and affordable test for diagnosis of pneumonia causing bacterial pathogens more compatible in Indian context.

Dr. Susmita and her team

Novel sample processing for the simple and rapid diagnosis of TB, MDR-TB and XDR-TB: Enhanced filtration device

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This collaborative project involves AIIMS and THSTI laboratories, TB Hospitals in New Delhi and Ambala and Industry. This project has a two-tier diagnostic approach for pulmonary TB, (i) to improve the safety and performance of existing direct smear microscopy test for TB diagnosis and (ii) at providing a rapid PCR-based method for TB, Multi-drug-resistant tuberculosis (MDR-TB) and Extensively drug-resistant tuberculosis (XDR-TB) diagnosis using DNA extracted from the slides used in smear microscopy. This project is ultimately directed towards making a kit for simple and rapid diagnosis of TB, MDR-TB and XDR-TB. A novel bio-safe processing solution will be used to process sputum samples and to enhance the sensitivity of sputum smear microscopy in simple laboratory settings. At the second and higher level, the smear microscopy slides will be transported to National Reference laboratory or equivalent laboratory for DNA extraction and real time PCR for the diagnosis of smear-negative TB as well as for the diagnosis of for drug resistant TB.

Tuberculosis (TB) is a public health problem of immense proportions in a large number of countries including India. The twentieth World Health Organization (WHO) Global TB report states that TB remains one of the world's deadliest infectious diseases. In 2014, worldwide, 9.6 million people were estimated to have fallen ill with TB with 1.5 million people dying from the disease. A simple, rapid and accurate low-cost diagnostic test for TB remains an unmet need and challenge ever since the smear microscopy test was developed ~125 years ago. The Revised National TB Control Programme (RNTCP) depends on direct smear microscopy for case detection. Direct smear microscopy is the mainstay of the TB control programmes in India and worldwide on account of its speed, relative simplicity and low cost. However, it has poor sensitivity (range, 20% to 80%), particularly in HIV- TB co-infected patients and pediatric subjects. Drug-resistant TB is a major threat for TB control worldwide and WHO estimates that 59% of reported TB patients estimated to have MDR-TB were not detected

in 2014. India holds the dubious distinction of harboring 23% of the global TB burden with 2.2% of its new TB cases and 20% of previously treated cases having MDR-TB. This project aimed towards providing a kit for more sensitive and safe smear microscopy than the existing smear microscopy and for the rapid detection of Multi Drug Resistant (MDR) and Extensively Drug Resistant (XDR) TB (Molecular DST). Current achievements are summarized below

Improved smear microscopy. At the first level, a novel bio-safe processing solution to liquefy and disinfect sputum has been developed at AIIMS. The liquefied sputum is concentrated by gravity filtration and bacteria are visualized in situ by LED fluorescence microscopy

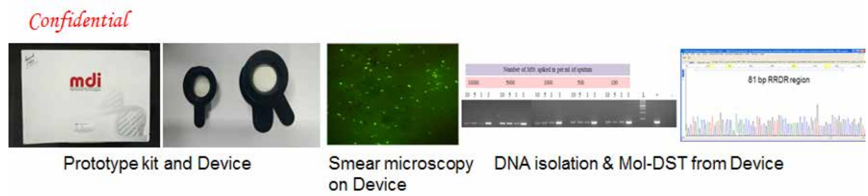

Figure-5: Novel sample processing for the simple and rapid diagnosis of TB, MDR-TB and XDR-TB: Enhanced filtration device

Molecular Drug susceptibility test. At the second level, filters are transported to the THSTI laboratory, DNA is extracted and used for PCR-based diagnosis of MDR-TB and XDR-TB and also to perform sequencing. Evaluation is in progress. (Fig.5)

Prototype Kits for smear microscopy and DNA isolation and PCR are under testing at AIIMS, THSTI, TB Hospital Ambala and NITRD Hospital. The standardized protocols are under evaluation.

Innovative and novel features

- A two-tier diagnostic approach is offered for pulmonary TB diagnosis using equipment free filtration device
- Approach combines sputum concentration, liquefaction and disinfection (biosafety) with in situ LED fluorescence microscopy and molecular DST.
- Rapid- sample processing to staining (~75 minutes) to Molecular DST (~120 minutes)
- The kit will have utility in the DOTS programme.

Dr. Sagarika and her team

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Dynamic Molecular Platform for the rapid detection of Drug Resistant TB

One of the major factors responsible for this current TB epidemic is the increase in various forms of drug resistant TB. MDR-TB is now widespread globally with an estimated half a million cases reported in 2011 and XDR TB has been reported in 84 countries. Only 19% of the total estimated numbers of MDR TB cases worldwide were notified to WHO in 2011 and less than 4% of MDR TB cases are currently diagnosed worldwide. The project proposes the establishment of a Central Molecular platform for Tuberculosis Drug susceptibility testing (Fig. 2). This involves the (i) development of an unique and efficient DNA isolation technology which will be compatible with pulmonary, extra pulmonary and paediatric TB samples; ii) Interrogation of all known mutations responsible for MDR and XDR-TB and iii) making the platform adaptable for detecting emerging mutations. The project has an important public health impact as the platform will also be linked to providing the result to patient within 1-2 days to start anti tubercular therapy as soon as possible to avoid further spread of resistance.

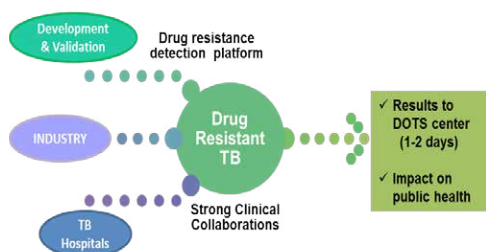

Figure-6: Dynamic Molecular Platform for the rapid detection of Drug Resistant TB

A novel DNA isolation methodology has been developed for isolating inhibitor free DNA from pulmonary TB sample smears. This unique lysis solution permeablizes mycobacteria to provide high quality DNA for further molecular testing. A plasmid DNA library database of all existing mutations for MDR-TB and XDR-TB is under preparation. HRM method for real time PCR is under development and validation. These will be used further for standardization of the Molecular DST assays. (Fig.6.)

Innovative and novel features

- Universal platform for diagnosing both MDR and XDR TB
- Public health impact as result to patient is provided in 1-2 days to start anti tubercular therapy quickly to avoid further spread of resistance.

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Utility of antigen detection for the accurate and rapid diagnosis of extrapulmonary TB (EPTB)

Tuberculous meningitis (TBM) is one of the most devastating manifestations of extra pulmonary tuberculosis with an estimated mortality of 1.5 per 100,000 population in India. Early diagnosis and prompt therapeutic intervention are essential for disease management and to minimize morbidity and mortality due to TBM. However, the accurate laboratory test for the diagnosis of TBM remains elusive, owing to a low bacterial load and the paucity of cerebrospinal fluid (CSF), especially from pediatric participants. Therefore, a rapid, low cost and accurate diagnostic test is urgently needed. We have earlier demonstrated that the detection of Mycobacterium tuberculosis GlcB/ HspX antigens/devR DNA in CSF improves the utility of existing algorithms for paediatric TBM

diagnosis and also hastens the speed of diagnosis (Haldar et al., 2012). Based on these encouraging results, we are assessing the utility of antigen detection in adult TBM diagnosis along with other forms of extra pulmonary TB. We are also exploring the avenue of exosome-based biomarkers for extra pulmonary TB diagnosis. Also, novel and specific reagents i.e. DNA aptamers for antigen detection-based TB diagnosis have been developed by one of the investigators (Dr Tarun Sharma). These aptamer reagents are currently being validated on appropriate clinical samples.

Progress: Currently efforts for standardization of the antigen detection assay for other forms of extrapulmonary TB are going on.

Innovative and novel features

- Antigen detection test for TB Meningitis with high diagnostic accuracy.
- Exosome biomarkers for TB diagnosis

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Generation of high affinity DNA aptamers for the detection of Tuberculous Meningitis

Tuberculous meningitis (TBM) is one of the most devastating manifestations of extra pulmonary tuberculosis (EPTB) and alone caused an estimated mortality of 1.5 per 100,000 population in India. Early diagnosis and timely therapeutic intervention are both essential for the effective management of TBM. However, the accurate laboratory diagnosis of TBM is very challenging owing to a low bacterial load and the paucity of cerebrospinal fluid (CSF), especially from pediatric participants. The current diagnostic tools suffer from one or other deficiency include to cite some specific example: inadequate sensitivity as in smear microscopy, adequate sensitivity but enormous delay in turnaround time as in culture, dependence on sophisticated instrumentation and proprietary reagents such as Gene Xpert. Therefore, to address the aforementioned challenges an accurate, rapid, low cost and simple test is urgently needed for the diagnosis of TBM.

We have generated a panel of high affinity ssDNA aptamer against recently reported potent TBM markers HspX and GlcB antigens that proved their utility to be used as potential TBM marker (Haldar et.al 2012). These ssDNA aptamer candidates were screened from a huge random DNA library (having ~10^{15} unique sequences) using subtractive SELEX (Systemic Evolution of Ligands by Exponential Enrichment) strategy. A number of novel aptamer candidates were obtained through SELEX. As obtained aptamer candidates were evaluated to determine their binding ability for HspX and GlcB. Further, cross reactivity of these aptamers were examined against various other mycobacterial antigens (ESAT-6, CFP-10, Ag85complex, MPT-51, GroES, HspX (for GlcB or vice versa), culture filtrate proteins and LAM). Based on the results best aptamer candidates were selected and used for determination of limit of detection and dissociation constants (Kd). All aptamer evinced Kd in low nanomolar and picomolar range. Truncation and mutation study was also performed to identify the actual sequence responsible for aptamer selection. Additionaly, aptamer selection is also performed against MPT-51and MPT-64 antigens of M.tb.

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Development of aptamer as a therapeutic agent for Tuberculosis

As a part of this project we have developed aptamer against Malate Synthase (GlcB) and HupB of Mycobacterium tuberculosis (M.tb.). GlcB is a good drug target inhibition of GlcB may inhibit the bacterial survival. We have isolated ssDNA aptamers with sub pico and nanomolar affinity that shows robust inhibition of catalytic activity of GlcB in vitro. In vivo studies are underway. We have also developed high affinity aptamers against HupB, a protein that facilitate the M.tb. invasion in cells. Thus we aim to block this invasion using aptamers

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Technology Platform for Simple and Efficient Production of Recombinant Antibodies and Antigens

Genesis and Objective

Most of the commercial in vitro diagnostic tests for the detection of pathogen specific antibodies, are based on the use of complex antigenic materials, such as cell lysate or partially purified fractions that suffer from cross reactivity, non-specific binding, lack of constant supply, batch-to-batch variation, safety issues (e.g. handling of pathogenic organisms) and economic feasibility. These issues can be considerably reduced with the use of correctly folded recombinant antigens. Interestingly the use recombinant antibody fragments in commercial IVDs for the detection of antigens is even scarce, despite having several advantages compared to full-length antibodies, largely due to production related issues. The objective of this project is to develop platform for convenient production of correctly folded recombinant reagents, which can facilitate the development of high quality diagnostic tests at relatively low cost.

Project Details

In this project we are developing strategies for efficient secretory expression of high-quality recombinant antigens and antibodies of diagnostic use in yeast Pichia pastoris. In this pursuit, we have developed novel method for the high-throughput expression screening of clones in 96-well format. The developed high-throughput expression-screening platform allows us to screen more than 1000 transformants in one round. We are evaluating several secretion signals for the efficient processing and secretion of different proteins of diagnostic utility. We are also evaluating culture conditions for efficient secretion and stability of recombinant proteins in shake-flask settings. A study

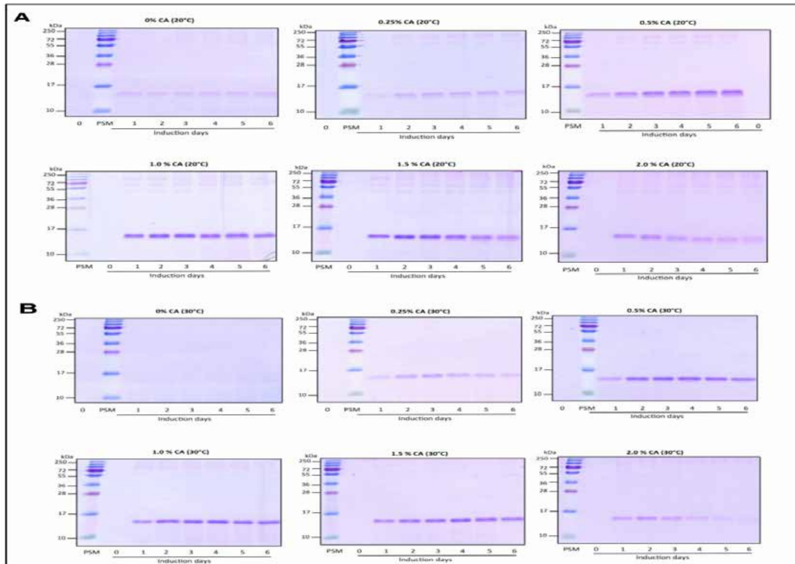

Figure 7

was performed to evaluate the effect, of induction temperature and supplementation of Casamino acids in minimal media, on the secretory overexpression of dengue virus envelope domain-III in P. pastoris. Temperature alone had a significant impact upon the amount of secretory EDIII. A bell-shaped correlation was observed between CA concentration and secretory EDIII titer. The maximum EDIII expression level was achieved under shake flask conditions with induction at 20 °C in the presence of 1 % CA. The overall increase in EDIII titer was ~9-fold compared to un-optimized conditions (Figure 7).

Diagnostic development for different etiologies tropical fevers

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Acute febrile illness (AFI) is common in the tropics and sub-tropics and can be caused by very diverse pathogens. The availability of reliable point of care test (POCT) that can quickly identify a pathogen from a group of pathogens that cause the similar symptoms is of paramount importance for patient treatment, surveillance and prevention of anti-microbial resistance. Despite the strong need, no multiplex POCT is available in the market that can be used in resource-limited settings for the detection of multiple etiologies of tropical fevers. Although, singleplex POCTs for different etiologies of fever are commercially available, but most of these tests are of poor quality. Because of the problems in available singleplex tests, we are working on the generation of high quality diagnostic intermediates/reagents that can be used for the development of high performance tests for routine diagnosis and surveillance purpose.

Details of some of the initiatives towards diagnostic development for different tropical fevers are listed below:

Anti-dengue virus (DENV) antibody detection for routine diagnosis and sero-surveillance:

Available diagnostic tests, for the detection of anti-DENV antibodies, suffer from the problem of cross-reactivity with antibodies induced by non-DENV flaviviruses including zika virus. Moreover, there is no simple diagnostic test available which can be used for the differentiation of antibodies induced by different serotypes of DENV. DENV specific (pan-serotype) and DENV-serotype-specific tests are also required for surveillance studies and during the DENV vaccine trials. This work aims to develop highly specific yet sensitive anti-DENV antibody diagnostic test without cross-reactivity problem with antibodies induced by other flaviviruses. Our intention is also to develop test that can differentiate antibodies induced by different serotypes of DENV. We are evaluating the use of several DENV antigenic fragments, isolated using

phage display library, as diagnostic intermediate for the detection of anti-DENV antibodies.

Dengue virus NS1 antigen detection for routine diagnosis and surveillance:

Dr. Gaurav and his team

The commercially available tests, for the detection of DENV NS1 antigen in human blood, suffer from poor sensitivity in the secondary DENV infection. Moreover, there is no commercial NS1 test available that can provide serotype information. To improve the sensitivity of pan-NS1 antigen assay in secondary infection and to develop serotype specific NS1 assay, in collaboration with University of Turku, we have isolated several anti-NS1 antibodies using human framework synthetic antibody libraries. The isolated antibodies have different specificities e.g. pan-flavi, pan-DENV and serotype specific. Studies are being done to identify the good antibody pairs and to covert selected ScFvs in more stable FAb format before assay development.

Fixed dose combinations of anti-TB drugs in Solid Lipid Nanoparticles and Nano Lipid Crystals formulations for enhanced protection in gastric pH environment

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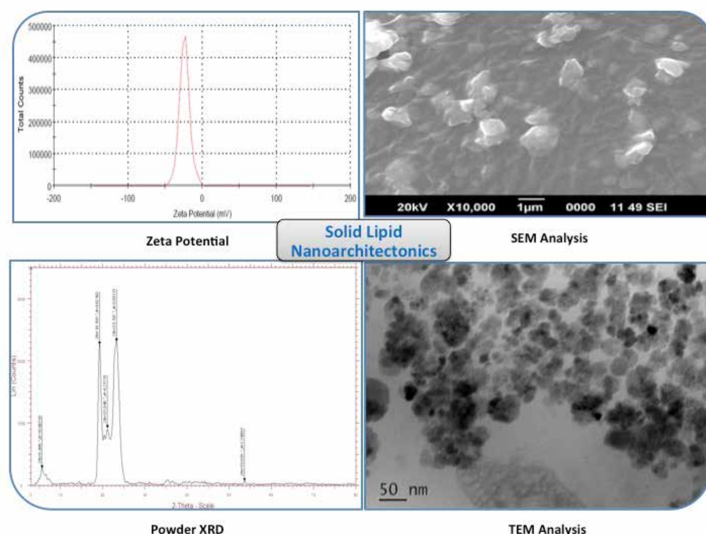
Globally in 2012-13, an estimated 4.5 Lakh people developed MDR-TB in addition to an estimated 1.7 Lakh deaths. Particularly for dual-drug oral formulations of first-line TB drugs, Rifampicin and Isoniazid, the degradation of drugs in the gastric environment results in loss of efficacy and chemical stability, decreased bioavailability of these drugs and the need for prolonged administration of large doses. This is a major factor causing patient non-compliance with the therapeutic regimen and eventually leads to the development of drug resistance.

The goal of this project is to develop a novel drug delivery system capable of protecting the drugs from in vivo degradation and imparting chemical stability to the oral dual-drug formulation. For this purpose, we are designing and developing novel lipid nanoparticle formulations (LNFs) for a fixed-dose oral formulation of both Rifampicin and Isoniazid.

We have successfully synthesized and thoroughly characterized LDC and LDC nanoparticle formulations of INH. Evaluation of the formulation using advanced analytical techniques to determine in vitro physico-chemical parameters such as total drug content, drug encapsulation efficiency, morphology (AFM, TEM), particle size analysis (DLS), thermogravimetry (DSC),

physical nature (XRD), drug-excipient compatibility (FTIR), pH dependent stability, in vitro drug release profile and has been completed. The mechanism of drug release kinetics is under evaluation.

In order to allow for the simultaneous determination and quantification of both drugs in bulk as well as in their LNF dosage forms, a novel RP-HPLC method has been developed as per ICH guidelines and validated as described in Fig 1-5 below. The method is sensitive enough to detect Rifampicin at a lower limit of detection (LOD) of 0.70 mg/ml and Isoniazid at an LOD of 0.27 mg/ml.



Work is in progress to establish the in vitro efficacy of these new formulations against free drug in both pathogenic and non-pathogenic strains of mycobacterium. Once the minimum inhibitory concentration is established, we will proceed to evaluating safety and efficacy in a small animal model. Successful demonstration of the therapeutic potential in vivo will pave the way for technology transfer or for future work related to regulatory approval. (Figure 8)

Figure-8: (A) TEM image of enriched OMVs from Mycobacterium Smegmatis. (B) Arrows point to the band of OMV's with and without Rifampicin, separated out in an Optiprep density cushion after centrifugation.

Lipid Drug Conjugates of hydrophilic drugs targeting pulmonary and extra-pulmonary TB

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It is well known that while hydrophilic drugs are easily soluble in aqueous media, they have significant difficulty in crossing hydrophobic membranes, including

those the gut and bacterial cell walls. A thorough literature search revealed that among first line TB drugs, Isoniazid (INH) is particularly problematic because of permeability issues, and suffers from extremely low bioavailability in serum. To compensate for this, large and frequent dosing is required, resulting in side effects and patient non-compliance. Further, this discouraged the use of this drug for treating extra-pulmonary TB as it is not able to permeate the blood brain barrier. Hence, we identified a need for enhancing the permeability of hydrophilic small molecule drugs that would enhance their bioavailability and allow for targeting of both pulmonary and extra-pulmonary TB.

While a lipid matrix may improve drug permeability, simple encapsulation is not feasible because of rapid partitioning of the hydrophilic moiety out of the matrix resulting in low drug loading. Hence, a direct covalent conjugation of the small molecule to a lipid chain is required to improved its overall hydrophobicity and bioavailability. This strategy results in the formation of a lipid-drug conjugate (LDC), wherein a hydrophilic drug is converted into water insoluble lipid drug conjugate. Further, formulation of such an LDC in a nanoparticle format (≤ 200 nm) would improve permeability of the drug across

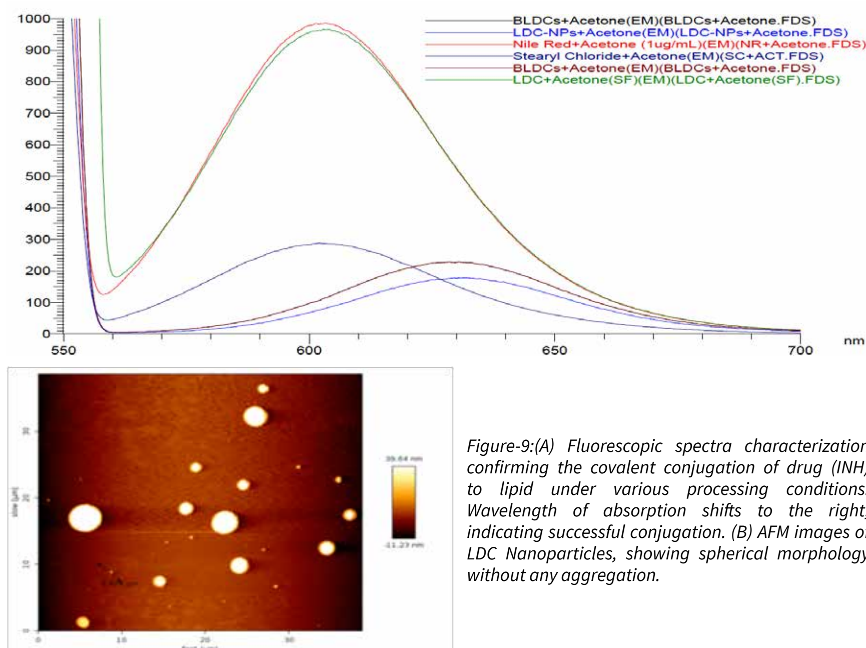

Figure-9:(A) Fluorescopic spectra characterization confirming the covalent conjugation of drug (INH) to lipid under various processing conditions. Wavelength of absorption shifts to the right, indicating successful conjugation. (B) AFM images of LDC Nanoparticles, showing spherical morphology without any aggregation.

the intestine and gastrointestinal tract while simultaneously reducing first-pass metabolism by transport of the drug through a lymphatic route to the systemic circulation. Such an LDC may be further processed into an oral, injectable or inhalable formulation.

We have successfully synthesized and thoroughly characterized LDC and LDC-nanoparticle formulations of INH. Work is in progress to establish the in vitro efficacy of these new formulations against free drug in both pathogenic and non-pathogenic strains of mycobacterium. Once the minimum inhibitory concentration is established, we will proceed to evaluating safety and efficacy in a small animal model. (Figure-9)

Bacterial Outer Membrane Vesicles as novel vehicles for targeted anti-microbial drug delivery

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Outer membrane-derived vesicles (OMVs) are nanoscale (approx. 40-300 nm) proteoliposomes produced naturally, mostly by Gram-negative bacteria but also by some Gram-positive species and mycobacteria. These bacterial “nanoparticles” are known to act as carriers for the long distance delivery of toxins, effector molecules antigens, virulence factors immunomodulators to name a few. The use of OMVs as delivery vehicles for antibiotics previously was reported in a study by Allan et.al. in which gentamicin from the MVs of Pseudomonas Aeruginosa was successfully delivered to Burkholderia Cepacia. Similarly, Gujrati and co- workers have recently demonstrated the successful use of OMVs for targeted delivery of siRNA. In this case, a mutant Escherichia coli strain that exhibits reduced endotoxicity toward human cells was engineered to generate OMVs displaying a human epidermal growth factor receptor 2 (HER2)-specificaffibody in the membrane as a targeting ligand.

Since a significant number of challenges with anti-tubercular therapy relate to drug delivery, and since the current outlook for new anti-TB drugs is extremely discouraging, it is important to explore novel methods of effectively targeting the bacterial with the available drugs. The objective of this project was to evaluate the use of targeted OMVs from non-pathogenic mycobacterial species as novel drug-delivery vehicles for first line anti-TB drugs.

We hypothesized that OMVs derived from non-pathogenic species of mycobacteria can be engineered to incorporate small molecule drugs. Further, we hypothesized that the physico-chemical composition, surface properties and organizational structures of these OMVs will be substantially similar to the outer membranes of more pathogenic species, thereby improving the surface absorption, permeability and intracellular bioavailability of their packaged

cargo. OMVs may be a particularly useful vehicle to enhance permeability and bioavailability of extremely lipophilic and poorly soluble drugs such as Rifampicin (RIF). Finally, targeting ligands naturally present or artificially engineered on the surface of these vesicles may provide host cells specific targeted delivery. All of these properties should synergistically combine to create a novel platform of drug delivery targeted against pathogenic mycobacteria, including MTb.

To test our hypotheses, we have successfully isolated OMVs from non-pathogenic Mycobacterium Smegmatis (M.Smeg.). These OMVs have been characterized using various tools including Dynamic Light Scattering (DLS), nanoparticle tracking analysis (NTA) and TEM. Further, we have successfully incorporated detectable quantities of RIF into the M.Smeg. OMVs by direct diffusion. Characterization of drug-loading capacity and evaluation of their therapeutic potential against pathogenic laboratory strains are currently under progress. This will be done both by direct dosing to bacteria as well as testing efficacy in bacteria engulfed by host immune cells. Simultaneously, we are also evaluating other approaches to enhancing drug encapsulation inside OMVs and engineering targeting ligands or homing peptides on their surface to allow selective targeting of pathogenic bacteria.

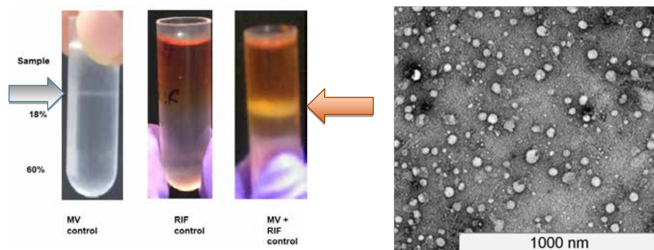

Figure-10: (A) TEM image of enriched OMVs from Mycobacterium Smegmatis. (B) Arrows point to the band of OMV's with and without Rifampicin, separated out in an Optiprep density cushion after centrifugation.

Once in vitro MICs and efficacy are established, we will test in vivo safety and efficacy in a small animal model. (Figure 10)

Microfluidic devices for isolation and evaluation of drug resistance of single bacteria

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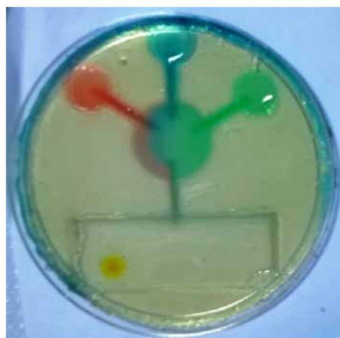
There are numerous reports in literature indicating that isolated copies of a clonal population of drug-sensitive bacteria may display genetic mutations leading to drug resistance. Since genetic mutation and sensitivity have been overwhelmingly studied in such clonal populations, detection of such isolated copies within a clonal population is typically missed. Researchers have recently employed microfluidic devices to isolate copies of a single bacterium and study cellular lifecycle and processes. However, the use of such devices to evaluate drug-sensitivity is not common practice yet. Microfluidic platforms therefore presents a unique tool for us to study genetic mutation and cellular processes under real-life conditions, including controlled exposure to various antibiotics and various carbon sources.

We envisioned a microfluidic platform designed to fulfill the following conditions. First, the device must be able to isolate a single copy of the bacterium from a clonal population, and anchor it to a suitable substrate. Next, it must be able to maintain the viability of the cell by allowing flow of carbon-enriched media. Finally, it should be capable of probing the bacteria with various stressors, including change of carbon source in the media and/or the introduction of various antibiotic drugs.

While this project has only been recently initiated, we have been able to create a prototype design that allows the introduction and mixing of three different fluids in a central well at microliter volumes. Work is in progress for finalizing device designs for creating microstructures to enable isolation of single bacterial copies. The prototype device has been replicated in both elastomer-

based as well as agar-based hydrogels to allow for testing the viability of bacteria post isolation.



Once the microscopic mold for the device is created using the photolithographic techniques described above, we will isolate various bacterial species and treat them with clinically relevant doses of antibiotics. The viable bacteria will then be evaluated using PCR to identify mutations against a control derived from the same clonal population. (Figure-11)

Figure-11: Prototype microfluidic design demonstrating mixing of three independent fluid streams in a central well. This design may be further refined to allow flow of various antibiotic and media solutions over bacterial copies isolated in the central well.

Social Innovation Immersion Program (SIIP) in Maternal and Child Health

PRINCIPAL INVESTIGATORS
Jonathan Pillai
Uma Chandra Mouli Natchu
(PBC, THSTI)

CONTRIBUTING SCIENTIFIC STAFF
Sumit Sharma
Lakshita Sagar
Aparna Rani

The SIIP was a BIRAC funded program was initiated in March 2015. This program was run as an internal collaboration between CBD and PBC, with P.I.'s from both center's serving as Fellowship co-directors. The objective of the program was to train a team of SIIP Fellows in applying the Biodesign process to producing social innovations in the area of MCH

At the beginning of this pilot program, 3 Fellows from a multi-disciplinary background were provided with a 6 month long clinical immersion to healthcare facilities at the community health, primary, secondary and tertiary levels. During this phase, the fellows made over 100 field visits to homes of rural patients, interacted with over 20 doctors and 200 patients and observed over 100 neonates in neonatal ICUs. From their observations, the fellows came up with over 140 unmet clinical needs.

Following the immersion phase, the fellows undertook a systematic needs filtering exercise. By adopting a series of progressively stringent qualitative and quantitative filters over 3 rounds of filtering, the fellows were able to arrive at the top 10 most impactful and clinically relevant needs. Some of the filters adopted for needs selection included morbidity, epidemiological data like disease incidence and prevalence, existence of competing products, market size and potential social impact.

The top 10 needs were further evaluated by a team of leading clinical experts including pediatricians, surgeons and obstetricians from our clinical partners at the Gurgaon General Hospital, Moulana Azad Medical College and the All India Institute of Medical Sciences. Based on the feedback provided by the clinical mentors, the fellows were able to short-list the Top 3 needs. Briefly, these clinical unmet needs included (i) a better way of proving thermoregulation for neonates during transport between referral centers, (ii) a cost-effective way of quantitatively detecting hypocalcemia in children at point-of-care, and (iii) a user-friendly, safe and reliable way to perform bone-marrow biopsy in children.

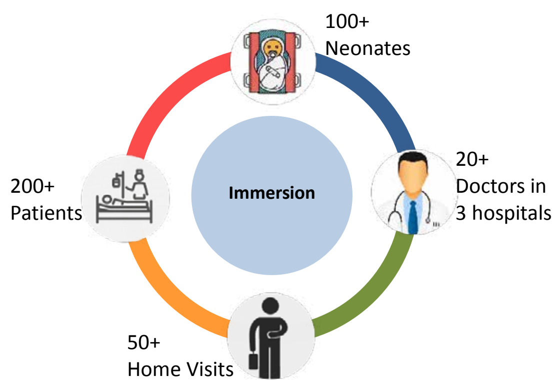
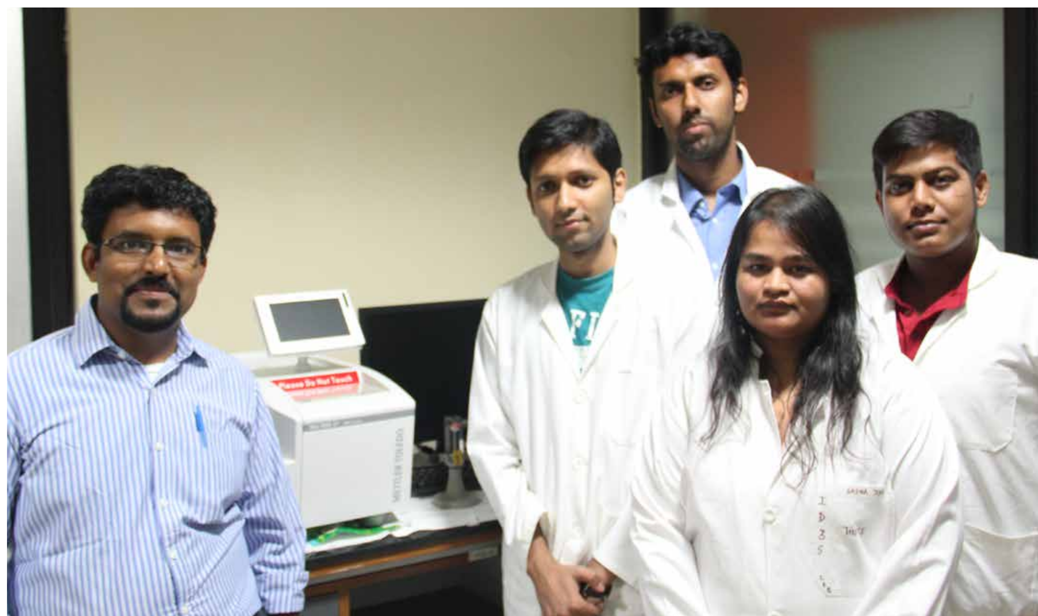


Figure-12: An overview of the outcomes from the 6-month clinical immersion phase of the Social Innovation Immersion Program.

These finalized top 3 needs will be adopted for further prototyping and product development in the second phase of this pilot program. Fellows will do a comprehensive brainstorming and concept selection process that will eventually lead to functional prototypes for each of the identified top needs. (Figure-12)



Dr. Jonathan and his team

Peer-reviewed Publications

1. Chaudhuri S, Maurya P, Kaur M, Tiwari A, Borth N, Bhatnagar S*, Kumar N*. Investigation of CHO Secretome: Potential Way to Improve Recombinant Protein Production from Bioprocess. J Bioprocess Biotech. 2015. (*Contributed equally).

2. Kumar N, Gupta DG, Kumar S, Maurya P, Tiwari A, Mathew B, Banerjee S, Haldar S, Pillai J, Bhatnagar S and Chaudhuri S. Exploring packaged microvesicle proteome composition of Chinese Hamster Ovary secretome. J Bioprocess Biotech 6:274.

3. Sharma, T. K*.; Bruno. J.G.; Cho W. (2016) The point behind translation of aptamers for point of care diagnostics. Aptamer and Synthetic Antibodies (Accepted, in press)

4. Weerathunge, P.; Sharma, T. K.; Ramanathan, R.; Bansal, V.; Nanozyme-based environmental Monitoring. Advanced Environmental Analysis: Applications of Nanomaterials, Volume 2 Chapter: 23 (Royal Society of Chemistry UK)

5. Kaushik, N., Rohila, D., Arora, U., Raut, R., Lamminmaki, U., Khanna, N., and Batra, G. (2016). Casamino acids facilitate the secretion of recombinant dengue virus serotype-3 envelope domain III in Pichia pastoris. BMC Biotechnol 16, 12.

6. Banerjee S., Pillai J. "Lipid nanoformulations for enhanced antituberculosis therapy". Book chapter in Nanoarchitectonics for Smart Delivery and Drug Targeting, Editors: Holman A. M. and Grumezescu A.M. Elsevier 2016, ISBN: 978-0-323-47347-7

Patents

Patent Application type:	Indian Provisional (1350/DEL/2015)
Title:	Production of recombinant Cytolethal Distending Toxin B protein and its uses as diagnostic tool thereof
Applicant:	THSTI
Date of filing:	May 14, 2015
Inventors:	Ashutosh Tiwari, Tarang Sharma, Anurag Sankhyan, Chandresh Sharma, Navin Khanna, Shinjini Bhatnagar

Patent Application type:	Indian provisional (2290/DEL/2015)
Title:	Method of improving stability of recombinant protein product in CHO batch culture and uses thereof
Applicant:	THSTI
Date of filing:	July 28, 2015
Inventors:	Niraj Kumar, Susmita Chaudhury, Ashutosh Tiwari

Patent Application type:	Indian provisional (2291/DEL/2015)

Title:	Human monoclonal antibodies specific to preS1 domain of Hepatitis B virus, and use thereof
Applicant:	THSTI
Date of filing:	July 28, 2015
Inventors:	Ashutosh Tiwari, Anurag Sankhyan, Navin Khanna, Subrat K Acharya, Subrata Sinha

Patent Application type:	Indian Provisional (201611001550)
Title:	Aptamer based system and method for the diagnosis of Tuberculous Meningitis (TBM)
Applicants:	THSTI, AIIMS-Delhi
Date of filing:	January 15, 2016
Inventors:	Tarun K Sharma, Jaya S. Tyagi, Abhijeet Dhiman, Chanchal Kumar, Ishara Datta

Patent Application type:	Indian Complete (683/DEL/2015)
Title:	Monoclonal Antibodies specific to Salmonella typhi flagellin, and use thereof
Applicant:	THSTI
Date of filing:	March 13, 2016
Inventors:	Ashutosh Tiwari, Tarang Sharma, Anurag Sankhyan, Chandresh Sharma, Navin Khanna, Shinjini Bhatnagar

Licensing Technologies

Licensed technologies covered under patent application no. 683/DEL/2015

Licensed technologies covered under patent application no. 1350/DEL/2015

Licensed technologies covered under patent application no. 201611001550

Faculty Entrepreneurship

Dr. Niraj and Dr. Susmita founded a company named Tritek Innovation Private Limited to commercialize the technologies they develop at CBD and the start-up company is incubated in THSTI Interim Incubator.

Dr. Tarun and Dr. Sagarika founded a company named Apta Bharat Innovation Private Limited to commercialize the technologies they develop at CBD and the start-up company is incubated in THSTI Interim Incubator.

Seminars and Conferences

Dr. Susmita Chaudhury and Dr. Niraj Kumar

Title of the Paper:	Investigation of CHO secretome: differentiation of classically and non-classically secreted proteins to develop strategies for improving performance of bioprocess.
Name of the Meeting:	Bioprocess India 2015
Place and Date:	Indian Institute of Technology, Madras (17-19 December, 2015)

Dr. Sagarika Haldar and Dr. Tarun Kumar Sharma

Title of the Talk:	Nucleic acid aptamer-based novel diagnostic tool for tuberculous meningitis
Name of the Meeting:	Tuberculous meningitis
Place and Date:	Vietnam (May 20 - 23, 2015)
Title of the Talk:	Nucleic acid aptamer-based novel diagnostic tool for tuberculous meningitis
Name of the Meeting:	Biosangam: An International Conference on Translational Biotechnology
Place and Date:	MNNIT Allahabad (February 4 - 6, 2016)

Dr. Sagarika Haldar

Title of the Poster:	Rapid detection of multi drug resistance in Mycobacterium tuberculosis directly from smear negative sputum samples by High Resolution Melt curve analysis. (Ist prize winning poster)
Name of the Meeting:	Integration of Genetics and Genomics in Laboratory Medicine
Place and Date:	Postgraduate Institute of Medical Education & Research, Chandigarh (March 12-13, 2016)
Title of the Talk:	Rapid detection of multi drug resistance in Mycobacterium tuberculosis directly from smear negative sputum samples by High Resolution Melt curve analysis
Name of the Meeting:	World TB day symposium 2016
Place and Date:	All India Institute of Medical Sciences on March 30, 2016.

Dr. Shubham Banerjee

Title of the Talk:	Fixed dose combinations of anti-TB drugs in Solid Lipid Nanoparticles and Nano Lipid Crystals formulations for enhanced protection in gastric pH environment.
Name of the Meeting:	Omics Conference (Pharma Expo)
Place and Date:	New Delhi (September, 2015)

Title of the Talk:	Fixed dose combinations of anti-TB drugs in Solid Lipid Nanoparticles and Nano Lipid Crystals formulations for enhanced protection in gastric pH environment.
Name of the Meeting:	Nanobio Interface Conference
Place and Date:	JNU, New Delhi (March, 2016)

Title of the Talk:	Fixed dose combinations of anti-TB drugs in Solid Lipid Nanoparticles and Nano Lipid Crystals formulations for enhanced protection in gastric pH environment.
Name of the Meeting:	7th Asian Association of Schools of Pharmacy
Place and Date:	Tapei, Taiwan (November, 2015)

Extramural Grants

Funding Agency:	SBIRI, DBT
Title of grant:	Novel sample processing for the simple and rapid diagnosis of TB, MDR-TB and XDR-TB.
Duration:	2.5 years (2015-2017)
Amount: Rs.	346.34 lakhs (total grant), Sanctioned to THSTI: Rs. 33.98 lakhs

Funding Agency:	DST
Title of grant: Dynamic	Molecular Platform for the rapid detection of Drug Resistant TB.
Duration:	5 years (2014-2019)
Amount:	Rs. 35 lakhs

Funding Agency:	Biotechnology Ignition Grant (BIG) from BIRAC
Title of grant:	Mycobacterium tuberculosis antigen-detection based point-of-care test using aptamer technology for tuberculous meningitis (TB meningitis)
Duration:	1.5 years (2016-2019)
Amount:	Rs. 50 lakhs

Funding Agency:	Dept. of Science and Technology, Govt. of India
Title of grant:	Fixed dose combinations of anti-TB drugs in Solid Lipid Nanoparticles and Nano Lipid Crystals formulations for enhanced protection in gastric pH environment
Duration:	
Amount:	Rs. 27 lakhs

Funding Agency:	BIRAC, Dept. of Biotechnology, Govt. of India
Title of grant:	Social Innovation Immersion Program (SSIP) in Maternal and Child Health
Duration:	September, 2015 - September, 2016
Amount:	INR 66 Lakhs

Honors and Awards

Dr. Sagarika Haldar

Recipient of the INSA INSPIRE Faculty award in Bio-Medical Sciences in January, 2014.

Received 1st prize in Poster session on Infectious Diseases in the 5th Annual Conference of the Molecular Pathology Association of India (MPAI) and International Symposium "Integration of Genetics and Genomics in Laboratory Medicine" held on March 12-13, 2016 at the PGIMER, Chandigarh. The poster was entitled Rapid detection of multi drug resistance in Mycobacterium tuberculosis directly from smear negative sputum samples by High Resolution Melt curve analysis.

Dr. Tarun Kumar Sharma

Editor of Aptamer and Synthetic Antibodies (Smith and Franklin UK)

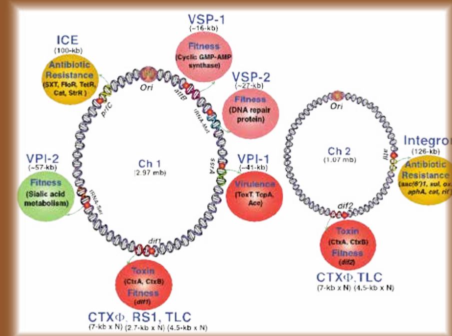
Dr. Subham Banerjee

Awarded the SERB Young Scientist Award (November 2015).

Won 1st prize for best oral presentation at Nanobio Interface Conference 2016.

High-end Instruments at THSTI

Centre for Human Microbial Ecology (CHME)



Vibrio Cholera Research

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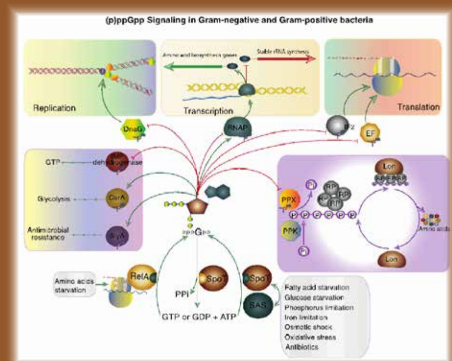
- Integration and excision mechanisms of integrative mobile genetic elements essential for Vibrio cholera pathogenicity 117

Gut and Vaginal Microbiota

- (p) ppGpp metabolism in bacteria and implications in gut microbiota homeostasis 118
- Multidrug resistance in enteric pathogens: Molecular insights into resistance traits of Indian isolates 120
- Understanding the diversity, dynamics and biology of vaginal microbiome of pregnant Indian women: A genomics perspectives 121
- MicrobDiab - Studies of interactions between the gut microbiome and the human host biology to elucidate novel aspects of the pathophysiology and pathogenesis of Type 2 Diabetes 122
- The Effects of Human Intestinal Microbiota on Immune Responses 123
- Gut microbiome and gut inflammation in severe acute malnourished Indian children 124
- Effect of environmental factors including diet and artificial sweeteners on gut microbiome and their consequences on type 2 diabetes 125



Regulatory T Cells

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An Overview

Dr. G. B. Nair

CHME was created as a niche Centre of THSTI on 26th July 2013 through an SFC grant from DBT. The main objective of the Centre was to explore the striking alliance between microorganisms and the human host and attempt to understand the role and effect of microorganisms in human health and disease. At present, CHME research is directed to investigate the role and impact of human microbiome in certain health disorders, which are directly linked to microbial richness, dynamics, and functional repository of the microbial genome. The major emphasis will be to take an ecological approach that considers the microbial community as a whole rather than as individual organisms as in the case of microbial pathogens. The processes include factors like: the richness and dynamics of the microbiome in human health and disease, Inter-relationships within the microbiome and its interaction with the host and importance of microbial metabolites in microbial and host physiology. In CHME, most of the research programmes use culture independent approaches to explore microbial community and their functional repertoires.

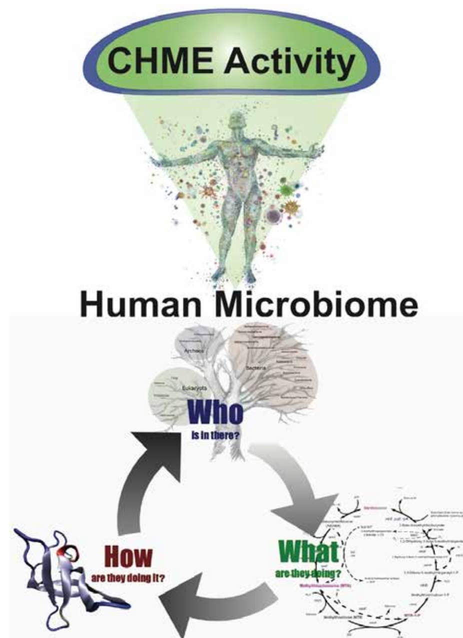


Figure 1: Schematic representation of the major questions that Centre for Human Microbial Ecology attempts to explore.

The culture independent exploration of microbial communities and their functional repertoires provide a good platform to predict microbial interactions and contribution of microbial functions to host physiology. On the other hand, high resolution candidate gene approaches, also known as functional metagenomics, provide evidence about the extent of microbial functions that influence host health and disease states. Functional metagenomics, the most promising part of human microbiome research, is emerging as highly important system that can be used to better understand the impact of microbial genomic repertoire on maintenance of healthy states. In CHME, structural and functional metagenomics are integrated to understand human microbiome and explore three basic questions; who is in there? What are they doing? How are they doing it? (Fig 1)

Major objectives of CHME

- Profiling and cataloguing human microbiota in different body sites with major emphasis on the gut microbiome.
- Study associations between the microbiome and clinical outcomes of global priority. Pregnancy outcomes and the vaginal microbiome.
- Understanding the detailed role of intestinal microbiota in under – nourished children with the aim of developing interventions like probiotics that could favorably modulate the intestinal microflora.
- Small molecule signaling systems of human gut pathogens.
- Investigate the effect of antibiotics on gut microbiota and study the restoration of microflora over time after antibiotic administration.

Ongoing Research Programs in CHME

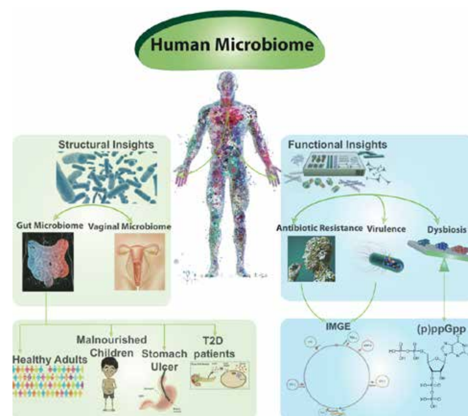
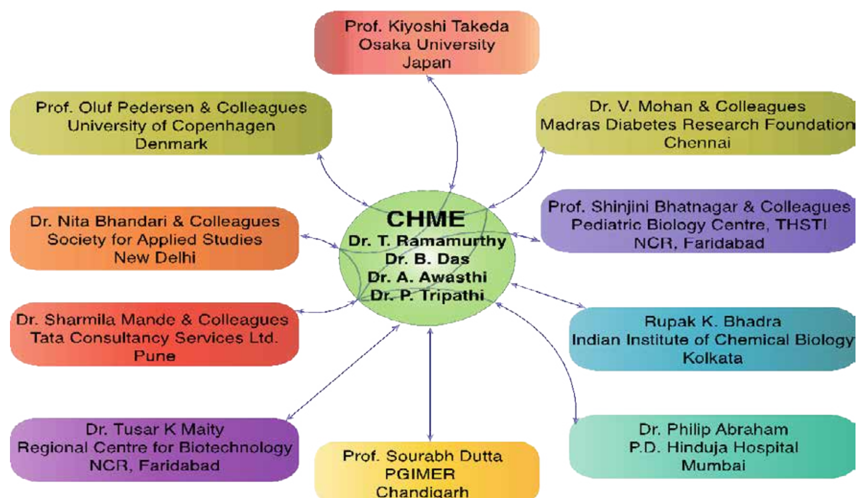


Fig. 2: A brief depiction of different ongoing research activities undertaken by different PIs working in CHME.

Currently, the human microbiome group at CHME is working on structural and functional metagenomics, and are emphasizing mainly on the gut and vaginal microbial communities (Fig. 2). CHME has successfully developed novel methodologies for community DNA extraction from human and environmental samples, established in house Next Generation Sequencing (NGS) facility, and isolated and cultured aerobic and anaerobic microbiota from different samples of human origin. The major research programs initiated and the progress made are elaborated below (Fig 2).

CHME Partners

Centre has developed National and International collaborations for different ongoing research programs. CHME has extensive collaborations at the Institute/Department/Scientist level. Apart from the intramural collaborations within the Institute with several Departments, following are the partners of CHME (Fig.3).



1. The Centre for Health Research and Development- SAS

The Centre for Health Research and Development, Society for Applied studies (CHRD, SAS), New Delhi is the regional Centre of the Society for Applied Studies (SAS), Kolkata. It is a not-for-profit research organization registered under the Societies Registration Act. Its entire functioning is typical of any research institute, which does research as a full time activity, disseminates its finding in the best journals in the field and contributes to policy, supported with evidence based research. SAS is a non-profitable research organization, working on childhood illness and on establishing neonatal services in district hospitals throughout the country. The CHRD in Delhi specializes in community based research, assessment and action, and product evaluation. CHRD interacts with other agencies to disseminate the knowledge it generates, helps visualize challenges in a scale up, build consensus on policies and implementation strategies and contributes with others to building skills in community research and action and improving delivery of child health programme. Scientists working in SAS is collaborating with CHME for subject identifications, anthropometric analysis, understanding disease biology and sample collection for gut microbiome analysis study of healthy adults as well as severe acute malnourished children.

2. Tata Consultancy Services (TCS), Innovation Labs (IL)

TCS IL was established in I981, when IT had barely emerged as an industry and provided an environment for sophisticated IT research in leading-edge technologies in various domains. Innovation Labs are connected to academic research centres on international repute for various collaborative research projects. These labs are customer aligned and metrics driven. Several innovative solutions emerging from these labs have won National and International awards. The research groups in Pune work on software engineering, process engineering, system research, application research, which includes computational life sciences (computational methods in life sciences, algorithms for metagenomic data analysis, human microbiome and health, system biology of tuberculosis, compression algorithms for Next Generation Sequencing data). TCS IL partnering with CHME for studying microbial metagenomics. They are performing computational analysis of metagenomics sequence data. CHME collaborates exclusively with Dr. Sharmila Mande, Principal Scientist and Head, Bio-Science R & D, TCS Innovation Labs, Tata Consultancy service Ltd.

3. Madras Diabetes Research Foundation (MDRF)

MDRF, established in 1996 by Dr. V. Mohan, internationally acclaimed diabetologist and research scientist and his wife Late Dr. Rema Mohan, an internationally known specialist in diabetic eye disorders. MDRF was founded with the vision of providing a world-class environment for research in diabetes and its complications. Within its short span of existence, MDRF has built up strengths in basic, clinical and epidemiological research. The quality of its research in diabetes and its complications is evidenced by numerous original publications in reputed peer reviewed journals. The institute also collaborates with several National and International centers.

As a non-profit organization; MDRF depends largely on private donors and government research support for its growth and activities. Its core departments have improved over the years with addition of state-of-the-art facilities. The major strength of the Institute lies in well-structured epidemiological studies that help the ongoing biochemical, genetic, molecular and cell based studies. Another main area where we focus is the definition of individuals at risk of diabetes or its complications by identification of risk markers. MDRF is working with CHME to explore the gut microbiome of diabetic patients and identifying microbial signatures for early prediction and possible intervention.

INVESTIGATOR
T. Ramamurthy

CONTRIBUTING SCIENTIFIC STAFF AND STUDENTS
Bhabatosh Das
Seema Mehra

COLLABORATORS
Shata Dutta and Asish K. Mukhopadhyay (NICED, Kolkata)

Dr. T. Ramamurthy

Evaluation of available rapid diagnostic tests for cholera

To evaluate the available rapid diagnostic tests for cholera along with conventional culture methods

A rapid diagnostic test (RDT) for cholera is available in India, but the diagnostic performance characteristics of this RDT in the community/field or at the bedside in a hospital has not been assessed adequately against similar tests produced by at least two other companies globally. In the proposed study, we will evaluate the diagnostic performance characteristics (efficiency, specificity and sustainability) of the available rapid detection kits in detection of Vibrio cholerae in the stool samples of patients with diarrhoea living in the cholera endemic region in India.

INVESTIGATOR
Bhabatosh Das

CONTRIBUTORS
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Ashok Kumar
Archana Pant

Dr. Bhabatosh Das

Integration and excision mechanisms of integrative mobile genetic elements essential for Vibrio cholerae pathogenicity

Objectives:

- To understand the integration and excision mechanisms of integrative mobile genetic elements (IMGEs) encoding virulence factors and antimicrobial resistance traits in the clinical isolates of Vibrio cholerae.
- Identify and characterize protein(s), which plays crucial role in the stability of IMGEs in the genome of enteric pathogens.

Comparative genomics of bacterial species reveals that genome of an organism can be divided into two parts: Core genome and horizontally acquired flexible genome. Core genome is highly stable and encodes all the essential functions, while horizontally acquired genetic elements are sporadic in nature and key for the genome fluidity of bacterial cells. Acquired genome is crucial for rapid evolution of bacterial pathogens and contributes significantly in bacterial pathogenesis, antimicrobial resistance, fitness and subsistence in

host associated and natural environment (Fig. 4).

The present study engineered the genome of a clinical V. cholerae strain, N16961, and measured real time integration and excision frequencies of Vibrio pathogenicity island-1 (VPI-1) in in vivo and in vitro conditions (Table 1). VPI-1 element is indispensable for V. cholerae colonization in the host intestine and production of cholera toxin and is, therefore, important for the development of diarrhoeal disease cholera.

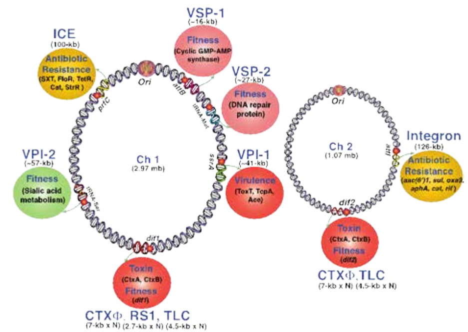

Fig 4. Integrative mobile genetic elements (IMGEs) reported in the Genome of epidemic V. cholerae clone. Relevant functions encoded by the IMGEs reported in the genome of V. cholerae are also specified.

VPI-1 element has two Tyrosine recombinases. The first time this study showed that integration of VPI-1 element is site-specific and both the integrases could mediate site-specific integration independently. We found that only 30 bp sequence of attB is sufficient for VPI-1 integration and excision and the integration and excision of VPI-1 element is recA independent. Using our reporter strain, we also first time isolated and characterized VPI-1 excised N16961 derivative strain SB3. We observed that, unlike MSHA and VGJf, absence of VPI-1 element has no influence on CTXf replication.

*Table 1: Excision frequency of VPI-1 element in clinical isolate of V. cholerae. Excision frequencies were measured after overnight growth in rich media (in vitro) or in rabbit ileal loop (*in vivo).*

Strain	Genotype	Excision frequency (%)
SB1	hapR-ve VPI-1DtcpA::Cm-SacB	1.04 x 10-4
SB1*	hapR-ve VPI-1DtcpA::Cm-SacB	<10-9
SB9	hapR+ve VPI-1DtcpA::Cm-SacB	2.86 x 10-4

INVESTIGATOR
Bhabatosh Das

CONTRIBUTORS
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D. Anbumani
Satyabrata Bag
Bipasa Saha
Ojasvi Mehta

(p) ppGpp metabolism in bacteria and implications in gut microbiota homeostasis

Objectives:

- Analyse canonical and novel (p)ppGpp synthetase/hydrolase in dominant human gut microbiota and model organisms of the representative phyla including Prevotella copri, Faecalibacterium prausnitzii, Escherichia coli and Vibrio cholerae.
- Identify environmental signals and dissect regulatory pathways of each (p)ppGpp synthetase in commensals and gut pathogens.
- Develop chromogenic model reporter strains that can aid prediction of differential expression of (p)ppGpp synthetase/hydrolase and identify bioactive compounds to precisely modulate the alarmone level by changing expression pattern of (p)ppGpp synthetase/hydrolase.

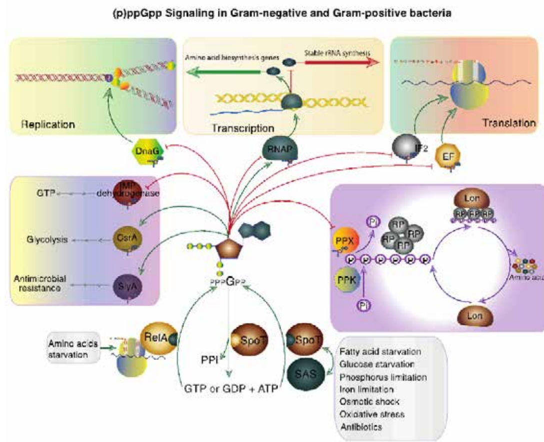

Fig. 5: Schematic representation of (p)ppGpp regulon in bacterial cell. Three different proteins directly linked to the (p)ppGpp homeostasis. It can synthesize and accumulates in the bacterial cells under various stress conditions. Elevated level of (p)ppGpp can induced or repressed expression of multiple genes. Arrowhead and inverse 'T' indicates activation and inhibition, respectively.

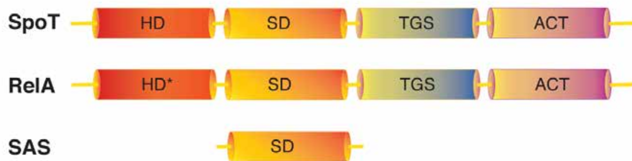

Fig 6. Multiple domains of three different (p)ppGpp synthetases/hydrolase reported in the genome in V. cholerae

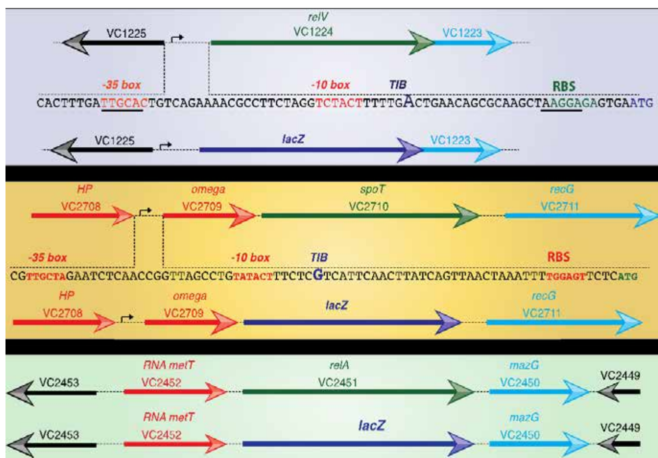

Fig 7. Schematic presentation of three different reporter strains engineered in this study. ORFs encoding RelA, SpoT and RelV were replaced by lacZ gene.

For an organism to survive in a complex ecology and compete with closely or distantly related multiple species for niche and resources, it is crucial to sense the surrounding environment and harmonize the cellular functioning accordingly. Guanosine penta- or tetraphosphate, collectively known as (p)ppGpp, the key intracellular small signaling molecule in bacteria, directly participates in the modulation of cellular physiology, upon sensing unfavorable environmental signals (Fig. 5). Quantitative differences in the intracellular concentration of (p)ppGpp determines the precise pattern of gene expression. We have validated functions of several (~13) (p)ppGpp synthetase/ hydrolase encoding genes in Prevotella copri, Faecalibacterium prausnitzii, Lactobacillus crispatus, Escherichia coli and Vibrio cholerae (Table 2). V. cholerae was chosen as model bacterium to explore the mechanistic insights of (p)ppGpp synthetase/hydrolase regulation because its genome encodes three different proteins that are directly linked to (p)ppGpp synthesis and hydrolysis (Fig. 6). We constructed a genomic library of V. cholerae and developed a high-throughput screening strategy to identify protein(s), which is essential for bacterial survival in the presence of (p)ppGpp. We have identified two proteins that are essential for bacterial survival in the presence of (p)ppGpp synthetase gene relV. For further understanding of the regulation of (p) ppGpp metabolism, we constructed three reporter strains, where relA or spoT or relV gene is replaced by a functional lacZ (Fig. 7). Using all three reporter strains, the signaling pathways and (p)ppGpp accumulation have been analyzed in enteric pathogens during several starvation conditions including antibiotic treatment and nutrient limitation. We observed that while spoT gene expressed constitutively, relA and relV expression was below the detection limit in nutrient rich environment (Fig. 8).

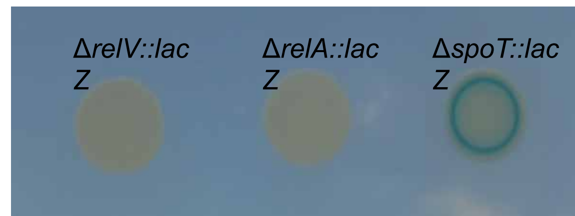

Figure-8: Expression profile of lacZ gene in nutrient rich medium fused with relV or relA or spoT promoters. Promoter fusion was done in the chromosome without any additional sequences.

Table 2. Functional validation of (p)ppGpp synthetases/hydrolases in homologous and heterologous genetic backgrounds. (p)ppGpp negative cells were complemented with the predicted (p)ppGpp metabolic genes and the growth of the complemented cells were monitored in non-permissive conditions.

Phyla	Bacteria	Host	ORF with native promoter	ORF with pBAD promoter
Proteobacteria	Vibrio cholerae	E. coli	Functional	Functional
Proteobacteria	Escherichia coli	V. cholerae	Functional	Functional
Proteobacteria	Vibrio parahaemolyticus	V. cholerae	Functional	Functional
Bacteroidetes	Prevotella copri	E. coli	Functional	Functional
Bacteroidetes	Prevotella copri	V. cholerae	Functional	Functional
Firmicutes	Lactobacillus crispatus	V. cholerae	Non-functional	Functional
Actinobacteria	Mycobacterium smegmatis	V. cholerae	Non-functional	Functional
Euryarchaeota	Methanosarcina acetivorans C2A	E. coli	Non-functional	Non-functional
Euryarchaeota	Methanosarcina acetivorans C2A	V. cholerae	Non-functional	Non-functional

Multidrug resistance in enteric pathogens: Molecular insights into resistance traits of Indian isolates

INVESTIGATOR
Bhabatosh Das

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Objectives:

- Understand the prevalence of Antimicrobial Resistance Traits (AMR) in the enteric pathogens isolated from different parts of India during 2008-2015.
- Reveal the molecular identity of AMR traits.
- Identify genetic elements and signaling pathways responsible for rapid dissemination of resistance traits.

Antimicrobial resistant Gram-negative bacteria have emerged as an urgent global health crisis and jeopardize effects of most, if not all, antibiotics commonly used to treat bacterial infections. Currently, there is no consensus on the description on prevalence of AMR pathogens of public health importance in Indian context. IN this project, we have isolated and examined AMR patterns of more than 1000 Gram-negative enteric pathogens. We further investigated complete genome sequences of 7 pandrug resistant (PDR) enteric pathogens and explored molecular identities and acquisition and dissemination mechanisms of resistance traits (GenBank Acc. No. KX302882 to KX302888).

The enteric pathogens were isolated from two Centers in India, Kolkata and Delhi. Morphology of seven representative PDR isolates was determined by atomic force microscopy. Antibiotic susceptibilities were determined by disc diffusion and broth dilution methods. High-throughput DNA sequencing was used to decode the genome of seven PDR pathogens. Functionalities of the resistance traits were confirmed de novo. Acquisition and dissemination of AMR traits of pathogens that are not in a parent-offspring correlation were investigated by natural transformation.

Present study found that >98% isolates are resistant against ≥ 2 antibiotics (MDR), >21% isolates are resistant against ≥ 10 antibiotics (XDR) and ~0.3% pathogens turned up resistant against ≥ 20 antibiotics. Thirty-two PDR isolates

showed resistance to nine different classes of antibiotics (b-lactams, aminoglycosides, amphenicols, quinolones, glycopeptides, sulfonamides, tetracyclines, macrolides, and pyrazinamide). Almost all the 32 PDR isolates harboured extrachromosomal genetic elements. Irrespective of the pathogen identity, the resistance traits are linked with replicative and/or integrative mobile genetic elements and could disseminate to other pathogens through horizontal gene transfer.

AMR enteric pathogens are highly prevalent in India and potentially threaten therapeutic efficacy of several drugs routinely used to treat diarrhoeal patients. The present study first time provided a comprehensive molecular genomics insights into PDR Indian isolates and might be useful to select preferable drugs in treating enteric infections.

Dr. Bhabatosh and his team

Understanding the diversity, dynamics and biology of vaginal microbiome of pregnant Indian women: A genomics perspectives

INVESTIGATOR
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(THSTI & WHO)

Objectives

- Understand the vaginal microbiome and biology of dominant vaginal microbiota prevalent in Indian women.
- Understand the role of vaginal microbiota, if any, in pregnancy outcomes.
- Possible use of vaginal microbiota in early prediction of preterm birth followed by an appropriate intervention to reduce preterm birth.
- Explore the possibility to use vaginal microbiota as potential probiotic candidate and identify potential bacteriocin against MDR pathogens, if any, from vaginal microbiota

Preterm birth (PTB) is a consequence of complex molecular process that triggers the quiescent uterus to expel the fetus before 37 weeks of gestation. Globally more than 10% babies are born preterm resulting 15 million preterm births annually. In India, the incident of PTB is around 13%. Multiple factors could affect PTB. While all risk factors are possibly important, we have recognized some risk factors that appear to be more important than others and regarding which there are critical gaps in knowledge. Multidisciplinary approaches, including metagenomics, genomics and proteomics are adopted to identify authentic risk factors and precise pathway that instigate PTB.

This study has adopted culture independent metagenomics approaches to elucidate diversity and dynamics of vaginal and gut microbiome in pregnant women. We are collecting high vaginal swabs (HVS) from pregnant women in four different time points and exploring diversity, dynamics and functions of bacterial component. Our initial focus will be to determine the difference in vaginal microbiomes in term and preterm birth women and define possible "core" microbiome with certain degree of variations within preterm birth women.

Progress:

- Genomic DNA was extracted from ~2000 HVS samples collected from four different visits of 1000 pregnant Indian women enrolled in the study "Inter-Institutional Program for Maternal, Neonatal and Infant Sciences: A translational approach to studying preterm birth".
- Sequencing 16S rRNA gene identified most dominated vaginal microbiota (GenBank Acc. No. KX057346 to KX057356).
- Five different Lactobacillus species were isolated from the HVS samples and the genomic DNA was extracted from all of them to explore the functional repertories.

MicrobDiab - Studies of interactions between the gut microbiome and the human host biology to elucidate novel aspects of the pathophysiology and pathogenesis of Type 2 Diabetes

INVESTIGATOR
Bhabatosh Das

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G. Balakrish Nair
(THSTI & WHO)

Objectives:

- Analyze the diversity and dynamics of gut microbiome of normal glucose tolerant (NGT), pre-diabetes and T2D patients of Indian and Danish origins.
- Asses how they associate with body composition, whole body insulin sensitivity, fasting and stimulated insulin secretion, inflammation markers, blood metabolomics, circulating microbial non-coding RNA and blood group markers.

The incidence of Type 2 Diabetes (T2D) has increased at an epidemic level and is accompanied by severe organ damage, which results in enormous costs on the health care system and lowers the quality of life and life expectancy of millions of people in India. Recent research indicates that altered gut microbiota composition and function may be involved in the pathogenesis of T2D and its co-morbidities. Therefore, there is a strong rationale to explore whether interactions between the gut microbiota as evaluated at the collective microbial genome level (the microbiome) and the host biology can provide novel insights into the pathophysiology and pathogenesis of pre-diabetes and T2D. The overall objective of the ongoing project is to identify gut microbiome signatures in Indian and Danish study participants which associate with pre-diabetes and T2D thereby enabling development of novel biomarkers for early diagnosis of people at high risk of progression to overt T2D.

Currently, we are (a) performing extensive phenotypes of 150 glucose tolerant individuals, 150 persons with pre-diabetes and 150 T2D patients from India and Denmark, a total of 900 individuals, (b) metagenomic approaches are adopted to identify phenotype-specific gut microbiome profiles at microbial

taxa and functional levels, (c) characterization of both common and ethnic specific gut microbiome patterns are going on to examine how they associate with the glucose tolerance state, insulin sensitivity, insulin secretion, inflammation markers, blood metabolomics, circulating microbial non-coding RNA and blood group markers, and (d) development and validation of microbiome markers that discriminate between individuals having various degrees of glucose tolerance.

Progress:

- Fecal and blood samples were collected from 450 Indian study subjects from three different groups with similar number of representation from glucose tolerant, pre-diabetes and T2D patients.
- DNA from 450 fecal samples was extracted using our laboratory-developed community DNA extraction method.
- Subsets of samples were used for determination of most abundant bacterial species in the stool samples. For the same, complete 16S rRNA gene was amplified, cloned and sequenced.
- Bacterial 16S rRNA genes of all the isolated DNA samples were amplified with adaptor and bar code tagged composite sequencing primers, specific for C1 and C5 regions. Purified samples were used for metagenomic sequencing using in-house 454 GS FLX+ pyrosequencer.
- 16S rRNA based metagenomic sequencing of 450 subjects is completed.
- Our initial analysis shown Gram-negative anaerobe Prevotella copri is the most dominated gut microbiota in all the Indian subjects regardless their physiological conditions.

The Effects of Human Intestinal Microbiota on Immune Responses

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G. Balakrish Nair
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Objectives:

- Explore the diversity of gut microbiome of healthy adult Indian and Japanese subjects.
- Understand the role of gut microbiota in maturation of gut immunity and protection against common enteric pathogens.

“Microbiome” refers to the ecological community of commensal, symbiotic, and pathogenic microorganisms living in a specific ecosystem. The distal gastrointestinal tract is the largest reservoir of microbes in the humans, and accommodates more than 1×1014 anaerobic and facultative aerobic microbial cells belonging to around 1000 distinct bacterial species. It is becoming increasingly evident that the gut microbiome has a role in defining states of health and disease. Furthermore, while a healthy subject typically harbors a distinct but fluctuating composition of microorganisms in the gut, the composition may be dramatically altered depending on the food habit, environmental exposure and host genotypes. Recent advances in the ability to conduct genomic analyses of microbiome by community DNA sequencing, along with access to large databases have opened up the prospect for conducting pioneering studies in this field. Currently, we are exploring the prokaryotic and eukaryotic microbial communities of the gut of healthy adult Indian and Japanese study subjects living in Osaka with the following objectives (a) Determine the prokaryotic and eukaryotic microbial

community structures in 50 healthy Indians and equal number of Japanese study subjects. (b) Determine the difference in gut microbiomes in Indian and Japanese individuals and define possible “core” microbiome with certain degree of variations within Indian and Japanese healthy people. (c) Based on the microbial composition predicts target population susceptible to enteric pathogens. (d) Study possible interactions in the gut microbiome. (e) Determine the presence of enteric pathogens in the gut of healthy subjects using highly efficient nested PCR approach. (f) Determine role of gut microbiome in host immune maturation.

Progress

- Faecal samples of 50 healthy adult Indians residing in the NCR region and 47 Japanese subjects living Osaka were collected.
- Communities DNA from all the collected samples were isolated. Bacterial 16S rRNA genes of all the isolated DNA samples were amplified with bar coded primers. Targeted metagenomic sequencing has been done for 97 samples projecting sequence variations in the V1-V5 regions. Distinct pattern of gut microbiota, both prokaryotic and eukaryotic, between Indian and Japanese subjects were observed.
- Presence of low level of enteric pathogens, including enterotoxigenic E. coli (ETEC), toxigenic V. cholerae and Shigella sp. in the gut of subset of healthy Indian subjects were determined.
- Sequences of O1 and O139 specific antigen encoding genes were detected in ctxAB positive stool samples.

Gut microbiome and gut inflammation in severe acute malnourished Indian children

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Objectives:

- Using metagenomics approaches explore cultureable and uncultured gut microbiota and envisioned interrelationships between the pattern of gut microbiome, level of gut inflammation and its impact on the nutritional status of children.

Malnutrition, characterized by symptoms like delayed growth, deficiencies in vital nutritional components etc., is a global health concern and affecting more than 300 million children worldwide. It is one of the major health concerns in India since 50% of children below the age of five suffer from various forms of malnutrition. In developing countries like India, malnutrition cannot be attributed to food insecurity alone. Efficient digestion of complex food ingredients and adsorption of nutrient are also crucial for normal development. The human gut microbiome, collective genomes of all the microbes residing in the gastrointestinal tracts, provides several metabolic functions that are not encoded in our own genome and plays important role in nutrient pre-processing, assimilation and energy harvest from ingested food particles. Consequently, dysbiosis of the gut microbiota has been implicated in malnutrition.

Currently, we are investigating gut microbiome and level of three different gut inflammatory markers (Neopterin, Calprotectin and Myeloperoxidase) in the faecal samples of 170 severely acute malnourished (SAM) children. Samples were collected at two different time points in a cohort of SAM children during enrollment and after treatment with antibiotics and ready-to-use therapeutic

food followed up by weekly up to 8 weeks or recovery, whichever is earlier. Gut inflammatory markers were measured by ELISA using commercially available kits. The level of Neopterin was detected by competitive ELISA, whereas the levels of Calprotectin and Myeloperoxidase were detected by sandwich ELISA. 16S rRNA based targeted metagenomics approach was adopted to investigate the gut microbiome of 340 samples collected from 170 SAM subjects.

Progress

- Community DNA from 340 fecal samples was extracted using our laboratory-developed community DNA extraction method.
- Bacterial 16S rRNA gene of all the isolated samples were amplified with adaptor and barcode tagged composite primers, specific for C1 and C5 regions. Purified samples were used for metagenomic sequencing using in-house 454 GS FLX+ pyrosequencer.
- All the stool samples were used to measure the levels of Neopterin, Calprotectin and Myeloperoxidase, three well-characterized biomarkers of gut inflammation.
- Levels of calprotectin and myeloperoxidase were determined by sandwiched ELISA whereas neopterin level was determined by competitive ELISA using commercially available kits.

Effect of environmental factors including diet and artificial sweeteners on gut microbiome and their consequences on type 2 diabetes

INVESTIGATOR
Prabhanshu Tripathi

Dr. Prabhanshu Tripathi

Consumption of sugary drinks is associated with increased risk of obesity and type 2 diabetes. Due to this set back, artificial sweeteners (AS) consumption became increasingly popular and were introduced largely in our diet in order to reduce calorie intake and normalize blood glucose levels without altering our taste for "sweetness". However, present literature on intake of 'AS' as a risk factor of type 2 diabetes are inconsistent. The aim of the present project is to determine the effects of diets, artificial sweeteners and medications on gut microbiota and consequences on host biology with emphasis on type 2 diabetes and establishment of possible therapies.

Type 2 diabetes is a metabolic disease primarily caused by obesity-linked insulin resistance. Both obesity and diabetes are characterized by a state of chronic low-grade inflammation with abnormal expression and production of multiple inflammatory mediators such as tumor necrosis factor and interleukins. In addition to well-established risk factors for type 2 diabetes including genetic predisposition, poor physical activity and obesity, an altered configuration of the microbial community in our gut 'the microbiota' have been linked to the increasing prevalence of type 2 diabetes. The gut microbiota includes members from all three domains of life (Bacteria, Archaea, and Eukarya) as well as viruses, but is dominated by anaerobic bacteria. The gut microbiota protects against pathogens and helps maturate and constantly help the immune system. It also plays a role for regulation of intestinal hormone secretion and for gastrointestinal nerve activity. Furthermore, members of the gut microbiota synthesize vitamin K and several B-vitamins including folate and vitamin B12 and they produce short chain fatty acids (SCFA) by fermentation of otherwise non-digestible carbohydrates.

Earlier studies in humans as well as in mice reported that obesity and

impaired glucose metabolism are associated with an altered ratio between the two major phyla in the human gut, Firmucutes and Bacteriodetes. It has been hypothesized that the microbiome may function by influencing fatty acid or carbohydrate metabolism, gut hormone concentrations, inflammatory pathway signaling, intestinal permeability, induction of proinflammatory states, and/or influencing the function of metabolically active liver and adipose tissues. The dissection of the relationship between the host and the microbiome is an exciting new field of research. If the microbiome can be shown to play a direct and important role in the predisposition for and development of diseases such as diabetes and obesity, it may also provide potential targets for treatment and prevention.

While some of the discrepancies between studies can be explained by ethnic or dietary differences other factors such as intake of medications are most likely to influence the bacterial composition and functional potentials. Very little information has so far been provided in any of the published studies on diet, malnutrition, artificial sweeteners and medication. Metformin, the first-line drug of choice for the treatment of type 2 diabetes, increases the levels of Akkamansia species in high fat fed mice in parallel to its beneficial effects on glucose metabolism. Supplying mice with A. muciniphila orally has been reported to result in improvements of glucose tolerance and metabolic dysfunctions such as metabolic endotoxemia and adipose tissue inflammation. These promising results not only suggest novel glucose-lowering mechanisms of metformin, but also provide future potential targets for altering glucose regulation by means of bacterio-therapy.

Dr. Prabhanshu and his team

The negative impact of consuming sugar-sweetened beverages on weight and other health outcomes has been increasingly recognized. Hence, many

people have started using artificial sweeteners like aspartame, sucralose, and saccharin as a way to reduce the risk of these consequences. However, upcoming evidences suggest that these artificial sweeteners may also be at increased risk of excessive weight gain, metabolic syndrome and type 2 diabetes. The aim of the present project is to determine the importance of the intestinal microbiota in regulation of glucose metabolism and their consequences. Moreover, the effects of diets (high fat or high fiber), artificial sweeteners and medications influencing the gut microbiota resulting in pathogenic conditions with emphasis on type 2 diabetes and possible therapies will be established.

Interplay between effector and regulatory T cells in the pathogenesis of intestinal inflammation

PRINCIPAL INVESTIGATOR
Amit Awasthi

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Dr. Amit Awasthi

In this project, we have proposed to understand the role of TGF-β3 in the induction and generation of pathogenic Th17 cells and how pathogenic Th17 cells are regulated in intestinal inflammation.

It is clearly and convincingly recognized that both pathogenic and nonpathogenic Th17 cells exist. Naïve T cells can be induced into Th17 cells in the presence of TGF-b1 plus IL-6 to become Th17 cells. These cells produce signature cytokines of Th17 cells like IL-17A, IL-17F, IL-21 and IL-22. In addition, Th17 cells induced by TGF-b1 and IL-6 also produce lot of IL-10 and IL-9 as well. Strikingly, these Th17 cells are non-pathogenic and unable transfer diseases like colitis and EAE in mouse models of IBD and multiple sclerosis indicating that the non-pathogenic nature of these cells, though they produce IL-17. However, once sensitized with IL-23, these non-pathogenic Th17 cells become pathogenic and induced high degree of tissue inflammation in colitis and EAE. Interestingly, IL-23-sensiized Th17 cells not only enhances the pathogenic markers of Th17 cells but also suppresses regulatory potential of Th17 cells by suppressing IL-10 production. Moreover, both IL-23- and IL-23R- deficient mice are completely resistant to developing EAE and colitis suggesting that IL-23 is not only required for inducing pathogenic Th17 cells but also critical for initiation of tissue inflammation in colitis and EAE.

To understand the role IL-23 in the induction of pathogenic Th17 cells as part of this project, we have performed microarray analysis of wild type Th17 cells and compared them IL-23R-/- Th17 cells, we have identified many genes which were differentially expressed between these two groups. We also identified the differential expression of TGF-β3 induced by IL-23 in Th17 cells suggested that this could be one of several genes essential for the development of pathogenic Th17 cells. We have further validated our results by various methods to identify the role of TGF-β3 in the induction and generation of pathogenic Th17 cells.

To further delineating the role of TGF-β3 in the induction and development pathogenic Th17 cells, we differentiated the Th17 cells in the presence of TGF-β3 plus IL-6 and compared them with TGF-β-induced Th17 cells. We found that both of these cell equally induced IL-17A and IL-17F genes. In addition, both of these cells induced similar level of RoRc, a master transcription of Th17 cells. However, TGF-β3-induced Th17 cells expressed very level of IL-23R expression as compared to TGF-β1-induced Th17 cells. This clearly suggested that TGF-β3-induced Th17 cells might be more pathogenic as compared to TGF-β1-induced Th17 cells as they express high surface expression IL-23R. To further validate our findings, we have compared both pathogenic and non-pathogenic Th17 cells by microarray analysis. Microarray analysis identified

the gene signature which are differentially expressed between TGF-β3 and TGF-β1 induced Th17 cells (Figure 9 and 10)

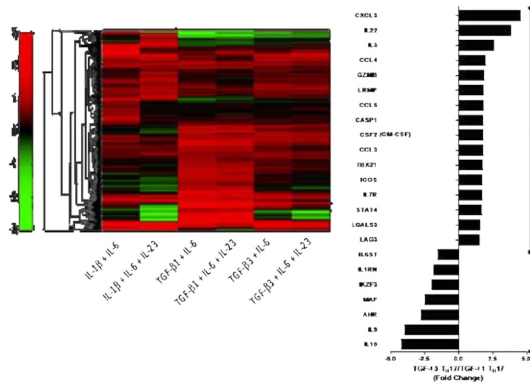

Figure-9: (a) Microarray analysis of TGF-b1/IL-6, TGF-b3/IL-6 and IL-1b/IL-6 with or without IL-23 treated sorted naïve CD4+ T cells. Expression of differentially expressed genes among these groups were shown, (b) differentially expressed genes in pathogenic vs non-pathogenic Th17 cells.

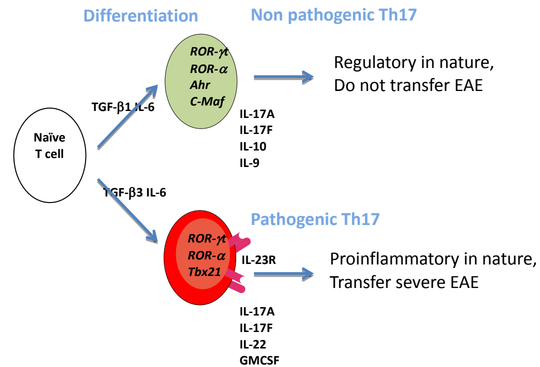

Figure -10: Understanding the induction of pathogenic TH17 cells. The antigenic stimulation of naïve T cells in the presence of TGF-β3, instead of TGF-b1, and IL-6 induce the development of pathogenic TH17 cells. While non-pathogenic Th17 cells express Ahr, c-maf, pathogenic Th17 cells express Tbx21. Non-pathogenic Th17 cells produce regulatory cytokines, IL-9 and IL-10, pathogenic Th17 cells produce Proinflammatory cytokines, Il-17 and GMCSF.

Dr. Amit and his team

IL-27 dependent regulation of Th17 and regulatory T cells

INVESTIGATOR
Amit Awasthi

In this project, we had proposed to determine the mechanism of IL-27-induced suppression of pathogenic Th17 cells.

Multiple mechanisms exist for IL-27-induced regulation of Th17 cells. IL-27 can suppress Th17 cells by directly targeting the expression of RoRβt. Moreover recently IL-27 was shown to induce the expression of PDL1 on T cell, which inhibits the development of Th17 cells through PD1-PDL1 interaction. These observations clearly indicated the existence of multiple mechanisms by IL-27 exerts its inhibitory functions on Th17 cells and Th17 cells associated tissue inflammation. To thoroughly study other additional mechanisms by which IL-27 regulated Th17 cells and the IL-23R expression, we performed a microarray analysis of TH17 cells in the presence or absence of IL-27. Comparing the two cell populations, we observe an overall high level of similarity (data not shown; Pearson r2=0.99, p<10-10) between the two treatment groups. Focusing on outliers, we observed that only a small fraction of the genes (~2%) showed a significant (>2-fold) change (either increase or decrease, Fig. 1A; Table 1).

These included Il17a, Rorβ, Ccr6, Il12rb2 and Tbx21. Our microarray data revealed that IL-27 induced expression of IL-12Rβ2 on Th17 cells, which could be essential for the suppression of Th17 cells development (Figure 11). Using IL-12Rβ2-deficient mice model, we further validated our findings that IL-27-inudced IL-12Rβ2 expression is essential for suppression of Th17 cells.

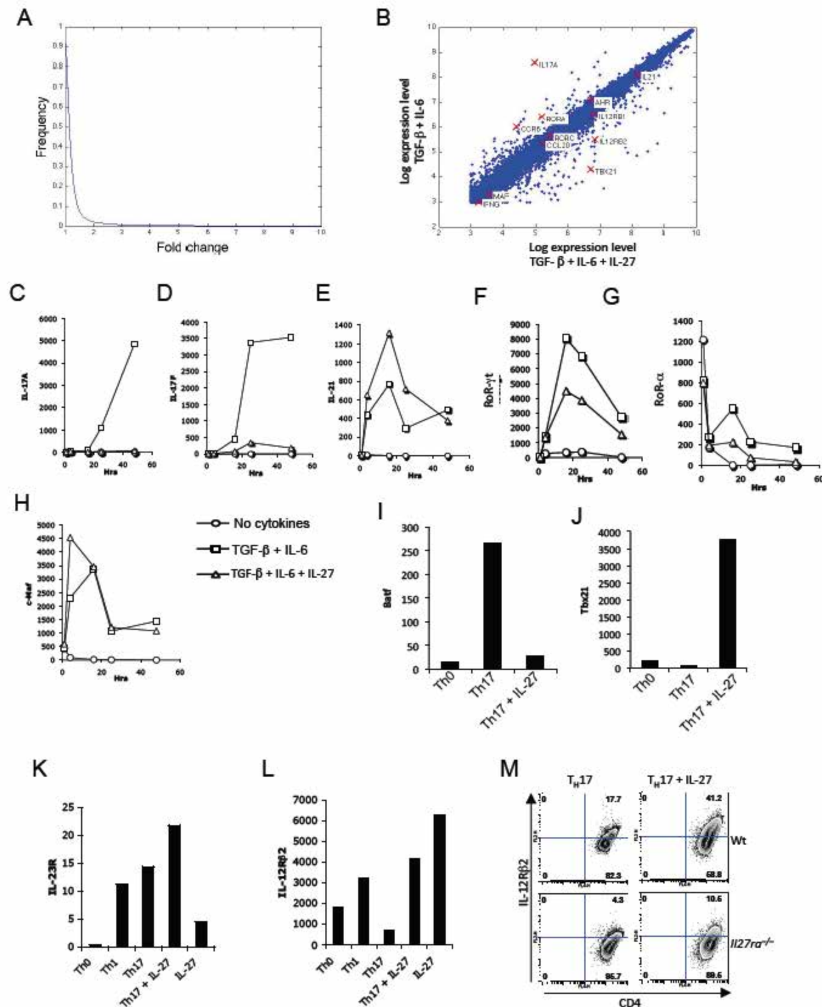

Figure 11. IL-27 induces the expression of IL-12Rb2 on Th17 cells

(a, b) Microarray analysis of Th17 cells treated with IL-27 and the expression of differentially expressed genes were determined. c-h, time kinetic analysis of indicated genes on Th17 cells treated with IL-27. i-l, expression of Batf, Tbx21, IL-12Rb1 and IL-12Rb2 was determined by qPCR, and the protein expression of IL-12Rb2 was determined on Th17 cells by FACS (m).

INVESTIGATOR
Amit Awasthi

Role retinoic acid in regulating inflammatory response

In this project we proposed to determine the following objectives:

a) Does retinoic acid generate reciprocally regulate the generation of effector and regulatory T cells in mice and human?
b) What are the micro-environmental conditions in human colonic mucosa, which nudge retinoic acid an active metabolite of Vitamin A to play a sustained pro-inflammatory role?

We have found that Retinoic acid significantly enhances the generation Foxp3+ Treg cells while suppresses the induction of Th9 cells. Interestingly, splenic DCs promote the induction and generation of Th9 cells while mesenteric lymph node (MLN) DCs suppress the development of Th9 cells and promote the induction of Foxp3+Treg cells. We further identified that MLN DCs mediate the suppression of proinflammatory T cells via retinoic acid.

In addition pro-inflammatory T cells like Th17 cells and Th1 cells in healthy human subject. We first determined as to how Retinoic acid enhanced or suppressed human CD4+ T cells differentiation into Th1 and Th17 cells as these are the two major effector T cells play are dominant role in inducing tissue inflammation in IBD. Culturing sorted naïve T cells into Th1 or Th17 culture condition in combination with Retinoic acid resulted enhanced Th1 and Th17 cells development.

We further tested as to how the Retinoic acid-treated dendritic cells (DCs) influence T cells differentiation into Th1 or Th17 cells. Interestingly, we found that Retinoic acid treated-DCs showed immature DCs phenotypes as compared to the DCs, which were treated with LPS alone. These RA-treated DCs enhanced the differentiation of Th1 and Th17 cells. After the determining the initial functions of Retinoic acid in T cells differentiation and DCs functions, now we will determine how the serum concentration of vitamin A in IBD pateints and its co-relation with inflammation, T cells differentiation and disease pathogenesis.

CHME Team

Peer-reviewed publications

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Patents

Patent Application type:	Provisional Indian Patent Application [2706/DEL/2015]
Title:	A Process for Preparation of Nanoparticles Encapsulated Antibacterial Drug and the Product Obtained thereby
Date of Filing:	August 28th, 2015
Inventors:	Amit Awasthi, Sakshi Malik, Ramendra Pati Pandey

Seminars and Conference

Dr. Bhabatosh Das

Title of the talk:	Antimicrobial Resistant Enteric Pathogens: Genomics Advances and Future Perspectives
Name of the Meeting:	3rd Biennial Conference & International Symposium on Stress, Microbiome & Probiotics
Date and Place:	March 11-13, 2016: NISER Bhubaneswar, Odisha

Title of the talk:	Antibiotic resistance: Molecular insights into resistance traits of human enteric pathogens
Name of the Meeting:	56th Annual Conference of Association of Microbiologists of India (AMI 2015)
Date and Place:	December 7-10, 2015, JNU, New Delhi

Title of the talk:	Placental microbiome and its role in pregnancy outcomes
Name of the Meeting:	Provocative ideas on Human Placental Biology; Department of Biotechnology, Ministry of Science and Technology, Govt. of India, New Delhi
Date and Place:	December 1-2, 2015, New Delhi.

Dr. Amit Awasthi

Role in the event:	Chairperson
Name of the event:	World Immunology Day
Place and Date:	THSTI, Faridabad (April 29, 2015)

Extramural Grants

Funding agency:	Bill & Melinda Gates Foundation
Title:	Evaluation of available rapid diagnostic tests for cholera
Duration:	November 2015 to October 2017
Amount:	$ 1,59,775

Funding Agency:	Department of Biotechnology (Govt. of India)
Title:	Effect of environmental factors including diet and artificial sweeteners on gut microbiome and their consequences on type 2 diabetes
Duration:	2016-2020
Amount:	Rs. 88 lacs

Funding agency:	DBT-Wellcome Trust India Alliance
Title:	Interplay between effector and regulatory T cells in the pathogenesis of intestinal inflammation
Duration:	2012-2017
Amount:	3.5 Cr. (Intermediate fellowship)

Title:	Vitamin A is the “micro-environmental cue” for triggering disease activity in patients with Inflammatory Bowel disease (Ulcerative colitis, Crohn’s Disease).
Duration:	2013-2016
Amount:	26, 62, 0000.00

Title	Human gastrointestinal immunology translational program (Glue grant)
Duration:	2014-2019
Funding agency:	Department of Biotechnology, Govt. of India
Amount:	46,10,800.00

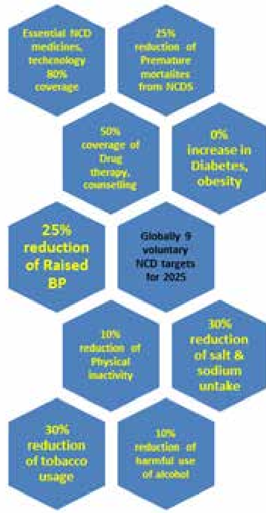
Honors and Awards

Dr. Bhabatosh Das participated in the World Health Organization (WHO) "Global Workshop on Strengthening Integrated Surveillance of Foodborne Diseases and Antimicrobial Resistance through the Whole Genome Sequencing methods" held in Thammasat University, Bangkok, Thailand during April 4-8, 2016.

Dr. Prabhanshu Tripathi got re-entry in Ramalingaswami fellowship from Department of Biotechnology (Govt. of India).

Dr. Amit Awasthi got membership of American Association of Immunologist (AAI).

Policy Center for Biomedical Research (PCBR)



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- Assessment for Global Strategy and Plan of Action on Public Health, Innovation and Intellectual Property for India 138
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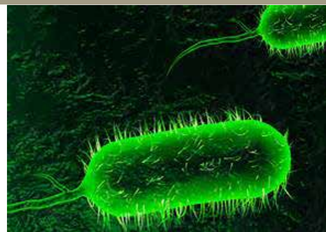

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An Overview

Dr. N. K. Ganguly

The Policy Center of Biomedical Research (PCBR) was conceptualized by the Department of Biotechnology (DBT) during the planning phase of THSTI and functioned in a project mode between 2007 and 2012, before becoming a centre of THSTI, providing inputs in the following areas: Suggesting intelligent Ideas for innovation; Exploring strategies where there are opportunities but no implementation; Technology diffusion and demand creation. PCBR research revolves around the following broad areas:

Suggesting intelligent Ideas for innovation: The centre looks at priority setting based on an appropriate matrix suitable for national, geographical and sub-geographical needs, in disease areas where burden is high. The needs assessment exercise is based on either a meeting of stakeholders or reaching a consensus through appropriately designed e-surveys, followed by analysis of data based on a globally acceptable approach.

Exploring strategies where there are opportunities but no implementation: There are opportunities in public health where interventions available globally are not being implemented in India. A division of the unit explores alternate/ innovative strategies to address implementation.

Technology diffusion and demand creation: This activity is executed by collaborating with appropriate stakeholders and communicating with policy-makers, parliamentarians, civil society organizations etc. to facilitate uptake of a product is useful for public health.

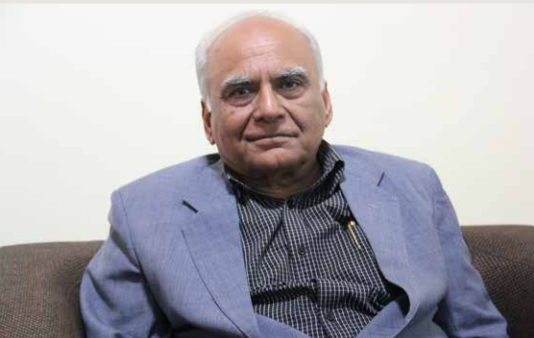
The center has created networks with the following agencies:
ICMR, MOH, BMGF, NICED, DOVE- JHU, RMRCBhubaneswar, PGIMER, RGCB, Trivandrum, NIE, WaSH @ UNICEF India, IDEA Asia, WHO GTFCC, Sabin Vaccine Institute, CDC USA,AHREF, GHS, MSD, State Health Departments of AndhraPradesh,Sikkim, Punjab and Delhi, FOGSI.

PCBR has forged partnerships with the following agencies:International Vaccine Access Center (IVAC),Global Health Strategies (GHS), New Delhi,Health Policy Unit of Institute of Economic Growth (IEG), DU, Center for Disease Control- India (CDC India), National Center for Disease Control (NCDC) and Manipal Center for Virus Research (MCVR), Manipal University.

The studies conducted by PCBR are:

INVESTIGATORS

Bratati Mukhopadhyay
N. K. Ganguly



Dr. N. K. Ganguly

Flagship Program on Point of Care (PoC) Diagnostics

Comprehensive analysis of health technologies on antimicrobial resistance (AMR) diagnostics to identify novel, indigenous, cost-effective platform technologies to facilitate integration at point of care.

Under the flagship program on PoC diagnostics for communicable diseases in India, current AMR diagnostics were landscaped.The challenge is to create an accurate, rapid, cost-effective and easy-to-use point of care test kit for bacterial infections to help reduce misdiagnosis and prescription. Challenges include the recognition of diagnostics as a critical tool in a larger AMR control strategy, better regulatory guidance on development of diagnostics and the harmonization of regulations.

PCBR has facilitateda Bangalore based investigator to develop and validate anindigenous, novel fast track test for 8 bacteria and 8 viruses in a panel appropriate for India. The investigator has been included under the ICMR pneumonia aetiology network for developing and validating the technology.

PCBR has started gathering data from laboratories across India on use of Rapid diagnostics tests (RDT)s for Cholera to assist an ongoing project of THSTI/ CHME by Prof. T Ramamurthy (PI)on comparative analysis of 4 RDTs in a Delhi based public hospital setting. The information will supplement to strengthen the claim that RDT for cholera diagnosisis a useful tool providing same day diagnosis and treatment options for outbreak and surveillance.

INVESTIGATORS

N. K. Ganguly
Bratati Mukhopadhyay

CONTRIBUTORS

Gautam Kumar Saha
Swati Verma
Nisha Arora

COLLABORATORS

Shah Hossain
Mayank Dwivedi
(CDC India)
G. Arun Kumar
(Manipal Virology Research Centre, Mangalore)

Dr. Bratati Mukhopadhyay

Laboratory mapping on public health, bio-surveillance and global security in order to prevent and tackle disease outbreaks

Survey of Biosafety and Biosurveillance practises in Public Health Laboratories in India to strengthen their capacity to tackle Infectious diseases outbreak, emergency responses etc.

A pan India exercise on the Laboratory mapping on public health, biosurveillance and global security was initiated in partnership with the ManipalCenter for virus research. A survey questionnaire were sent to all academic, diagnostic and R & D organizations carrying out work on infectious diseases. Analysis is being carried out prior to report dissemination. Of the 129 respondents, 75% were in the public sector. (Fig.1)

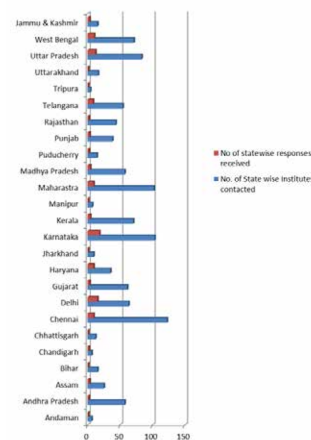


Figure-1: Number of Institutes contacted vs number of responses received on the Biosafety mapping from Indian States /UT

The survey covered participation in established national surveillance networks such as: Polio, TB/RNTCP, HIV/NACO, Vector Borne Diseases/NVBDCP, IntegratedDisease Surveillance Program/IDSP, Influenza,Measles/ Other Vaccine Preventable Diseases,Global Foodborne Infection Network,Animal Disease Surveillance Network. It evaluated presence of external quality assurance, biosafety and types of samples handled and showed that the majority deal with human pathogens, a limited number handle both animal and human pathogens, few receive human, animal and environmental pathogens. Per the survey, there is a

significant gap in the ability of laboratories in India to handle food pathogens.

Conclusion: Mapping is a useful tool to assess preparedness and it is evident that in the context of tackling national health security, surveillance, handling of public health threats and emergency responses, a strategic engagement is needed. The Virus Research and Diagnostics Laboratory Network of ICMR is a potentially useful information source with 36 centres.

Assessment for Global Strategy and Plan of Action on Public Health, Innovation and Intellectual Property for India

INVESTIGATOR
N. K. Ganguly

CONTRIBUTORS
Bratati Mukhopadhyay
Sanjukta Sen Gupta
Gautam Kumar Saha
Swati Verma
Nisha Arora

COLLABORATORS
Manisha Sreedhar (WHO SEARO)
Madhur Gupta (WCO, New Delhi)

Who requested a situation analysis for India on Global Strategy and Plan of Action on Public Health, Innovation and Intellectual Property announced at the World Health Assembly as resolution WHA 61.21 and published in 2011.

Conclusion: A report analysing 8 different yet interlinked elements on Health R & D has been prepared and submitted to the WHO, and can be shared more widely once approved.

Research strengthening in the WHO South East Asian Region

INVESTIGATORS
N. K. Ganguly
Dipika Sur

CONTRUBUTORS
Bratati Mukhopadhyay
Sanjukta Sen Gupta
Gautam Kumar Saha
Swati Verma
Nisha Arora

PCBR was assigned to draft a plan for discussions to identify, prioritize for strategic actions, and list the steps needed to accomplish the objectives necessary to create health research systems to support the implementation of health programmes within the Region. The plan will support outstanding research teams within as well as encourage collaboration among Member States.

Figure-2: NCD targets by WHO for 2025 (Source http://www.who.int/nmh/global_monitoring_framework/en/)

The potential drivers identified by the technical committee were strengthening the major flagship programs in the region with need for research capacity strengthening for i) elimination program for NTDs in the region by 2020 and elimination of vaccine preventable diseases with focus on measles & rubella, ii) In Bhutan, Timor Leste and Maldives major impetus is needed to provide universal health care needs for the population with respect to health programs, financial assistance for development of human resources and infrastructural assistance, iii) the control of rising NCDs, antimicrobial resistance, providing emergency response in disease outbreaks and iv) the need for providing Universal Health Coverage (UHC). Health systems function best with high coverage of UHC and the best functioning systems are supported by R & D that helps create appropriate responses to the emerging health problems. The major area of focus to improve UHC is through providing access to drugs, addressing health workforce issues (e.g training & retaining them), developing methods for continuous monitoring of UHC andequal importance being given to both formulation and implementation of projects.

The document prepared served as background document /agenda for ACHR

meeting held in New Delhi in Dec 2015.

Conclusion: To promote health research among the member nations it is imperative to have a consensus to build a robust frame; where each Member State would update institutional research action plans, will be equipped with knowledge on the regional research framework and country cooperation teamwork, based on above priorities. WHO will continue to support this process to assist countries to strengthen their capacities and implement research activities byRD's initiatives.(Fig.2)

Enteric fever in India- a retrospective review of existing data on surveillance for enteric fever in Asia project (SEAP Phase I)

INVESTIGATOR
N . K. Ganguly

CONTRIBUTORS
Dipika Sur
Bratati Mukhopadhyay
Swati Verma
Nisha Arora
Radhesh Nautiyal
Prity Kapur

COLLABORATORS
Denise Garrett and Caitlin Bercume
(Sabin Vaccine Institte (SVI), USA)

Typhoid fever caused by Salmonella typhi remains a serious systemic infection in the developing world, but there is a lack of accurate data on true burden of disease of typhoid fever. This study will collect two years of hospital based retrospective data from 5 hospitals in India.

The protocol was finalized, SVI collaborators and PCBR coordinators visited the hospitals sites, checked the archived medical records and finalized the sitesand site PIs.Five hospitals across India namely Medanta Medicity Hospital, Gurgaon, Apollo Hospital, Kolkata, PGIMER, Chandigarh, CMC, Vellore and Kasturba Hospital, Manipal, Managalore have collected data following IRB clearance and appropriate training with online data entry.

Conclusion: Data analysis is ongoing. The project will highlight the latest disease burden, age distribution of laboratory confirmed enteric fever cases and will facilitate policy decisions on prevention and control of the disease, ranging from strengthening of surveillance sysytem, better and effective treatment options , safe water and hygiene interventions and introduction of newer typhoid conjugate vaccine in India.

Creation of a Roadmap for Cholera prevention and control in India

INVESTIGATORS
G. B. Nair (till Oct 2016)
N. K Ganguly
Sanjukta Sen Gupta

EXPERTS & CONTRIBUTORS
G. B Nair
(as Advisor, since Nov 2016)
Dipika Sur
T. Ramamurthy
Shanta Dutta
(NICED)
Mohamad Ali & David Sack
(JHU)
Nisha Arora

Creation of roadmap is in progress which included collation and analysis of published and unpublished data on cholera as well as on water, sanitation and hygiene in India to landscape current activities and prepare for future design of interventional strategies to prevent and control cholera in country.

An Expert Group for Cholera in India was set up to guide the creation of a national plan. This group includes subject experts, members of the immunization division in the Ministry of Health and Family Welfare, UNICEF, SEARO, BMGF and DOVE at John Hopkins University, apart from representatives of industry and key institutes that work on Cholera.

Dr. Sanjukta Sen Gupta

The project is ongoing, data on number of cases and deaths due to cholera have been obtained from IDSP for the years 2012-2015. Analysis for morbidity and deaths, identification of "hotspot for cholera" in conjunction with other data at district level viz. coverage of clean water & sanitation, literacy, indicators of wealth etc. that help assess the risk for cholera and identification of laboratory capacity for cholera surveillance have been carried out.The decision making process, financing vaccine supply, vaccine dosing schedule, economic implications on trade and tourism etc. from countries that have used OCV, have been complied. Further analysis is in progress. A policymakers meet has been planned in the first quarter of 2017.

Flagship program on Mother and Child Health

INVESTIGATOR
Dipika Sur

CONTRIBUTORS
Swati Verma
Nisha Arora

Dr. Dipika Sur

Objective: The main objective of this flagship program is to identify the gaps in innovations and landscape the successful technologies/solutions currently being implemented in different parts of the world to create affordable, sustainable solutions for promoting maternal & child health and achieve the MDG goals 4 and 5.

Role of mHealth and eHealth in the reduction of maternal and newborn morbidity and mortality in different regional countries have been studied and following outcomes are suggested:

- The catalytic impact of the investments will translate into clear survival benefits for mothers and newborns at risk of dying from preventable and curable diseases each year.
- It will reduce the delays that prevent timely access and utilization of needed services for mothers and newborns.
- Strategic integration of eHealth and mHealth at the most appropriate delivery points along the maternal newborn continuum of care will strengthen the health systems.
- Capacity of frontline health workers who deliver life- saving interventions in the most remote, hard –to- reach populations, will be enhanced.

International Meetings organized by the Center

One of the major activities of PCBR is to trigger discussion and get a concensus on a particular subject through organisation of multi-stakeholder meetings in which national and international particpants from academia, government (both state and central), NGOs as well as industry participate. We also have representatives of major funding and coalitions for technical support in global health like WHO, UNICEF, GAVI, BMGF etc. participate in these meetings. The concensus recomendation of such meetings is then circulated for consideration for priority setting and in decision making.

ORGANIZER

Bratati Mukhopadhyay
N. K. Ganguly

PARTNER

U.S. National Academy of Sciences

A workshop organized on "An Introduction to Educational Institutes for Responsible Science"

The above mentioned workshop was jointly organized by PCBR with the U.S. National Academy of Sciences on May 5-6, 2015 at National Institute of Immunology, New Delhi meant for the faculty/university staff who have been working on Science teaching.
The goals of this event were:

- Introduce the concept of Responsible Conduct of Science through Active Learning
- Expand the number of Indian scientists across a variety of institutions affiliated with higher education and research who know about the Institutes
- Listen to and record impressions/feedback from experiences in India
- Identify key interested persons and institutions for further engagement
- Identify options/needs for next steps in India

A meeting report has been prepared.

ORGANIZERS

N. K. Ganguly
Sanjukta Sen Gupta

COLLABORATORS

International Vaccine Acess Center
UNICEF
Immunisation Technical Support unit
Global Health Strategies, New Delhi

International Symposium on Pneumococcal conjugate vaccines in India: A roadmap to introduction, Taj Mahal, Mansingh Road New Delhi (16-17 Nov 2015).

An International symposium was organized by the PCBR with partners to build consensus on the evidence-based policy roadmap for PCV introduction in India and lay out options for PCV introduction in India. 135 participants from around the globe attended the meeting. Discussions focussed on the global status and regional experience with the introduction and use of PCV, impact of PCV on pneumococcal disease from regional and worldwide studies, design options for pneumococcal surveillance and impact evaluations, pneumococcal diagnosis, issues in PCV procurement, supply, delivery and implementation, sustainable financing and communications and advocacy. A roadmap that created by a group of experts was discussed in a working group meeting during the symposium.

Peer-reviewed Publications

1. Hajela N, Ramakrishna BS, Nair GB, Abraham P, Gopalan S, Ganguly NK. Gut microbiome, gut function, and probiotics: Implications for health. Indian J Gastroenterol. 2015 Mar;34(2):93-107. doi: 10.1007/s12664-015-0547-6. Epub 2015 Apr 29.PMID: 25917520

2. The State of the World's Antibiotics 2015. Helen Gelbrand, Molly Miller-Petrie, Suraj Pant, SumanthGandra, Jordan Levinson, Devra Barter, Andrea White, RamananLaxminarayan, N K Ganguly, Samuel Kariuki, Linus Ndegwa, Eveline Wesangula, BetuelSigaúque, EsperançaSevene, Buddha Basnynat, Paras Pokharel, Sameer Mani Dixit, SantoshiGiri, Adriano Duse, Olga Perovic, Kim Faure, Said Aboud, Robinson Mdegela, Khadija Msami, Denis K Byarugaba, Donna A Kusemererwa, James Lakony, Nguyen Van Kinh, Heiman Wertheim, Do Thuy Nga. Wound Healing Southern Africa 2015;8(2):30-34

3. Balasegaram M, Bréchot C, Farrar J, Heymann D, Ganguly N, Khor M, et al. (2015) A Global Biomedical R&D Fund and Mechanism for Innovations of Public Health Importance. PLoS Med 12(5): e1001831. doi:10.1371/journal.pmed.1001831

4. News Paper Article : A vaccine Can Stop The Zika Virus: N K Ganguly; Hindustan times 28th Feb 2016

5. Editorial Board member of Book published: Excellence in hospital leadership. Published by IIM Ahmedabad.

6. Acknowledged for contribution to book "Global Population Health Well-Being In the 21st Century: Towards New paradigms, Policy and Practice" by George R. Lueddeke.

G K Saha

Contributed to G-finder Survey Report 2015: Neglected Disease Research & development: The Ebola effect: Published by Policy Cures in Dec 2015

Seminars and Conferences

Dr. Prof. N. K. Ganguly

Title of the presentation: "Changing Patterns of Cardiovascular Diseases in Developing Countries" on Cardiac Diseases
Name of the meeting: National Health Seminar
Place and Date: Delhi, July 2015, Delhi

Title of the presentation: Antimicrobial Resistance in Developing Countries
Place and Date: Geneva in Nov, 2015

Title of the presentation: STEPS "India-Pneumo Summit"
Place and Date: October 2015 in Mumbai

Title of the presentation: "Value of Vaccines"
Name of the meeting: IMMUNOCON 2015, Indian Immunology Society
Place and Date: Oct. 2015, RMRI, Patna

Title of the presentation:	The use of technology platform to tackle the various outbreaks of India
Name of the meeting:	Society for Biotechnologists (India) Lakeshore Hospital and Research Centre Ltd.
Place and Date:	Kochi, Kerala ,December, 2015.

Title of the presentation:	Orphan Drugs are Needed
Name of the meeting:	Meeting - Rare Disease
Place and Date:	IHC, Delhi Jan, 2016

Title of the presentation:	Emergency Structure To Tackle Public Health Emergencies In India
Place and Date:	Seychelles, 2016

Title of the presentation:	Probiotics & Vaccine
Name of the meeting:	PAi Conference
Place and Date:	Bhubaneshwar March, 2016.

Title of the presentation:	India's perspective on antibiotic resistance
Name of the meeting:	17th International Congress on Infectious Disease (ICID)
Place and Date:	Hyderabad, March, 2016

Dr. Sanjukta Sen Gupta

Title of the presentation:	Activities in cholera control in India.
Name of the meeting:	4th IDEA Asia meeting
Place and Date:	New Delhi , 1st April 2015

Title of the presentation :	Progress in the strategies against Cholera in Asia
Name of the Meeting :	4 th IDEA Africa
Place and Date:	Cotonou in Benin, Africa (5-9 Oct, 2015)

Dr. Dipika Sur

Title of the presentation:	Challenges in new vaccine introduction in National Programme in India
Name of the meeting:	National Conference of Indian Public Health Association in HIMS
Place and Date:	Dehradun, February, 2016

Extramural Grants

Funding Agency:	Manipal Virology Center, Manipal
Title of grant:	Laboratory mapping on public health, bio-surveillance and global security in order to prevent and tackle disease outbreaks
Duration:	April-September, 2016.
Amount:	13 Lakhs

Funding Agency:	Bill and Melinda Gates Foundation
Title of Grant:	Creation of a road map for cholera prevention and control
Duration:	2015-2017
Amount:	68 Lakhs

Funding Agency:	WHO-Country Office, New Delhi.
Title of Project:	Global Strategy plan of action on PH, IPR for 8 elements
Amount:	27.7 Lakhs

Funding Agency:	WHO-SEARO, New Delhi,.
Title of grant:	Collaborate with technical department, identify drivers for promoting a vibrant research culture in SEA ACHR and provide a roadmap to strengthen the research in SEA region
Amount:	8.16 Lakhs

Title of grant:	Enteric fever in India- a retrospective review of existing data on surveillance for enteric fever in Asia project (SEAP)
Funding Agency:	Sabin Vaccine Institute, USA
Duration:	April-September, 2016.
Amount:	80 Lakhs

Awards

Dr. Prof. N. K. Ganguly

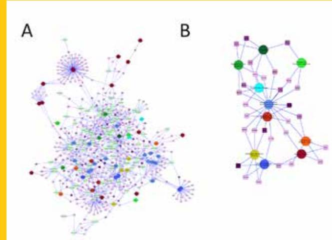
- Dr. D Sundaresan Memorial Lecture Award on 11th February 2015 at NDRI, Karnal: on Probiotics and Vaccine
- Padmabhushan Professor M.V. Pylee Life Time Achievement Award 17th December, 2015 awarded by Society for Biotechnologists, India.
- Helmholtz International Fellow Award (2015): Awarded by the Helmholtz Center for Infection Research, Germany.
- Distinguished Scientific Award by Yakult Central University

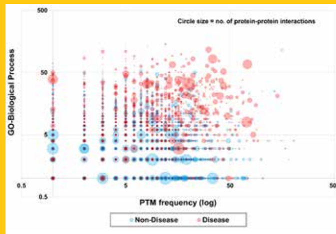
Dr. Sanjukta Sen Gupta

Invitation to participate in the 9th International Conference on Typhoid and Invasive NTS Disease orgnaised by Coalition against Typhoid (CaT) of Sabin Vaccine Institute at Bali, Indonesia (April 30- May 3 ,2015) to deliberate on development on Vi typhoid conjugate vaccine.

PCBR team

Drug Discovery Research Center (DDRC)





Integrating a platform for disease interrogation and drug target identification

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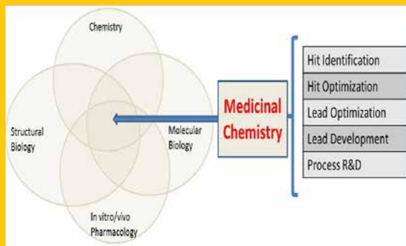

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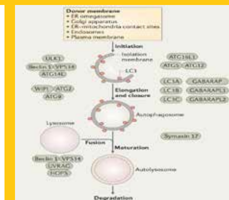

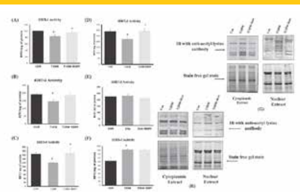

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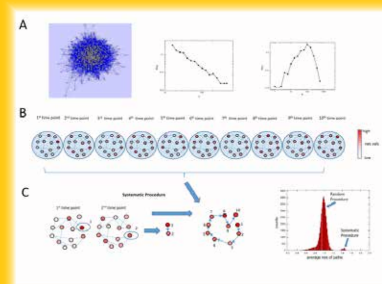

An Overview

Dr. Kanury V. S. Rao

The Drug Discovery research Centre (DDRC) has been designed as a multi-disciplinary unit that integrates basic with translational research in the field of drug discovery. Its overall mission is to synthesize diverse disciplines to generate a robust and innovative pipeline for drug discovery research. Convergence between varied disciplines such as mathematics, computational sciences, and cell and molecular biology is employed for interrogating disease-specific molecular network properties and delineation of regions of sensitivity. Seamless linkage of this activity with downstream capabilities in high-content screening, structural biology, synthetic and medicinal chemistry and pharmacology then anchors a facile workflow from target identification to lead development and optimization. The expertise domains that DDRC incorporates are the following:

- Assay development and High-content screening: Development and standardization of robust, sensitive, and reproducible platforms for high-content, medium-throughput screening.
- Synthetic and Medicinal Chemistry: Strong capabilities for organic synthesis and SAR optimization, coupled with medicinal chemistry expertise.
- Cell and Molecular Biology: Expertise in developing new tools and approaches for analyzing the basis of disease-specific phenotypic perturbations in cellular systems. Research emphasis on integrating high-throughput experimental approaches with the tools of systems biology to delineate disease-specific networks.
- Structural Biology: Cloning, expression and purification of proteins/ protein complexes. Biochemical characterization through activity and kinetic assays. Biophysical characterization by SPR, ITC, co-elution, GPC and SAXs. Structure determination of protein, protein-ligand, and protein-protein complexes.
- Computational and Mathematical Biology: Incorporate and develop expertise in all aspects of high-throughput data analysis, network biology, and mathematical modeling of network dynamics. Capabilities for data handling extend across all molecular components and processes of a cell, complemented by strong expertise in the modelling of complex systems behavior. The latter is synergistically approached from both network-based and purely mathematical strategies. Additional areas of expertise include cheminformatics, bioinformatics, in silico drug design and computational mass spectrometry.

Research Focus at DDRC

The present area of focus of DDRC is the metabolic syndrome (MetS). MetS is a chronic progressive disorder that has become a global public health concern, in addition to emerging as an epidemic of 21st century India. The etiology of this syndrome is complex as it its pathophysiology. In addition to genetics, the contributory factors include ageing, diet, low physical activity, stress, disrupted chronobiology/sleep, mood disorders, excessive alcohol intake, and smoking. While MetS was traditionally thought to afflict the elderly, recent years have seen an increasing incidence in the younger population. This is largely a result lifestyle changes where the effect of reduced physical activity is compounded by increased consumption of high calorie diets.

The operative paradigm at DDRC is that a better understanding of MetS can only be achieved by taking both its temporal features and its multivariate character into account. Given that MetS finds differential expression in terms of clinical symptoms in the human population, our view is that capturing the underlying variables, and defining their relationship to the eventual outcome will be central to eventually gaining a grip on the problem. Broadly therefore, the aim is to extract the dynamical network that captures initiation, progression, and development of MetS; and then exploit this data for the purposes of vulnerable target identification and subsequent drug development. To this end, research at DDRC is presently being conducted under four broad themes. These are:

- Integrating a platform for disease interrogation and drug target identification
- Lead discovery and development
- Early translation
- Discovery research

Research under each of the above themes combines both experimental and theoretical approaches that converge through the collaborative participation of multiple investigators. Although collaborative in nature each program is, however, intellectually driven by a specific Team Leader who is also responsible for maintaining focus and momentum of the project.

Theme 1: Integrating a platform for disease interrogation and drug target identification

1.1 Mathematical Biology

1.1.1 Use of models trajectories to understand the regulatory mechanisms underlying MetS

TEAM LEADERS
Samrat Chatterjee
Kanury Rao

CO-INVESTIGATOR
Shilpa Jamwal

RESEARCH PARTICIPANT
Rajat Anand

Metabolic disorders such as obesity and diabetes develop gradually over time in an individual. Recently, systematic experiments tracking disease progression are conducted giving a high throughput complex data. There is a pressing need for developing methods to analyze this complex data to capture disease development at molecular level. The broad aim of the present project is to develop tools to capture the mechanism for a disease development over time. If we can link the initial molecular perturbations to the final disease state

Dr. Samrat Chatterjee

molecular profile, it will help us to design preventive therapeutic strategies. To generate the methodologies for such complex analysis we have been employing microarray data from the mouse model of diet induced MetS that had been previously generated. In these experiments C57/Bl6J mice were kept either on normal diet (ND), or on a diet containing high levels of fat and sucrose (HFHSD) for a period of eighteen weeks. Periodic monitoring of these animals revealed that, in contrast to the former group, mice in the latter developed the classical symptoms of MetS. This included a marked gain in weight along with the development diabetes, dyslipidemia, and chronic systemic inflammation. Starting from 24 hrs after initiation of the feed, and stretching up to the 18-week time point, different tissues (including liver, adipose tissue, skeletal muscle, hippocampus etc.) were periodically collected from these mice. RNA was extracted from these tissues and the gene expression pattern determined through a microarray analysis. We at DDRC have first taken the liver-specific gene expression data for developing methods that capture disease initiation, progression and establishment.

In the micro-array data, each probe had three replicate samples and their respective geometric mean with p-value. The gene expressions in the list were given in log2-scale ratio between different groups with ND group. A preliminary analysis was first conducted in order to reduce noise from the data and select only those genes that were significantly perturbed at one or more time points with high confidence. This exercise generated a matrix with significantly perturbed genes, and their temporal expression patterns.

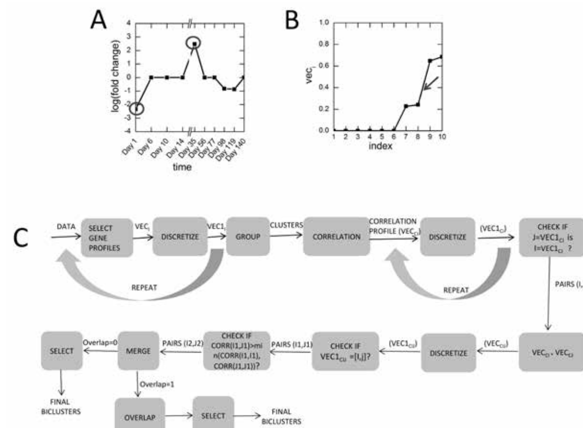

Figure-1: Discretization and clustering of co-expression genes

From this we developed and employed a novel cluster analysis method to group the genes based on their temporal pattern (co-expression conditions), and then probed for the functional classes or the pathways shown by those clusters. Iterative rounds of this exercise have now identified a cluster of 50 highly perturbed genes showing perturbation for a significant time period of disease progression (Figure 1).

Gradual development of disease over time takes place through different perturbations of the biological processes (Figure 2).

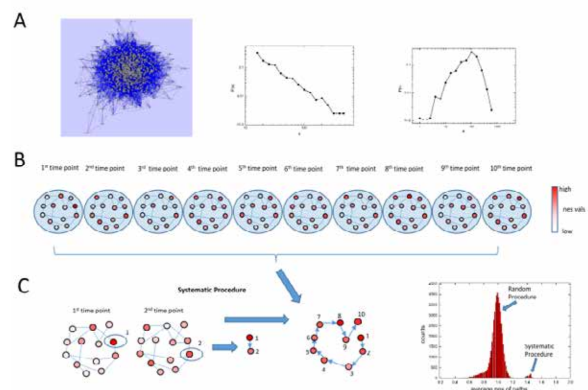

Figure-2: Figure showing schematically different concepts of the algorithm to link different biological processes.

The perturbed biological processes usually work in an interdependent way. To capture this, we constructed a network between biological processes through common genes (Figure 3) and then analyzed the data to obtain perturbed biological processes at each time point. Finally, we used the biological process network to find links between these perturbed

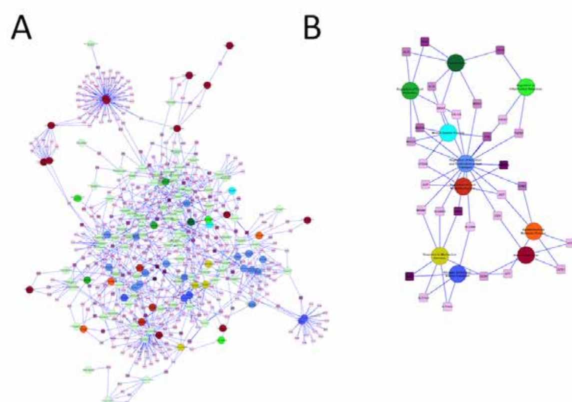

Figure-3: Biological Process (BP) network showing interlinked BP perturbed t different time points.

biological processes. This enabled us to identify paths linking the initial to the final perturbed processes and thus capture disease progression. Using statistical analysis, we established that the connecting genes present in these paths might contain some biological information and thus can be used further for mechanistic studies. Additionally we have derived a mathematical formula to score genes and identified significant set of genes regulating such a complex process network. These significantly perturbed genes at early time points could be possible target for at least mitigation of disease progression.

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1.1.2 Developing metabolome-based models for prognosis and diagnosis of metabolic syndrome (MetS)

The aim of this project is to develop a model based on serum metabolite profiles profile for both prognosis and diagnosis of MetS. For this we employ a mass spectrometry based untargeted metabolite analysis. As the first step towards this goal we are analyzing sera samples from a 5-year cohort of 6000 T2DM samples, longitudinally collected every alternate year in Delhi and Chennai. This work is in collaboration with Prof. Nikhil Tandon, AIIMS – Delhi. The samples are divided into six groups having baseline samples as normal or pre-diabetic, and subsequent samples remaining either remain normal or becoming pre-diabetic/diabetic on follow-ups. Our aim here is to capture the signatures of perturbations that co-evolve with the different phenotypic transitions. To achieve this we are constructing a regression model using metabolic profiles and different body parameters such as glucose levels using machine-learning tools. To start our model building we have completed a pilot study comprises of ~ 200 human sera samples collected from patients having diabetes and CVD.

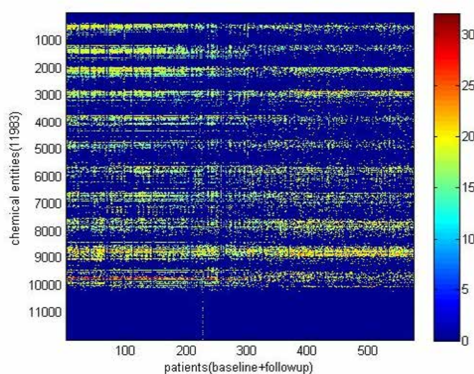

Figure -4: Heat map of the matrix with 11983 chemical entities for each patient and total patient is 287 with baseline and follow-up profile.

Once the data for the untargeted metabolites is obtained, the first challenge is to identify the ions and then represent them in a matrix (Figure 4) to compare their qualitative change over the disease state. This will help us to capture the important metabolites that drive different phenotypic transitions. Once we build our matrix where the elements represent unique metabolic ions, the next step is to build regression model using machine learning tools like support vector machine (SVM), partial least square regression (PLSR) etc to extract a multi-dimensional correlation between the metabolites

representing a disease state, and the body parameter like blood glucose of the corresponding state. This can be used as a molecular signature, which will be further linked to capture the trajectories defining different phenotypic transitions. These trajectories will then be used to build the predictive model for prognosis and diagnosis of metabolic syndrome (Mets).

1.1.3 Exploiting genome-scale metabolic models to capture disease progression and identify drug targets

TEAM LEADERS

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Shyam Lal Bagaria

The aim of this project is to capture tissue specific metabolic cues for the development of metabolic syndrome and its progression. To begin with, we have standardized a panel composed of nearly 200 metabolites that are collectively reflective of all the biochemical pathways of a cell. This panel will be utilized to monitoring quantitative variations in metabolome of the relevant tissue samples that reflect a specific disease phenotype. Once we have data for this targeted 200 metabolites for different tissues under different disease conditions, we will super-impose them on a global metabolic network build from genomic and bibliomic data. This genome-scale metabolic network consists of around 2600 metabolites and 1700 enzymatic proteins. Once we identify the perturbed metabolites from our data, we can use the network to capture the perturbed sub-pathways (Figure 5). This will be followed by flux balance analysis (FBA) to capture the distribution of the fluxes and, further, using numerical simulation we can identify the choke points regulating the flux flow. The corresponding enzymatic proteins, controlling these reactions, can then be targeted to manipulate the regulation of the fluxes. As a final step we will use a directed protein-protein interaction network (DPPI) to capture the core module of the proteins responsible for the perturbations of all the sub-pathways in the metabolic network under different disease states.

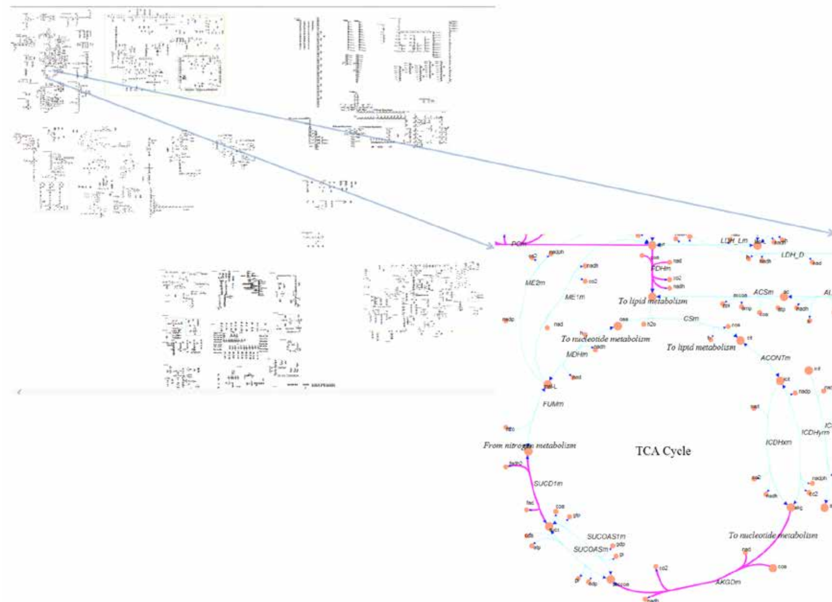
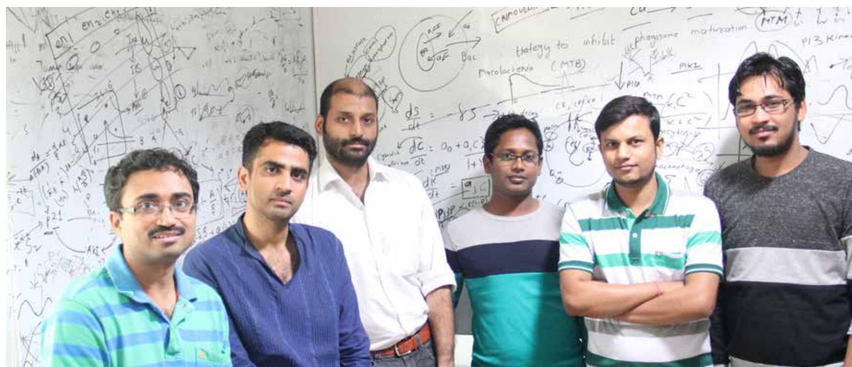


Figure-5: Example of a genome-scale metabolic network capturing the sub-pathways perturbed from a metabolic data

At present we are interrogating the metabolite profiles of liver tissue from diet induced SD rats as disease model for diabetes. The sampling of the rat tissues was done at specific time intervals over a period of twenty weeks. Here we

considered the metabolic profiling of each rat separately to capture the entire range of the possible values that each metabolite can take under a particular disease state. Superimposing the values in the metabolic network will give rise to a distribution function at each steps of the flux flow. The challenge here is to analysis this flux flow distribution function (instead of a single value) for identification of choke points and possible regulatory proteins.



Dr. Samrat and his team

1.2. Computational Proteomics & Mass Spectrometry

1.2.1 Hyperplexing integrated with BONCAT enables the study of temporal dynamics of newly translated proteins

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PARTICIPANTS

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Dr. Amit Kumar Yadav

To address the low throughput in sample multiplexing in proteomics, we have developed 18plex Hyperplexing using SILAC (3-plex) and iTRAQ (6-plex) combined together. Using a model system, we have employed several sets of SILAC (3-plex) with iTRAQ (6-plex) to increase the multiplexing capacity to 18-plex, termed Hyperplexing (Dephure et al, Sci. Sig, 2012) and integrated it with bio-orthogonal click chemistry (BONCAT) to fish out newly translated proteins (figure 6). These sets of the 18-plex data were run on high resolution ABI 5600 triple-TOF mass spectrometer.

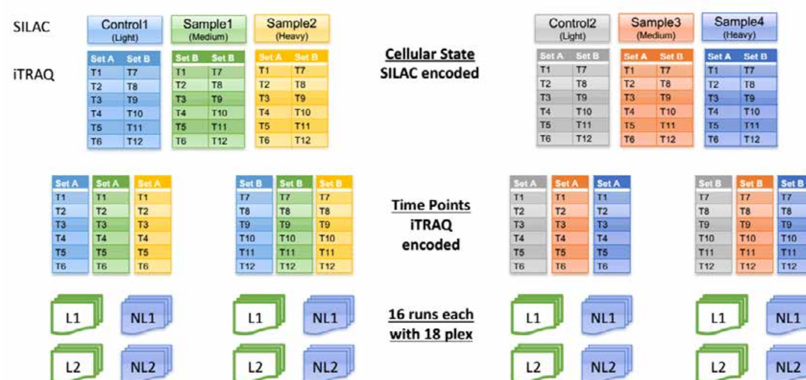

Figure-6: Overview of Hyperplexing to study temporal dynamics of proteomes

We have developed analysis pipelines and iTRAQ analysis tool QuantWizIQ to deal with such multifarious data. Our tool shows high correspondence with ProteinPilot (R2=0.99) and iTRAQAnalyzer (R2=0.99) on independent public datasets.

The identifications from MaxQuant and quantitation from QuantWizIQ have

been integrated together for accurate quantitation across each temporal dimension through the in-house developed HyperQuant pipeline. The data was then integrated from peptides to proteins to calculate accurate ratios. We are analyzing the temporal profiles of newly synthesized proteins in differently perturbed cells.

Hyperplexing is a generically applicable technique and pipeline that will enable large scale monitoring of samples in high throughput with substantially reduced instrument time. Integrated with BONCAT, we can monitor newly translated proteins in response to drugs, stress or other perturbations. The in-house developed analysis pipeline and use of mzJSON for interactive data visualizations with high-end compute infrastructure makes the highly complex data amenable for analyses. This technique can then be applied and integrated with other teams of DDRC utilizing quantitative proteomics for example – understanding ubiquitin degradation system as a potential for drug target exploration (with Sameena Khan).

1.2.2 Emergent properties of the human disease PTM-ome

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Sakshi Pareek

Human proteome is being interrogated to understand the role of post-translational modifications (PTMs) in development and disease. Analyzing data from highly curated neXtProt database, we found multiply modified proteins to be associated with diseases. These proteins are majorly non-housekeeping in nature and are found to have high functional diversity. We defined the functional diversity index, FDI based on number of biological processes, PPIs and PTM frequency and used this as an indicator for disease susceptibility (figure 7). We also observed that evolutionarily PTMs, PPIs and Isoforms are interlinked in an intricate manner such that proteins having high number of functions (moonlighting proteins) address this diversity either through PTMs, PPIs or number of isoforms. The relation between isoform frequency and PTMs appears to be inversely correlated.

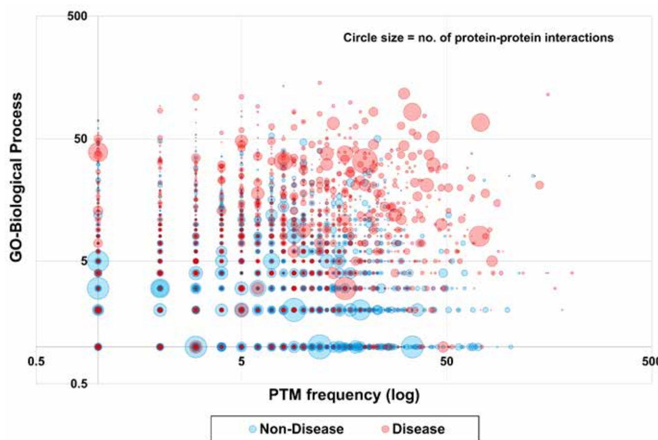

Figure-7: Disease propensity of the human proteome

The PTM based data can be useful to reconstruct networks and integrating with metabolic networks. Mathematical modeling of metabolic reconstructions can be carried out with greater precision integrating PTM data (Samrat Chatterjee). Other DDRC members are exploring this data for leads into role of PTMs in diabetes and other metabolic disorders (Shilpa Jamwal and Sanjay Banerjee). Shailendra Asthana will explore this data for structural insights into PTM for their role in PPI in interaction interfaces of binding pockets and active sites by docking and simulations. Sameena Khan will be exploiting this information for ubiquitination and protein degradation.

1.2.3 mzJSON- a data standard for browser based interactive visualization for proteomics data

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Data visualization is an important step in analysis and interpretation of large-scale proteomics studies. Standard XML data formats for raw, identification results and quantitation data are continuously becoming burgeoning and unmanageable. We have proposed mzJSON, a data format based on JavaScript Object Notation (JSON) for data exchange and browser based dynamic, interactive visualizations using applications developed in JavaScript (example shown in figure 8). Since these applications can run in any modern web browser, data visualization becomes easy and platform independent and any web developer can exploit JavaScript libraries like D3 for creating their own custom applications. We have developed some applications from mzJSON format and these are in use for data analysis by our mass spectrometry group. We can extend these functionalities in future for specific analysis needs of DDRC group.

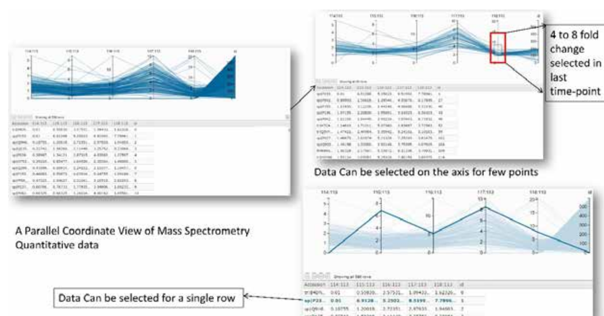

Figure-8: Example interactive application to explore Hyperplexing temporal dynamics data

1.2.4 Large scale PTM identification and quantitation

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PARTICIPANTS
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Suruchi Aggarwal
Pallavi Mahajan

Large scale analyses of PTMs from blind search of tandem mass spectrometry data is an attractive but challenging computational task. There are several computational and statistical bottlenecks in correct interpretation of MS/MS data for finding unknown PTMs all collectively leading to poor identification rates and high error rates. We are developing computational pipelines and techniques in two ways - blind PTM searches from database search or spectral library searches. In future, we also plan to explore hybrid approaches combing the two. For database search, we have built a pipeline upon a fast blind PTM tool MODa, named as MODa Pipeline that invokes multiple runs for arbitrary number of files with single click. The pipelines runs the tool in automated fashion, corrects for mass errors and helps in identifying modifications, corrects for PTM masses for accurate identification in later step.

We are also devising accurate site localization with a scoring function borrowed from MassWiz algorithm, which inherently incorporates rules for accurate site localization for modified peptides. Knowledge of correct site of PTM occurrence is of high importance in understanding signal propagation and

Dr. Amit and his team

transduction in signaling networks and the functional roles of PTMs. Currently we are also exploring spectral libraries as a source for identifying PTMs in blind search mode and developing specific custom spectral libraries for the purpose. These can be useful to map proteomic and metabolic perturbations in rat models of diabetes progression in a timely and accurate manner.

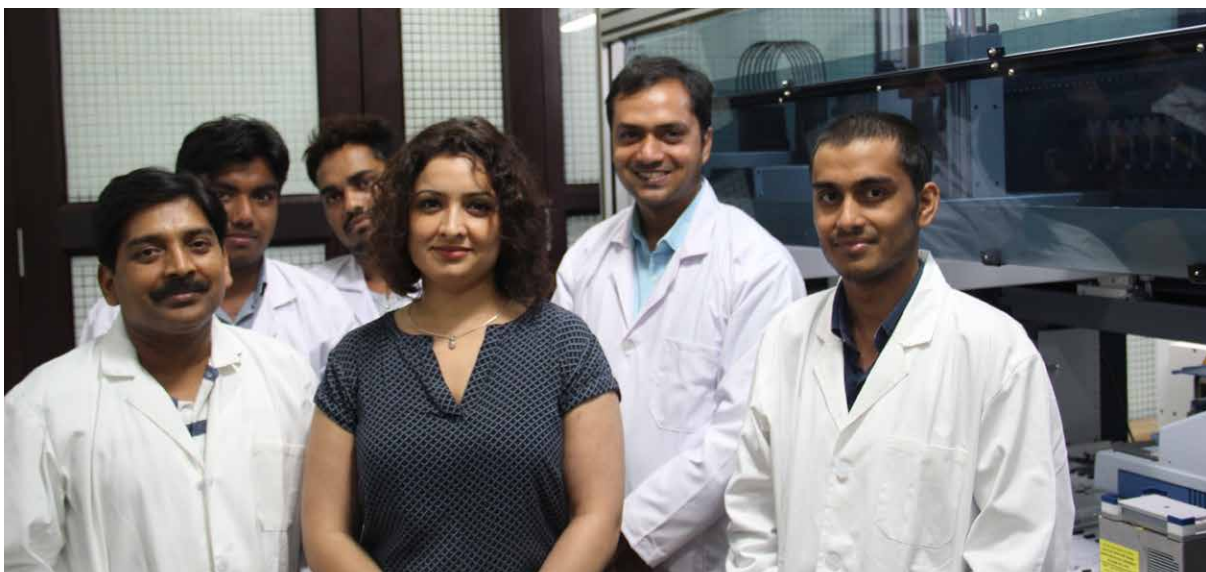
Theme 2: Lead discovery and development

TEAM LEADER
Shilpa Jamwal

Dr. Shilpa Jamwal

2.1 High-content screening platform

As a biologist at DDRC, my role essentially is to provide core support to Lead-to-drug development activities. My Core responsibility is to establish the platform for High Content Screening along with designing, optimization and operation of high content screening methodologies. Though progress is severely hindered due to untimely release of funds, we have been able finalize purchase of high content screening system that is highly sensitive and competent, expecting to reach by this June end. The integration of automation with imaging will extend our capability to generating data in a medium throughput pace. Meanwhile, the efforts are focused towards designing and optimization of the cell lines for generating disease models. These sensitive and robust in-vitro models will serve to screen, identify as well as validate the targets of the lead molecule. For generating model systems, cell lines that are metabolically relevant are being employed. These include hepatocytes, adipocytes, macrophages, smooth muscle cells, cardiomyocytes and pancreatic beta-cells. These models are intended to replicate and create the molecular dynamics and phenotypic profile of tissues representing the pathology of disease in focus. Currently, our model system is human hepatocyte cell line (HepG2) that is being optimized to represent the Insulin resistance in these cells. In order to do so, multiple methodologies are being employed and tested for several cellular phenotypic and molecular markers. We are also enabling multiplexing of screening parameters which will be used for large scale screening. We are hopeful that with soon to arrive High content screening platform in place, our efforts will pace up and provide much required support to core DDRC programs.


Dr. Shilpa and her team

TEAM LEADER
Dinesh Mahajan

RESEARCH PARTICIPANTS
Varun Kumar
Nidhi Sharma
Prasant Deb

Dr. Dinesh Mahajan

2.2 Synthetic and Medicinal Chemistry

Synthetic & medicinal chemistry group just started with the real action in laboratory and still working towards to be fully functional. The group is physically equipped with state of the art chemistry hoods for 10 chemists, high resolution NMR, HPLC and LCMS. The LC capabilities include preparative chromatography as well as chiral analytical chromatography.

The major focus of this group is to support ongoing drug discovery and development efforts at DDRC. Medicinal chemistry group brings basic understanding and expertise of chemistry towards early and late pre-clinical drug discovery & development. The focus spans around, identification of hits for new identified targets at DDRC; optimization of hits to identify lead with drug like properties; and further optimization of lead to identify a candidate for Investigational New Drug (IND) studies; and process optimizations of the lead candidates. These early pre-clinical activities involve frequent, intense and dynamic interactions with team of structural biologists, computational biologists, molecular biologists, in vitro/vivo pharmacologists. The approach of the team is to address key safety issues upfront when striving for high potency during designing or synthesis of molecules. The basic understanding such as, key pharmacophore (based on SAR); liabilities associated with ligand structure (ADME optimization); target or off target based potential safety issues, will help in stay focus on designing of new molecules and their synthesis in lab. This approach has a potential to provide high success rate from hit identification to IND candidate identification.

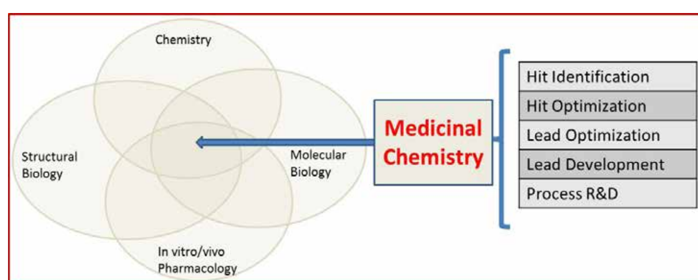


Figure-9: Medicinal Chemistry focus overview

New target identification is another key mandate of DDRC as a basic or early discovery area especially in therapeutic area of metabolic syndromes. The scientists at DDRC are exploiting system biology approach along with metabolome based models to understand the onset and progression of metabolic syndromes. This will eventually lead to identification of novel targets. Medicinal chemistry team will support these early discovery activities around new target identification by providing chemical tools/ligands to study, elucidate or validate novel targets or biochemical pathways.

Dr. Dinesh and his team

The other area that we are focusing is development of new synthetic methodologies and processes for cost efficient synthesis for target molecules of interest i.e. new identified leads, existing APIs or natural products.

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Dr. Shailendra Asthana

2.3 Protein-protein interaction: Protein-Protein Interaction Interface Depot of Secondary Structures: a Systematic Non-Conventional in silico Approach to Convert Undruggable to Druggable Binding Sites

Proteins are the social molecule of the cell, and often accomplish their function as part of large molecular machines, whose action is coordinated through intricate regulatory networks of transient protein–protein interactions (PPIs). It is thus the inter-relationships between molecules, rather than the individual components, that will ultimately determine the behavior of a biological system. PPIs are known to mediate fundamental signaling pathways and cellular processes of biological systems. Although PPIs are highly promising pharmaceutical targets, they are not preferred targets in conventional drug discovery because of their extended flat, shallow interfaces. In particular, compound libraries for high-throughput screening that offer attractive lead compounds for enzymatic targets lack the topological and functional complexity necessary for PPI modulation. Therefore, the development of small modulators of PPIs (undruggable sites) is a fundamental challenge.

The large experimental resources have been devoted to unveiling protein inter-relationships in a high-throughput manner in several model organisms, including human. However, interaction discovery experiments can only indicate that two proteins interact, but do not reveal the molecular details or the mechanism of binding. This atomic level of detail, often needed to understand function, is only captured in high resolution three dimensional (3D) structures, in which individual residue contacts are resolved and the interaction interfaces characterized. Indeed, the number of 3D structures of proteins stored in the Protein Data Bank (PDB), is exponentially increasing, though the structural data are very scarce in comparison to PPI data, and a considerable fraction of the proteomes of model organisms are not yet covered. Henceforth, by combining sequences and structural features such as amino acid composition, residue conservation, solvent accessibility and secondary structure, the in silico methods are robust enough to predict PP interface sites. Here, we devised a strategy for generating PP complexes and their quantitative and qualitative analysis. For the same, the molecular docking methods evolve as a reliable methodology. Docking programs tend to generate many potential models for a complex, which are then scored and

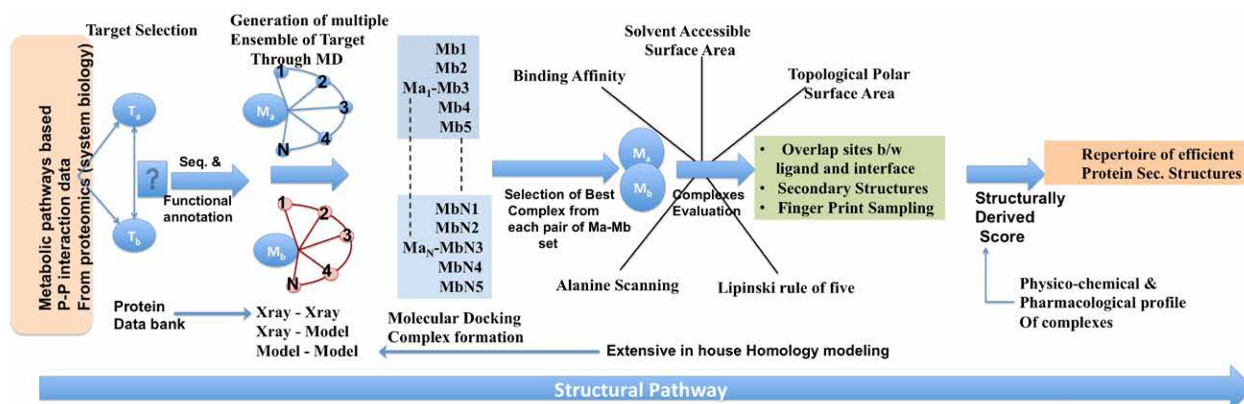

Figure-10: structural flow (pipeline) for identification of key secondary structure and residues at interface of PPIs

ranked. From the literature, it revealed well that docking programs are fairly good at identifying interface site, with average precision. To get a better understanding of PPIs, it would be fundamental to merge basic biological data into a systematic in silico view that allows us to move between resolution limits: from the physical wiring of a cell to the molecular bases of individual interaction, to establish a 3D interactome (annotated with structural information). We are developing a pipeline to identify PP complexes that are potentially amenable to disruption by synthetic mimics of protein secondary structures. The successful completion is tremendously gratifying and opens new avenues of drug discovery.

Dr. Shailendra and his team

2.4 Target validation

TEAM LEADER
Sameena Khan

RESEARCH PARTICIPANT
Sudha

Dr. Sameena Khan

Target validation is an essential step for any drug development process and we are building a team that will integrate to serve this pipeline at DDRC. This team will establish the spectrum of capabilities that are required for defining molecular details of small molecule-protein interactions. Biophysical techniques will be combined with X Ray crystal structure resolution, and further complemented with the tools of cell and molecular biology. Specific areas of expertise that the team will incorporate are:

Gene cloning, Protein Expression and Purification: Selected genes will be amplified and cloned into different expression systems. The entire expression space will be screened (using different vector systems, temperatures, inducer concentrations and different host strains) for optimal expression, while different chromatography techniques will be employed to purify the proteins to homogeneity.

Biochemical characterization: Target proteins will be characterized for their biochemical features and functioning. Activity assays and kinetic analysis will be performed. Further characterization will include an analysis of the oligomeric status and solution behavior of the protein of interest. Biophysical techniques will also be employed to study affinity and stoichiometry of interaction using SPR, ITC, co-elution, GPC. Biochemical assays, combined site directed mutagenesis, will also be utilized to characterize the active sites, and monitor reaction kinetics.

Defining biological roles of the target protein: Substrate proteins for the selected targets will be captured using pull down/Co-immunoprecipitation experiments and later identified using mass spectrometry. This data will be integrated with the other relevant information from molecular and cellular studies to decipher the biological role of the protein, and the degree of redundancy in its functioning.

Structure determination: Attempts to determine structure of selected proteins using X-ray crystallographic techniques. This will involve screening the entire crystallization space to hook the condition where good quality crystals can be obtained. Atomic level information such as active site volume, key interacting residues or the overall milieu will provide the mechanistic insight into the basis of interaction of the protein with its substrate.

Dr. Sameena and her team

Theme 3: Early translation

TEAM LEADERS

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Shailendra Asthana
Sameena Khan
Shilpa Jamwal
Kanury Rao

3.1 Developing a novel class of drug-like molecules that trigger autophagy

Autophagy is one of the fundamental eukaryotic pathways. It provides a mechanism to cell to eliminate intracellular microorganisms (pathogens) as well as degradation of cytoplasmic material through lysosome degradation pathway (Figure 11). Additionally this cellular process also generates precursors needed for several metabolic pathways. Owing to its inherited function, autophagy has multiple effects on cellular immunity.

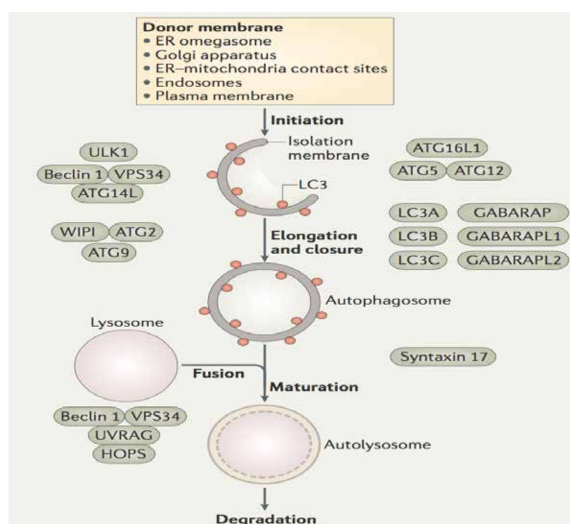

Figure-11: Broad outline of the autophagy pathway (www.nature.com/reviews/immunol)

The diversity of cellular roles for this pathway has highlighted its potential for range of therapies pertaining to disorders/diseases. Role of autophagy in infectious diseases, metabolic syndromes and autoimmune disorders is one of the hot areas of research in drug discovery.

DDRC has a deep interest in identifying new host targets i.e. individual proteins of complex of proteins and corresponding chemical ligands/disruptor to develop new therapeutic methods for metabolic syndromes and infectious diseases. Owing to this interest, the team at DDRC focused their efforts on identification of a small molecule as a hit and a host protein complex as a novel target to induce autophagy in in vitro model. We designed and identified a virtual library of molecules based on structural biology and computational chemistry approaches. This was a work done in collaboration with an external lab. Few of these virtual molecules were selected for synthesis and physically evaluated for their autophagy induction in cellular assays. Two specific lead-like molecules were identified based on their cellular potency and novel IP space.

The identified small molecule as a hit has demonstrated the induction of autophagy by disrupting the function of a specific cellular protein complex in host cell. This small molecule induced autophagy is found to be independent of mTOR activity. This was determined and established by analysis of activity of mTOR downstream proteins with or without treatment with identified hit and using Rapamycin as a positive control. The key success of this approach revolves around induction of autophagy by disruption of host protein complex by a small molecule resulting in boost of cellular immunity without targeting mTOR pathway. It is expected that, host based target and identified chemical lead will help host cell to fight against different infections. This approach can have potential merits of safety over mTOR inhibition approach, as mTOR is one of the key proteins for housekeeping function at cellular level.

The identified lead like structures are found to have IC50 around 50nm to 500nm when screened against CEM-GFP cells infected with HIV-1 based on ELISA readout.

In-silico strategy to discover small-molecule activators for autophagy:

The growing appreciation that autophagy may prevent the occurrence, delay the progression; calls for development of autophagy modulators that can induce and/or enhance autophagy. A small peptide called Tat-beclin, derived from key autophagy regulator Beclin 1 was found to enhance autophagy by binding to GAPR, an endogenous inhibitor of Beclin 1. Leveraging the existing knowledge about specific peptide and its target, we want to discover small molecules to activate autophagy. To achieve the same, using various advanced computational methods, we planned three parallel alternative routes (figure 12). First, we performed Protein-peptide interaction study to find the preferable Tat-beclin binding site on GAPR using different protein-protein docking algorithms and determine the key residues of Tat-beclin, involved in the interactions. We would then design small molecules that mimic the key residues using peptido-mimetic and fragment-based approaches. Secondly, we use the protein-protein docking algorithms to obtain theoretically favored interaction poses (other than Tat-beclin) of beclin 1-GAPR complexes, to identify an alternate peptide that might bind to GAPR with a high affinity. Third, by identifying the binding site on GAPR, we are performing an extensive virtual screening of small molecules databases (~50,000 compds) to find potent inhibitors of GAPR. Moreover, to avoid any bias, a blind virtual screening (taking whole protein instead of selected binding site) is also conducted.

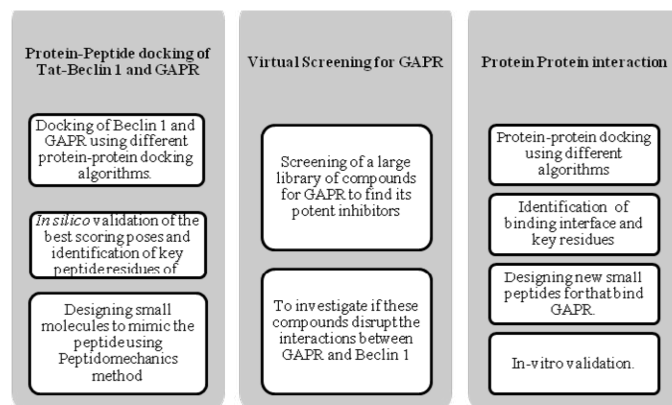

Figure-12: Three parallel computational strategies for identification of hit against GAPR

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Dinesh Mahajan

CO-INVESTIGATORS
Sanjay Banerjee
Shilpa Jamwal
Sameena Khan
Kanury Rao

3.2 Inhibitors of SGLT2: Developing lead molecules against Sodium Glucose Transporter 2 (SGL-2s): A target for the control of hyperglycemia in type 2 diabetes.

The treatment of diabetes has been mainly focused on maintaining normal blood glucose concentrations. Insulin and hypoglycemic agents have been used as standard therapeutic strategies. However, these are characterized by limited efficacy and adverse side effects, making the development of new therapeutic alternatives mandatory. Inhibition of glucose reabsorption in the kidney, mediated by Sodium glucose transporter (SGLT), represents a promising therapeutic approach. The most prevalent and functionally important SGLT in the kidney is SGLT2 as this transporter accounts for approximately 90% of glucose reabsorption in the kidney. SGLT2 is the low-affinity and high-capacity transporter, which is present at high density on the brush-boarder membrane of the S1 segment of the proximal convoluted tubule. SGLT2 binds with both sodium and glucose in the tubular filtrate and then translocate across the apical cell membrane. Nowadays, SGLT2 is considered as a novel and established targets for diabetes. Hypoglycemia, which occurs more primarily with most of the anti-diabetic drugs, SGLT2 inhibitors are free from this effect. As SGLT2 inhibitors act independently of insulin-dependent mechanism, intense hypoglycemic effect was not expected.

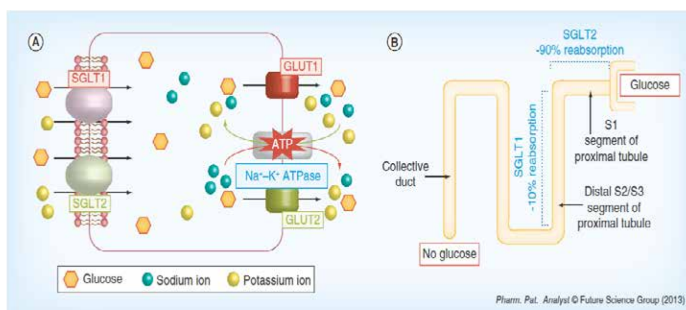

Figure-13: Mechanism and location of glucose absorption through SGLT2. (A) A hypothetical cell representing both renal and gastrointestinal cells which transport or reabsorb glucose through SGLT2. (B) Location and distribution of SGLT1 and SGLT2 in nephron and its reabsorption capacity (Pharm. Pat. Analyst, 2013, 2:77–91).

With recent approval by FDA to drugs based on SGLT2 inhibition provided clinical validation for this target. We are working on a discovery project to come up with a new structure class and a lead molecule having therapeutic benefit based on selective inhibition of SGLT-2 over other isoforms, SGLT1. For this purpose, a structure-based computational approach was employed, which encompasses homology modeling of SGLT protein, molecular dynamics, virtual screening, pharmacophore- ligand- structure- and fragment based screenings, to design novel inhibitors with high potency and affinity.

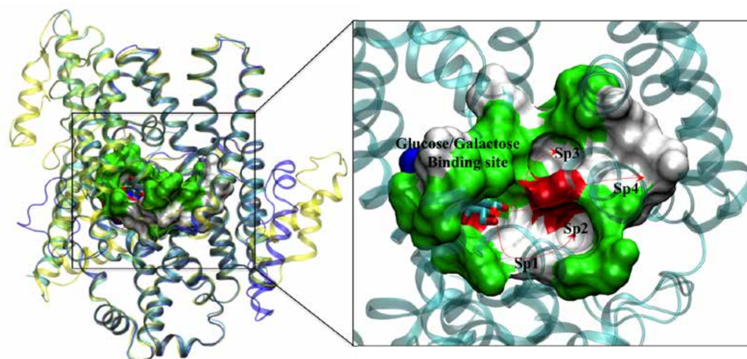

Figure-14: Superimposed modeled structure of SGLT1 and SGLT2. The glucose/galactose binding site is shown. Newly identified neighboring, small pockets (Sps) of glucose binding site are used for structure based drug discovery approach.

A library of molecules was identified as virtual hits based on these computational studies. In parallel, a pharmacophore model was generated for existing lead molecules, which are in clinical studies or approved for market. The virtual hit generated were screened around the pharmacophore model generated from binding understating of clinical candidates for further refinements based on interaction map. The selected molecules coming out of this comparative pharmacophore binding models were further evaluated based on traditional medicinal chemistry parameters such ease of synthesis in lab, druggable structure and patent scope. The molecules scoring high on these parameters were selected for further synthesis in chemistry lab. Synthesis of these new chemical entities and their in vitro screening to identify a hit is a work in progress. The team at DDRC has also identified and established an industrial collaborator to support

the hit identification to lead optimization efforts of this project. The final aim of this project is to deliver a novel lead with new IP space having PoC in animal model when dosed orally.

Theme 4: Discovery Research

TEAM LEADER
Sameena Khan

RESEARCH PARTICIPANTS
Ishita Gupta
Kanika Singh

4.1 Defining regulatory mechanisms that drive cell-fate decisions.

Understanding regulatory mechanisms that control cell-fate is one of the key challenges in biology today. This question is of fundamental importance in chronic diseases such as the metabolic syndrome where altered homeostatic states are established, and then maintained, through functional reprogramming of key components of the cellular machinery. Although resolution of the etiological basis for disease-specific perturbations in cellular pathways remains a challenging task, emerging evidence implicates a central role for modulation in turnover rates of proteins. It is now recognized that timely degradation of functionally significant proteins is central to maintenance of cell health, and dysregulation of this process underlies several human chronic diseases. Therefore, it is our view that interrogating protein turnover in cells can offer and alternate, and potentially useful, strategy for delineating disease-causing mechanistic perturbations, and consequently also facilitate identification of protein targets for drug development.

The Ubiquitin Proteasome System (UPS) constitutes the principal non-lysosymal system for degrading proteins in mammalian cells. This system is involved in the complex regulation of most proteins in a cell and is, therefore, central to regulation of almost all cellular processes. Thus, it is our thesis that delineation of the altered substrate specificities of the UPS in a given disease state can provide important mechanistic insights that can eventually lead to drug target identification. Selective UPS-dependent degradation is

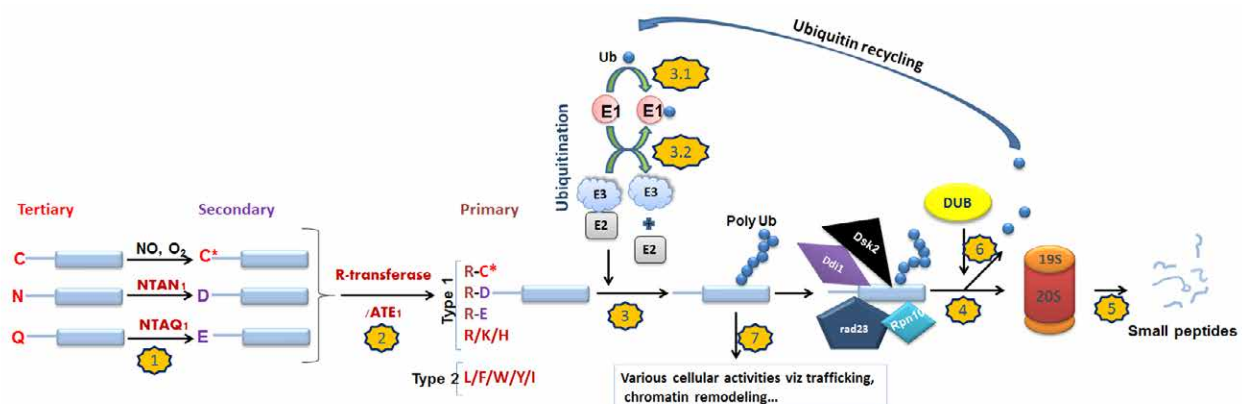

Figure-15: Map of Ubiquitin mediated degradation machinery in human's is depicted. Only N-end mediated UPS pathway is shown and single letter code is used for amino acid description. Tertiary destabalising residues N, Q and C on the N-terminal of substrate protein (blue rectangle) are modified by NTAN1 (N-terminal N-amidase) and NTAQ1 (N-terminal Q- amidase) enzymes to yield secondary destabilizing N-terminal residues D and E. Oxidized Cys derivatives (C) also serve as secondary destabilizing residues in mammals. Secondary destabilizing residues D, E and oxidized Cysteine derivatives (C*) on the substrate protein N-terminal are conjugated by Arg-tRNA transferase (R-transferase) to R (a primary destabilizing residue). Destabilizing N-terminal basic (R, K, H) and bulky hydrophobic (L, F, Y, W, I) residues are bound directly by N-recognin (also called E3 Ubiquitin (Ub) ligase), which mediate substrate selectivity by recognizing N-terminal signal. A complex of E3 Ub ligase and E2 ubiquitin-conjugating enzyme (E2) transfers Ub molecule to Lys residue on substarte protein. Ubiquitin is activated by E1 ubiquitin-activating enzyme (E1) and is transferred to E2. Polyubiquitinated substrate can be targeted to the proteasome via shuttle proteins (Dsk2, Ddi1, rad23, rpn10) and destroyed by complex 26S proteasome machinery (20S core + 19 S regulatory particle). Finally, deubiquitinating enzymes (DUB) recycle ubiquitin proteins. Poly or monoubiquitylation can also be an activation/repression signal that modulates the substrate activity in several cellular processes such as trafficking or chromatin modeling.*

executed indistinctly in three steps: recognition of the protein degradation signal sequence (degron), marking this target protein for degradation via ubiquitin (Ub) conjugation, and finally degradation by the proteasome. UPS mediated pathway confers constitutive and conditional metabolic instability on a protein by temporal control and selective degradation. The destabilizing N-terminal residues, called N-degrons are a class of protein degradation signals that dictates the in-vivo half-life of proteins. Substrates of this pathway are often generated by proteolytic cleavage or N-end modifications. UPS pathway known to control the levels of key proteins regulates almost all of the cellular activities including cell cycle progression, DNA replication and repair, transcription, protein quality control, immune response, and apoptosis. UPS has been explored for its link to cancer, autoimmune disorders and the age related, neurological disorders and metabolic disorder. Details of the typical mammalian N-end UPS is presented in Figure 15.

Our interest is to investigate the role of Ubiquitin proteasome system (UPS) in diseases of the metabolic syndrome such as diabetes and cardiovascular diseases. We are currently focused on decrypting the fine balance between ubiquitination and deubiquitination processes, and to probe how this fine-tuning regulates apoptosis. We aim to identify the E3s and DUBs that regulate apoptosis of cardiomyocytes, and subsequently delineate those that crosstalk with the anti-apoptotic, insulin-dependent signaling pathway. We have initiated our studies toward this goal and a crosstalk between the two pathways has been established. Our subsequent goal is to link this crosstalk with the UPS pathway proteins. This will be complemented by an analysis of the pattern of ubiquitinated proteins in normal cardiomyocytes, and establishment of their linkages with the E3 and DUBs. The regulatory roles of E3 ligases and DUBs are being studied through a multi-disciplinary approach that combines biophysics and structural biology, bioinformatics and network analysis, mass spectrometry, and various tools of biochemistry and cell and molecular biology. The larger aim in this specific program is to integrate structural biology with the tools of cell biology and systems biology for drug development efforts.

TEAM LEADER
Shilpa Jamwal

4.2 Delineating cellular pathways that initiate the metabolic syndrome

The escalating cases of metabolic complications have exposed a strong association between obesity, inflammation, diabetes and adipose dysfunction. The potential of adipose tissue, consequential of its endocrine behavior and metabolic regulation is being recognized globally. Current therapies for metabolic complication emphasize primarily on management of disease rather targeting the route cause. Along with our focus to develop intervention therapy at DDRC, we also recognize the need and value of developing new approaches that either prevent initiation of MetS, or mitigate its progression. We aim to understand the progression of adipose tissue dysfunction by capturing information of early mechanisms that mediate disruption of critical tissue funtioning. To define the early perturbations that characterize initiation of disease, diet-induced rat model of MetS have been employed wherein the temporally collected visceral white adipose tissue samples be analyzed for multiple disease parameters that essentially will include immunocyte infiltration, biochemical changes, and modulations in the proteo-metabolome flux and so on. Also the focus in this direction is the perturbations induced in visceral white adipose tissue (VWAT), as the free fatty acids (FFAs) liberated

from this site represent at least one of primary events that initiates/drives MetS. Additionally, adipocytes also link between obesity and diabetes, with adipocyte-derived factors playing a critical role in regulating insulin sensitivity of other peripheral tissues.

The animal experiments have been conducted, and serially collected VWAT over multiple time points has been generated. Additional tissues collected include liver and skeletal muscle, and the first objective is to analyze each of these to determine the time point at which insulin resistance first appears. Taking this time point as the window for establishment of the process towards MetS (i.e. pre-diabetic condition), a detailed temporal analysis of VWAT will be performed. Parameters to be studied will. While providing a framework for subsequent analysis of the disease initiation process, these findings are also aimed at generating an assay platform for developing and testing lead compounds capable of inhibiting/suppressing MetS development.

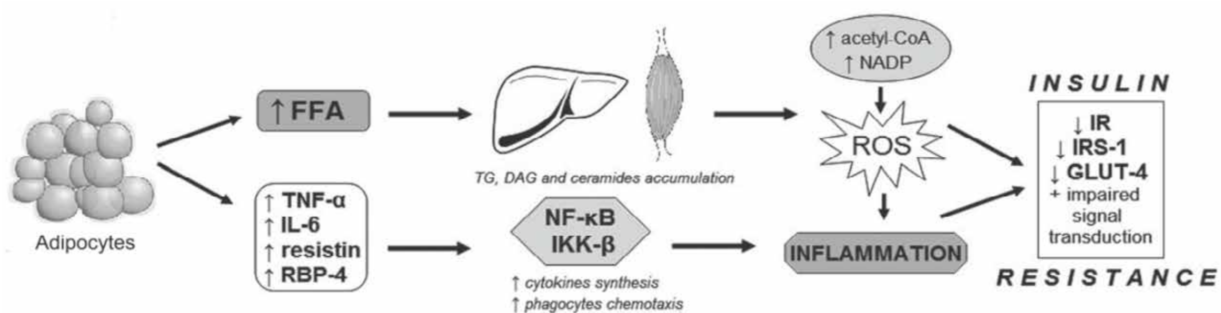

Figure-16: Role of adipose tissue in insulin resistance (Cardiovascular Diabetology 2014, 13:103)

4.3 Understanding the Pathophysiology of Cardiac Complication in Diabetes

TEAM LEADER
Sanjay K Banerjee

RESEARCH PARTICIPANTS
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Ramu Adela
Hina Lateef
Paramaeshwar Katare
Soheb Anwar
Bugga Parmesh
Roshan Kumar

Dr. Sanjay K Banerjee

Since the prevalence of diabetes in India is expected to increase in ever-alarming proportions, cardiovascular disease stands to become the most frequent cause of death. The growing incidence of type 2 diabetes and the increased mortality due to its cardiovascular complications gained wide attention among researchers to understand the complexity of the problem. Cardiovascular complication accounts for more than 80% of diabetic deaths. Diabetic patients have almost twice the rate of mortality because of congestive heart failure than non-diabetic individuals. Several etiological factors including hyperglycemia and insulin resistance are responsible for increased risk of cardiac complications. The overwhelming effect of all these factors leads to cardiac abnormalities including myocardial dysfunction, myocardial hypertrophy, impairment of contractile proteins, accumulation of extracellular matrix proteins and decreased left ventricular compliance. Alteration of cardiac muscle in diabetes has led to the recognition of a new cardiac disease termed “diabetic cardiomyopathy”. The complexity and pandemic proportions of heart disease and diabetes call for a comprehensive understanding of the pathophysiological mechanisms involved and look for a therapeutic strategy to prevent this. As one of the focuses of biology group of DDRC is to look the metabolic disease progression and identify novel target for therapeutic intervention, we have developed animal models of diabetes and cardiovascular disease and looked the molecular mechanisms.

4.3.1 Role of Sirtuins on Diabetic Cardiomyopathy

Recently, the involvement of posttranslational modification in disease biology has gained wide interest and focus of our center. Acetylation of histone and non-histone proteins is considered to be one of the important post-translational and epigenetic modifications. The role of acetylated protein in disease biology is still in its infancy and its role in malfunctioning diabetic heart is poorly understood. Among several mechanisms, posttranslational modifications of proteins play a crucial role in diabetic cardiomyopathy. Acetylation plays vital role in metabolic regulation and has a strong link to metabolic disease like diabetes. We are trying to understand the expression and activity of situins, a class of protein deacetylases, on diabetic cardiomyopathies. There are seven sirtuins present in cell, whose regulation in diabetic heart is not clearly known. We focused to find out the expression and regulation of all sirtuins in diabetic heart with the effect of sirtuin activator, resveratrol administration on them. We have induced type 1 diabetes (T1DM) rat model using steptozotocin and type 2 diabetes (T2DM) rat model by feeding high fructose diet for a period of eight weeks and analyzed the myocardial changes. Increased oxidative stress and cardiac phenotype alterations shows the induction of cardiac abnormalities in both models. We have observed decreased SIRT-1 and increased SIRT-3 activity in the T2DM rat heart. Moreover, in case of T1DM, gene and protein expression of all sirtuins was down, except SIRT-2 whose protein levels were increased. Administration of resveratrol prevented the alteration in SIRT-1 in T2DM and SIRT-1, 2, 3 and SIRT-5 in T1DM rat heart. Altered level of protein acetylation was observed corresponding to the changes in sirtuins. Our data clearly indicated that sirtuins are perturbed in both types of diabetic heart and can be considered as druggable target for therapeutic intervention. We have shown that SIRT-1, which deacetylates NFkB-p65 at lysine 310 and histone 3 (H3) at lysine 9 position, play important role disease pathogenesis of diabetic cardiomyopathy in T2DM. SIRT1 activation leads to decreased binding of NFkB-p65 to DNA and attenuated cardiac hypertrophy and oxidative stress through reduced transcription of NADPH oxidase subunits. We also confirmed that knockdown or inhibition of SIRT1 in H9C2 cells increased acetylation of NFkB-p65 K310 and H3K9. Our data demonstrated that SIRT-1 activation attenuates cardiac oxidative stress and complications in diabetes. Further work is going on to identify the role of SIRT3 in diabetic cardiomyopathy.

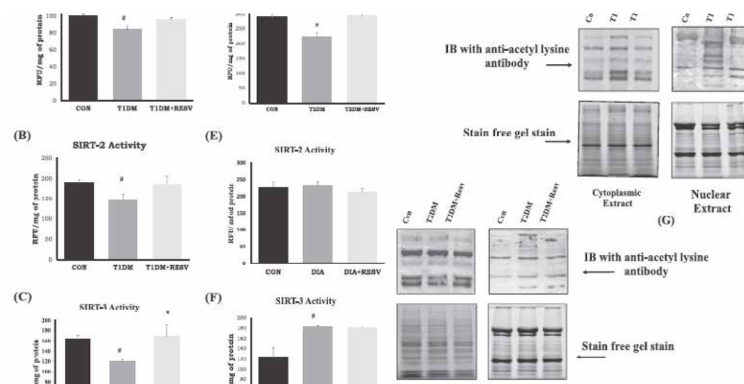

*Figure-17: Effect of resveratrol on activity of sirtuins and protein acetylation status in diabetic rat heart. A) SIRT-1 activity in T1DM, B) SIRT-2 activity in T1DM, C) SIRT-3 activity inT1DM. D) SIRT-1 activity in T2DM, E) SIRT-2 activity in T2DM, F) SIRT-3 activity in T2DM, G) Extent of protein acetylation in cytoplasmic and nuclear extract of T1DM, H) Extent of protein acetylation in cytoplasmic and nuclear extract of T2DM. Data shown as Mean ± SEM, #p < 0.05 vs Con; *p < 0.05 vs Dia. (Bagul et al., Journal of Nutritional Biochemistry, 2015, 26;11:1298–1307.)*

4.3.2 Delineating the Molecular Mechanism of Hypertrophic Cardiomyopathy and its Prevention by Pharmacological Intervention

The onset of heart failure is typically preceded by cardiac hypertrophy, a response of the heart to increased workload, a cardiac insult such as a heart attack or genetic mutation. Hypertrophic growth termed as hypertrophic

cardiomyopathy accompanies many forms of heart disease, including ischemic disease, hypertension, heart failure, and valvular disease. Treatment strategies for heart failure commonly include diuretics, angiotensin converting enzyme inhibitors, angiotensin II receptor blockers and β-blockers; however, mortality rates remain high. Here, we discuss new therapeutic approaches. To accomplish this goal, it is essential to identify molecular events involved in the hypertrophic process and to identify the signaling systems that promote pathological hypertrophy. Cardiac hypertrophy is induced by administration of isoproterenol at the dose of 5mg/kg body weight/day subcutaneously for 14 days through alzet minipump. Slow release of isoproterenol continuously from alzet pump causes cardiac hypertrophy without any cardiac injury. This model simulates chronic activation of sympathetic nervous system, which releases adrenaline or noradrenaline in blood to activate beta 1 receptor. Chronic activation of beta 1 receptor makes cardiac hypertrophy. Cardiac hypertrophy markers like heart weight and body weight ratio, heart weight and tail length ratio; cardio-myocytes size; ANP, BNP, beta-MHC mRNA expression were measured for the confirmation of cardiac hypertrophy model establishment. A reduction in the activity of NADH dehydrogenase (Complex I), MTT reduction (all dehydrogenases) and cytochrome C oxidase (Complex IV) of the respiratory chain along with reduction of mitochondrial biogenesis following isoproterenol treatment in rats is clearly indicative of reduction of cardiac mitochondrial complex activities. We have explored whether raw garlic and its one active compound, diallyl disulfide (DADS) could inhibit hypertrophy through H2S and/or mitochondrial biogenesis. Aqueous garlic homogenate and DADS were fed to rats with cardiac hypertrophy for 14 days at a dose of 250mg/kg/day and 50mg/kg/day respectively. Garlic and DADS reduced cardiac hypertrophy markers and normalized mitochondrial electron transport chain-complex activities, mitochondrial enzyme activities and mitochondrial biogenetic and apoptotic genes expression. Garlic and DADS enhanced eNOS and p-AKT level in rat heart along with increased NRF2 protein level and Tfam gene expression. In conclusion, garlic and one of its active compounds, DADS induces mitochondrial biogenesis and ameliorates cardiac hypertrophy via activation of eNOS-Nrf2-Tfam pathway in rats. This mechanism needs to be explored further for therapeutic intervention of cardiac hypertrophy.

Dr. Sanjay and his team

TEAM LEADER
Shailendra Asthana

RESEARCH PARTICIPANTS
Mritunjay Singh
Charu Suri

4.4 Modulation of Sirt1 activity by targeting unstructured disordered N-terminal

Drug discovery efforts are focused in identifying new leads that inhibit enzyme function, which is challenging, however, it is rare to encounter small molecule enzyme activator. Discovering activator can provide a means to better understand the endogenous methods of enzyme activation and underlie allosteric mechanism. Some biological problems like metabolic disorder where stabilizing enzymes through activator is more promising for better health. In metabolic disorder, sirtuins are the attractive therapeutic target as it is implicated in multiple metabolic and age-associated pathways and disorders. Sirtuins generally catalyze the deacetylation of modified lysine residues in protein substrates coupled with the breakdown of NAD+ into nicotinamide (NAM) and 2'-O-acyl-ADP-ribose. Small molecule sirtuin-activating compounds (STAC) have been developed which increase the catalytic deacetylation of specific Lys residues by SIRT1 in multiple substrates, resulting in a variety of biological responses. However, the molecular mechanism of SIRT1 activation by STACs remains controversial.

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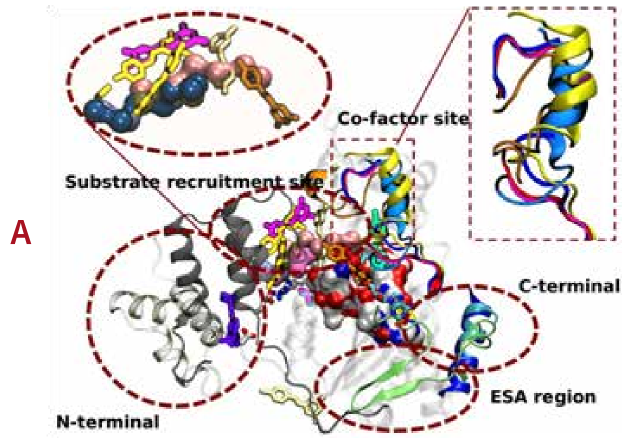
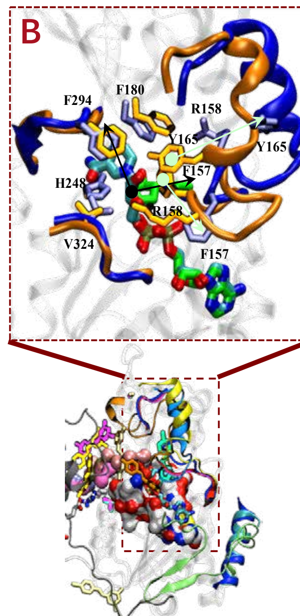


Structural characterizations of SIRT1 fragments have shed light on the inhibitor binding and key regulatory element, however, the location for endogenous and/or exogenous modulator's site/s which trigger activation is still elusive. To obtain the molecular details that govern the binding of STACs to SIRT1 is difficult due to lack of crystal structure of full-length enzyme, as its certain regions like N- and C-terminal, which supposed to involved in various biological processes, are highly flexible and disordered. To address this problem, we developed a fragment-based protocol, which is the combination of different algorithms (HMM, profile-profile search, fold recognition) to generate the complete modeled structure of full-length SIRT1. From multi-step docking studies we found that the C-terminal region of SIRT1 mainly influence the inhibitory activity for sirtuin inhibitors. However, the N-terminal (in multiple orientations depend on size and shape of substrates) might involve



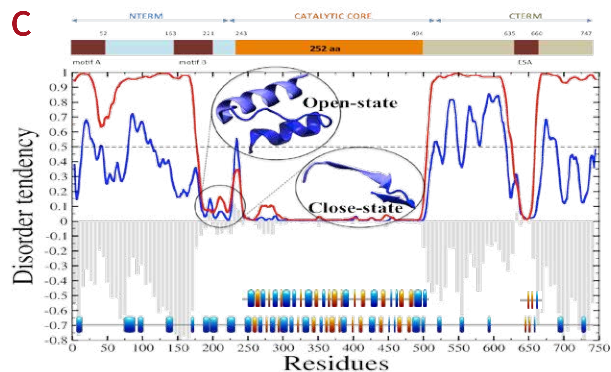


*Figure-17: Effect of resveratrol on activity of sirtuins and protein acetylation status in diabetic rat heart. A) SIRT-1 activity in T1DM, B) SIRT-2 activity in T1DM, C) SIRT-3 activity inT1DM. D) SIRT-1 activity in T2DM, E) SIRT-2 activity in T2DM, F) SIRT-3 activity in T2DM, G) Extent of protein acetylation in cytoplasmic and nuclear extract of T1DM, H) Extent of protein acetylation in cytoplasmic and nuclear extract of T2DM. Data shown as Mean ± SEM, #p < 0.05 vs Con; *p < 0.05 vs Dia. (Bagul et al., Journal of Nutritional Biochemistry, 2015, 26;11:1298–1307.)*

in activation. Together, this work highlights the unique properties of the SIRT1 and its implications for the development of SIRT1-specific regulatory molecules

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Patents & Copyrights

Copyrights

QuantWiz[IQ]:	Diary No. 2190/2016-CO/SW on 22.02.2016. Suruchi Aggarwal, Amit Kumar Yadav.
MODa Pipeline:	Diary no. 2186/2016-CO/SW on 22.02.2016. Manu Kandpal, Suruchi Aggarwal, Amit Kumar Yadav.
HyperQuant:	Diary no. 5027/2016-CO/SW on 26.04.2016. Suruchi Aggarwal, Amit Kumar Yadav.

Patents

Patent Application Type:	PCT Application [WO2015IN00226]
Title of the Invention:	Novel Compounds as Anti-Tubercular Agents
Date of filing:	June 1, 2015
Inventors:	KVS. Rao, Varshneya Singh, Dinesh Mahajan, Sundeep Dugar, Santosh Kumar Rai

Honors and Awards

1. Private-Public Partnership program with revelations Biotech Pvt. Ltd.: DDRC has entered into a collaborative research program with revelations Biotech Pvt. Ltd. to develop new approaches for diagnosis and treatment of diabetes. The duration of the program is 5 years.

2. Partnership Centre with Stanford University, Stanford Linear Accelerator Centre, and NCCS: A joint centre, "Centre for Integrative Biology of Non-communicable Diseases", between DDRC, NCCS-Pune, Department of Radiation Oncology, Stanford University and the Stanford Linear Accelerator Centre has been awarded the prestigious Indo-US Science and Technology Forum Award 2015. The Department of Radiation Oncology, Stanford University and DDRC will act as the nodal host institutions for this Centre.

3. Recognition of DDRC by Jadavpur University for the PhD program in Mathematical Biology: Through its Centre for Mathematical Biology and Ecology, Jadavpur University (JU) has granted recognition to DDRC for registering students under the JU PhD program in Mathematical Biology.

4. Dr. Sameena Khan has been awarded the INSA Medal for Young Scientists for the Year 2015. This highly competitive award is given to early career scientists in recognition of their creativity and excellence in research, and for the promise that they show for the future. Dr. Khan's award recognized her body of work providing key insights into the biology of the malaria parasite Plasmodium falciparum, and the identification of novel strategies for drug development.

5. Dr. Shailendra Asthana, has received the Young Investigator Award-2015 for his research work titled, "Small molecule modulation of Sirt1 activity by targeting its unstructured disordered N-terminal" at the Conference on Bioinformatics and Computer Aided Drug Design (CBCADD)-2015, recently held at IIT Guwahati, from Dec. 6-8, 2015.

6. Dr. Shailendra Asthana has been awarded the DST-SERB Start-Up Young Scientists Research Grant-2015 for his project titled, "Functional characterization of finger loop with/without inhibitors in HCV RNA-Dependent RNA Polymerase: A microscopic picture through computational simulations and its application for new antiviral drug development".

7. Dr. Sanjay K Banerjee has been awarded with the Prof. Shailendra K Vajpeyee Award, 2015 for his research work titled "Role of Nitric Oxide on Hypertension in Diabetes: A Study from bench to Bed".

8. Dr. Amit Yadav was selected for the "Scientist Lecture" at the Proteomics Society of India Meeting, Vellore, 2015.

9. Ms. Suruchi Aggarwal, Senior Research Fellow, was selected for the "Young Scientist Lecture" at the Proteomics Society of India Meeting, Vellore, 2015.

High-end Instruments at THSTI

Population Science Partnership Centre (PSPC)

- A phase iii randomized, double blind placebo controlled trial to evaluate the non-interference in the immune response of three doses of orv 116e to antigens contained in childhood vaccines and to assess the clinical lot consistency of three production lots 172
- Phase iii, multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of live attenuated bovine-human rotavirus reassortant pentavalent vaccine (brv-pv) against severe rotavirus gastroenteritis in healthy indian infants 174
- The Effects of Human Intestinal Microbiota on Immune Responses 175
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An Overview

Dr. Nita Bhandari

The Population Science Partnership Centre (PSPC) is collaboration between Translational Health Science and Technology Institute (THSTI), Haryana and Centre for Health Research and Development, Society for Applied Studies (CHRD-SAS), New Delhi. The two institutes have complementary skills and expertise; THSTI has the laboratory infrastructure and CHRD-SAS has vast field research experience.

The centre's mission is to utilize strengths and resources of the two institutes to create a potentially cost saving, scientifically productive and health impact enhancing collaboration. The aim is to pursue collaborative research and innovation in population-based science, focused on development, evaluation and diffusion of affordable technologies and solutions for improved health and nutrition including conduct of clinical trials; emphasize on solutions and technologies of public health importance to India and to the poorest; promote utilization of under-used existing technologies through appropriate modification and evaluation.

The overall vision is to incorporate hypothesis-driven scientific questions in population cohorts and clinical trials related to diseases of major public health importance. The knowledge gained will provide insights for prevention, diagnosis and treatment of fetal, neonatal and childhood disorders that contribute most to the disease burden in the country.

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Nidhi Goyal

COLLABORATORS

CHRD-SAS
THSTI
Department of Biotechnology, Govt. of India
PATH
Bharat Biotech International Limited

A phase III randomized, double blind placebo controlled trial to evaluate the non-interference in the immune response of three doses of ORV 116E to antigens contained in childhood vaccines and to assess the clinical lot consistency of three production lots

Rotavirus infections are the leading cause of diarrhea-associated mortality in developing countries. Rotavirus infections are estimated to cause ~527,000 deaths annually, predominantly in developing countries. In India, by age 5, nearly every child will have an episode of rotavirus gastroenteritis. The Indian rotavirus vaccine based on a neonatal rotavirus strain 116E developed as a Public-Private Partnership (PPP), under the Indo- US Vaccine Action Program and coordinated by the Government of India (Department of Biotechnology) has recently completed a multicentre phase III clinical trial in India. The licensure has been obtained by Bharat Biotech International Limited in January 2014.

This study is a phase III, randomized, double blind, placebo-controlled trial to assess non-interference of ORV 116E to the childhood vaccines and clinical consistency in the immune responses to the three production lots of ORV 116E.

The study is being conducted in the urban neighborhoods of Govindpuri-Sangam Vihar-Tigri-Dakshinpuri and Tuglakabad in South Delhi, India.

Ethical clearances were obtained from THSTI-IEC and Western Institutional Review Board. The study was approved by the office of the Drugs Controller General (India) and is being conducted as per the protocol, Schedule Y and Good Clinical Practices.

Prior to study initiation, the study clinic was set up. The clinic is manned by paediatricians and physicians, open 24x7, equipped to handle all emergencies and there are systems in place to escort subjects to the hospital, if required. All categories of staff were trained on the protocol, filling case report forms, standard operating procedures and good clinical practice.

Subjects were enrolled into the study at 6 weeks of age after obtaining consent under audio visual recording and subsequent screening at the study clinic. Subjects were given 3 doses of the ORV 116E/Placebo along with childhood vaccines at 6, 10 and 14 weeks of age. A baseline blood specimen of ~1.5 mL was collected from all subjects for rotavirus IgA assays. Around 6mL of post immune blood specimens was collected 28 days after the third dose of the Test Article/Placebo for assessing immunogenicity to the childhood vaccines.

The study team made daily contacts for 14 days after each of the three doses till four weeks after the third dose to ascertain serious adverse events and signs and symptoms of intussusception. Additionally, after four weeks of the third dose, the subjects were contacted weekly till the age of one year for signs and symptoms of intussusception and events of death, if any

If the SAE was diarrhea, stool specimens were collected and sent to Translational Health Science and Technology Institute for RV detection and typing. If a confirmed RV positive report was obtained, an aliquot of the stool specimen to the Wellcome Laboratory, Christian Medical College, Vellore for detection of diarrheagenic E. coli and Shigella. For assessing clinical lot consistency, blood specimens were collected 28 (±5) days after the third dose of ROTAVAC®/placebo. The serum anti-RV IgA assay was performed by the central laboratory at THSTI where clinical lot consistency was assessed by the GMTs of IgA antibody.All serious adverse events were reported to the regulatory authorities and THSTI-IEC within 24 hours of coming to know of the event.

The study was initiated in May 2014. Enrolment of 1356 subjects was completed in September 2014 and follow up in August 2015. Enrolled subjects have received three doses of the vaccine. All subjects are being followed up till the age of one year. Data analysis has been completed and the Clinical Study Report was submitted to the Project Management Committee on March 5, 2016.

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Phase III, multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of live attenuated bovine-human rotavirus reassortant pentavalent vaccine (BRV-PV) against severe rotavirus gastroenteritis in healthy indian infants

According to the WHO-coordinated Global Rotavirus Surveillance Network, 37% of deaths are attributable to diarrhea and 5% of all deaths in children under 5 years. Five countries accounted for more than half of all deaths attributable to rotavirus infection; India alone accounted for 22% of deaths (98,621 deaths) in this study. Although commercial rotavirus vaccines are currently available and have been demonstrated to be safe and effective in low-income, high-burden populations, they are not affordable in developing countries. Serum Institute of India is developing a live attenuated bovine-human (UK) reassortant pentavalent rotavirus vaccine for oral vaccination against human rotavirus gastroenteritis in healthy infants and plans to establish proof of vaccine efficacy in the proposed efficacy trial, which will enroll infants in six sites in India representative of different demographic, climatic and socio-cultural factors. In Delhi, it is being conducted in the urban slums of south Delhi.

Prior to study initiation, the study clinic was set up. The clinic is manned by pediatricians and physicians, open 24x7, equipped to handle all emergencies and there are systems in place to escort subjects to the hospital, if required. All categories of staff were trained on the protocol, filling case report forms, standard operating procedures and good clinical practice. Ethical clearances were obtained from THSTI-IEC and Western Institutional Review Board. The study was approved by the office of the Drugs Controller General (India), ethics committees of each of the participating sites Government of India's Health Ministry Screening Committee and the State Governments.

Subjects were enrolled into the study at 6 weeks of age after obtaining informed consent under audio visual recording and subsequent screening by a physician at the study clinic. Subjects are given 3 doses of the BRV-PV/Placebo at 6, 10 and 14 weeks of age along with OPV and Pentavalent vaccine (containing DPT, HepB and HiB) primary doses and Measles, MMR and booster vaccines according to the national immunization schedule. Active surveillance for Gastroenteritis, Intussusception and other illnesses is done by weekly contacts with the participating infants starting from the time of the first vaccination until infants reach two years of age. Once an episode of gastroenteritis is identified, study personnel maintain close contact with the parent until the child's illness is resolved.

Diarrhea Diary Cards are being used by the parents during gastroenteritis episodes to record number and duration of looser than normal stools, axillary temperature, number and duration of vomiting episodes, any treatment given and duration of hospital stay, if any. Stool samples are collected from all subjects having gastroenteritis. All Serious Adverse Event are reported to the regulatory authorities, Ethics committee and Sponsors, as per the applicable guidelines, throughout the study period.

The study was initiated in August 2014 and completed enrolment 2100 subject in April 2015. All subjects have received dose 1 of BRV-PV/Piacebo; 1934 subjects received dose 2 and 1878 subjects received dose 3. Subjects will be followed up till the age of two years. The last follow up of the youngest subject will be in Q1, 2017. A subset of the participants has been enrolled in

an "immunogenicity cohort" to assess immune response to the vaccine. Blood samples were obtained just before vaccination and 4 weeks (+/- 7 days) after the third vaccination in the infants in this cohort. The sera will be tested for anti-rotavirus IgA at the Wellcome Trust Research Laboratory in Vellore. In addition, the sera may be tested for poliovirus antibodies.

The Effects of Human Intestinal Microbiota on Immune Responses

INVESTIGATORS
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There is recent evidence indicating the influence of different environments and diets on microbial ecology of the human gut. This study assumes that the composition of gut microbiota varies between people living in Japanese and Indian communities, and hypothesize that resistance to intestinal infection in Indian populations is regulated by their unique intestinal microbiota.

In this study, fecal samples of Indian and Japanese subjects were collected, and the intestinal microbiota were analyzed by molecular techniques. Fecal samples were collected from 50 healthy Indian adults residing in low socioeconomic dwellings in urban and rural areas of Delhi and Haryana states. These areas were typical urban resettlement neighborhoods and rural areas in South Delhi and Urban Faridabad and Rural Haryana, in India. The study population from Japan were comprised of healthy adults residing in high or average socioeconomic dwelling in Osaka prefecture. This area was typical urban area with good hygienic environment. The study was conducted in two phases. The first phase included collection, processing and transportation of fecal specimen from healthy adults in India and Japan to the testing laboratories in Japan. The specimens underwent specified analysis of the intestinal microbiota in the laboratory. In the second phase the analyzed fecal specimen will be fed to germ-free mice and the development of immune responses in these mice were tested.

DNA was extracted from 60 fecal samples and subjected to the Ion PGM sequencer to analyze the intestinal microbiota. Fecal suspensions were also subjected to high liquid performance chromatography (HPLC) to measure the concentration of short chain fatty acids (SCFAs). The principal component analysis showed that the plots of Japanese samples were clearly separated from those of Indian ones, but there were no distinct characteristics within the different areas. Hierarchical clustering showed that 60 subjects were broadly divided to 2 clusters: cluster I and II. All Japanese samples were grouped in the cluster I, which was characterized by the high abundance of Bacteroides genus, whereas Indian samples were in the cluster II, characterized by high numbers of Prevotella genus. The Indian samples were further subdivided to 2 clusters, IIa and IIb, mainly based on the differential proportion of Prevotella. Some Indian samples showed unique bacterial profilings. For examples, the sample from IND-47was dominated by Klebsiella, which is known to be the enteric pathogen. HPLC analysis showed that concentrations of total SCFAs and propionic acid in Indian samples were significantly higher than those in Japanese samples. In addition, concentrations of total SCFAs and propionic acid in cluster IIa were significantly higher than those in cluster IIb

The specimens from the Indian volunteers were collected in July 2013. Specimens have been collected from additional Japanese volunteers and analysis of the specimens is ongoing. Additional specimens were collected from 10 of the Indian volunteers in August 2014 and the results of the specimen analysis were shared by the Japanese laboratory partners. It was noted that

Prevotella was dominant in Indians and that Bacteroides was dominant in the Japanese participants. It was also found that genus Candida, especially C.albican and C.tropicalis is frequently detected in Indians whereas many Japanese harbour Saccharomyces cerevisiae as the dominant fungi, and that he detection ratios of Candida in Japanese are very low.

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Gut Inflammation Markers as Determinants of Response to Treatment and Recovery in Children with Uncomplicated Severe Acute Malnutrition Undergoing Community Based Rehabilitation

Malnutrition is a global health problem affecting more than 300 million pre-school children worldwide. It is one of the major health concerns in India since around 50% of children below the age of two suffer from various forms of malnutrition. The gut microbiome plays an important role in nutrient pre-processing, assimilation and energy harvest from food. The human gut microbiome, collective genomes of all the microbes residing in the gastrointestinal tracts, provides several metabolic functions that are not encoded in our own genome. These functions facilitate the pre-processing of dietary nutrients and efficient harvest of dietary energy for the host. Consequently, dysbiosis of the gut microbiota has been implicated in malnutrition. Malnourished children may have underlying inflammation of the gut as a part of an enteropathy. This leads to malabsorption of processed dietary compounds. In addition, inflammatory molecules could affect gut homeostasis and there can be critical nutrient diversion.

The Society for Applied Studies coordinated a multicentre study to evaluate the impact of novel therapeutic foods embedded in various potentially usable feeding regimens for management of uncomplicated severe acute malnourished children (SAM) in children aged 6-59 months. SAM contributes to 25% of under 5 deaths. Preliminary results showed that nearly half the children do not respond to treatment with ready to use therapeutic foods (RUTF). We hypothesize that exposure to microbes and dietary deficiencies cause chronic gut inflammation and immune activation that contribute to poor response. A substudy was designed to ascertain presence of gut inflammation through measurement of specified biomarkers and examine the relationship between the gut microbiome, inflammatory markers and the response to treatment

The study was conducted in three sites - urban slums and resettlement colonies in the national capital region, rural (predominantly tribal) Rajasthan, rural and urban Tamil Nadu - where the prevalence of .SAM is above the national average of 6.4%. Study participants were children aged 6 to 59 months with weight for height less than -3 SD of WHO standard or oedema of both feet or both. Children with complicated SAM were excluded and referred to a hospital. All enrolled children were randomized to one of the three groups centrally produced RUTF, locally produce RUTF and augmented home prepared foods. These children were followed up weekly upto 16 weeks or recovery whichever was earlier and for 16 weeks after completion of the treatment phase. Faecal samples were collected at enrolment and at 8 weeks or recovery whichever was earlier. Blood sample was also collected at enrollment for analysis of high sensitivity CRP, Tissue Trans Glutaminase Antibody, Serum IgA, Serum Albumin, Platelet count and urine for Microalbuminuria. Total bacterial DNA sample was extracted in the laboratory from fecal sample, isolated DNA sample used for targeted metagenomic analysis using 454 GS FLX plus Pyrosequence. Inflammatory markers Calprotectin, Neopterin and Myeloperoxidase were

analysed by competitive or sandwich Elisa.

The follow up of the children is over and analysis of specimen for microbiome has been conducted by TCS Research, Tata Consultancy Services Ltd, Pune; the results are awaited.

Peer-reviewed Publications

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5. Prinja S, Bahuguna P, Mohan P, Mazumder S, Taneja S, Bhandari N, van den Hombergh H, Kumar R. Cost Effectiveness of Implementing Integrated Management of Neonatal and Childhood Illnesses Program in District Faridabad, India. PLoS One. Jan 4, 2016;11(1):e0145043.

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12. Lamberti LM, Fischer Walker CL, Taneja S, Mazumder S, Black RE. The Influence of Episode Severity on Caregiver Recall, Care-seeking, and Treatment of Diarrhea Among Children 2-59 Months of Age in Bihar, Gujarat, and Uttar Pradesh, India. Am J Trop Med Hyg. 2015 Aug;93(2):250-6.

13. Taneja S, Bahl S, Mazumder S, Martines J, Bhandari N, Bhan MK. Impact on inequities in health indicators: Effect of implementing the integrated management of neonatal and childhood illness programme in Haryana, India. J Glob Health. 2015 Jun;5(1):010401.

Seminars and Conferences

Title of the Workshop: Causality Assessment for Clinical Trials in the Indian Scenario
Date and Place: SenseCR in Gurgaon (April 2015)
Presenter: Dr. Temsunaro Rongsen-Chandola, Dr. Nidhi Goyal, Dr. Vikash Kedia

Title of the Workshop: Accreditation for Sites. Investigators and Ethics Committees
Date and Place: SenseCR in Gurgaon (April 2015)
Presenter: Tarun Batra, Alok Arya

Title of the Workshop: Current Regulatory Requirements for Members of Institutional Ethics Committee (An Awareness Program)
Date and Place: THSTI, NCR Biotech Science Cluster, Faridabad (April 2015)
Presenter: Tarun Batra

Title of the Workshop: Short Course in Biostatistics & Epidemiology for Clinical and Public Health Research using STATA
Date and Place: CMC, Vellore (July 2015)
Presenter: Dr. Ravi Upadhyay, Dr. Bireshwar Sinha

Title of the Workshop: Demystifying Indian Regulations for New Drug Approval; BIRAC-CDSA Regulatory Workshop series in South India
Date and Place: Hyderabad (September 2015)
Presenter: Tarun Batra, Alok Arya

Title of the Workshop:	Diagnostics
Date and Place:	THSTI, NCR Biotech Science Cluster, Faridabad (November 2015)
Presenter:	Dr. Ranadip Chowdhury, Dr. Bireshwar Sinha
Title of the Workshop:	Practical Consideration in Conduct of Community Trials" and "Data Management Requirements for Clinical Trials
Name of Meeting:	India Vaccinology Course (INDVAC)
Date and Place:	Christian Medical College, Vellore (September, 2015)
Presenter:	Dr. Nita Bhandari, Dr. Sunita Taneja
Title of the Workshop:	Mentoring PhD students on Clinical Research Methodology
Date and Place:	THSTI, NCR Biotech Science Cluster, Faridabad (October 2015)
Presenter:	Dr. Nita Bhandari, Dr. Temsunaro Rongsen Chandola
Title of the Workshop:	Essentials of Statistics and Clinical Data Analysis using R
Date and Place:	THSTI, NCR Biotech Science Cluster, Faridabad (December 2015)
Presenter:	Dr. Ranadip Chowdhury, Kiran Bhatia, Girish Chand Pant
Title of the Workshop:	Self Controlled Case Series Methodology Workshop for Early implementation of roll out Rotavirus vaccine in the public health system under monitoring
Date and Place:	(March 2016)
Presenter:	Dr. Nita Bhandari, Dr. Sunita Taneja, Dr. Temsunaro Rongsen Chandola, Dr. Nidhi Goyal Dr. Dr. Ranadip Chowdhury, Dr. Bireshwar Sinha

High-end Instruments at THSTI

Clinical Development Services Agency (CDSA)

An Overview

Dr. Sudhakar Bangera

CDSA was launched in September 2009 as an extramural unit of THSTI. It was created to facilitate development of affordable healthcare products for public health diseases. Registered in September 2010 as an autonomous, not-for profit research society by the Registrar of societies, Delhi, under the Societies Registration Act XXI of 1860, it aims to develop an eco-system for training and learning and work with public sector institutions, and small and medium enterprises (SME) to translate innovative technologies into medical products for public good.

CDSA has a simple governance structure with a 14-member Governing Body and an Executive Management Committee that provides operational oversight. In 2015-2016, CDSA had a core staff team of 13 employees comprising of Program Director, Director Training, Director of Clinical Portfolio Management, Associate Medical Director, Biostatistician, Administrative Manager, Finance Manager, IT administrator, HR Associate, Medical Writer, Account Associate, Clinical Database Designer, Training Coordinator and two consultants, one in the field of Regulatory Affairs and other in Vaccines. In addition, CDSA employs data coordinators, quality managers and monitors on projects.

Focus Areas

Main objectives of CDSA

1. As a training academy, CDSA aims to build capacity and capability in the area of clinical development and translational research. We conduct training programs for young clinical researchers, ethics committee members and other personnel to support development of effective and efficient clinical research professionals
2. Monitor public health studies for compliance to Schedule Y regulations, CDSCO-GCP guidelines, Study Protocol and other requirements.
3. Support Investigators and SMEs by providing Clinical Study Support Services like regulatory consultation, project management, medical monitoring, audit, data management and biostatistics
4. With 5 Centers of Excellence (CoEs), CDSA has formed a collegium of Centers of Clinical Research Excellence for collaboration in education, training and capacity building and Collaboration in Research, Innovation, and Clinical Development Support Services. The institutions are: KEM Hospital, Pune; Society of Applied Sciences (SAS), New Delhi; Center for Chronic Disease Control (CCDC), New Delhi; JSS University, Mysore and CMC Vellore.

Departmental Activities

Dr. Sucheta Banerjee Kurundkar

Training Department

Team Members: 02

Dr. Sucheta Banerjee Kurundkar (Director Training)

Ms. Neha Mishra (Training Coordinator)

CDSA's Training Department has carved a niche of its own across the professional community. The testimonials it receives from the participants stand proof of its commitment towards excellence. This has kept the small team motivated to stand by the commitment to work on the national mandate to build capacity and capability in the area of clinical development and translational research. From 3 trainings in 3 years (FY 2009-12) to 21 trainings in a year (FY 2015-16), this department has set its aim high.

Table 1: Trainings at CDSA: A snapshot

	2009-12 (3 yrs)	2012-13	2013-14	2014-15	2015-16	Total
Trainings	3	10	14	17	21	65
Faculty	11	112	146	175	233	710
Participants	41	436	894	1241	1906	4518
Institutions Represented	10	117	222	428	536	1313
Cities	2	5	10	10	9	36

This year we completed a national awareness series to strengthen our Institutional Ethics Committees in India. CDSA reached out to all cities with its aim to provide a learning opportunity on Ethics and Regulations in India. We completed 15 programs covering 1700 participants with strong support from CDSCO, ICMR and several medical colleges. It is considered as a benchmark program in ethics at this scale and was commended by Late Padmashree (Dr.) Ranjit Roy Choudhury and recently applauded by Dr. G. N. Singh, DCG(I), CDSCO.

With BIRAC, we completed a four program South India Series on *Regulatory Awareness* attended by several SMEs, innovators or incubators, academicians and industry on four core area (new drugs, biopharmaceuticals, medical device and *in vitro* diagnostics and phytopharmaceuticals). All programs (except Bangalore) were held at CDSCO regional offices supported by Deputy Drug Controllers like Shri. Prasad (CDSCO, Hyderabad), Dr. Bangarurajan (CDSCO, Mumbai), Dr. Manivannan (CDSCO, Chennai), addressed participant queries related to regulation.

We also had a very interesting forum, '*Meet the Regulators*' organised with C-CAMP, Bangalore. This allowed us to provide a platform, where innovators could meet one-to-one with the senior regulators and seek regulatory guidance.

The online registration and online exams that we had initiated had made us shift from conventional system to IT driven platforms. From next year, we will

gear up towards conducting webinars, online courses (using our eLearning module).

The following is the list of all training conducted:

Table 2: Trainings Conducted by CDSA (2015-16)

S.No.	Date	Program Name	Funding	City	No. of Faculty	No. of Partici-pants	No. of Attendee Institutions
1	Apr 09, 2015	Current Regulatory Requirements for Members of Institutional Ethics Committees (An Awareness Program)	THSTI	THSTI, Faridabad	05	10	06
2	Apr 22, 2015	Good Laboratory Practice – An Awareness Program (Only for PTB Team, THSTI)	CDSA - THSTI	THSTI, Faridabad	01	51	02
3	May 19-20, 2015	Current Regulatory Requirements for Members of Institutional Ethics Committees (Awareness Program)	CDSA	Father Muller Medical College, Mangalore	17	94	09
4	Jun 03, 2015	Workshop on Human Research Protection and Good Clinical Practice	RCB	RCB, Faridabad	04	19	05
5	Jul 14-15, 2015	Current Regulatory Requirements for Members of Institutional Ethics Committees (Awareness Program)	CDSA	BMCRI, Bangalore	17	190	51
6	Aug 18, 2015	Good Clinical Practice (GCP) [An Awareness Program]	CDSA	Govt. Kilpauk Medical College, Chennai	06	179	45
7	Aug 19-20, 2015	Current Regulatory Requirements for Members of Institutional Ethics Committees (Awareness Program)	CDSA	Govt. Kilpauk Medical College, Chennai	13	216	49
8	Sep 04, 2015	BIRAC-CDSA Workshop Series in South India: “Demystifying Indian Drug Regulations for New Drugs Approvals”	BIRAC	CDSCO Bhawan, Hyderabad	11	55	28
9	Sep 15-16,2015	Current Regulatory Requirements for Members of Institutional Ethics Committees (An Awareness Program)	CDSA	BBD University, Lucknow	17	120	12

S.No.	Date	Program Name	Funding	City	No. of Faculty	No. of Partici-pants	No. of Attendee Institutions
10	Oct 15, 2015	BIRAC-CDSA Workshop Series in South India: "Regulatory Requirements for Biopharmaceuticals – From Science to Commercialization"	BIRAC	NCBS, Bangalore	13	43	25
11	Oct 16, 2015	Workshop on Regulatory Requirements & Clinical Development for Med-Tech Innovators	C-CAMP	C-CAMP, Bangalore	05	39	21
12	Sep 10 - Nov 07, 2015	Manipal University - CDSA Bioethics Certificate Course	Collaborative Program with Manipal University	Online Program with Contact sessions at Manipal	39	49	10
13	Nov 30, 2015	Presentation & Communication Skills Training for Scientists	CDSA	CDSA, Faridabad	01	17	04
14	Dec 01-04,2015	Essentials of Statistics and Clinical Data Analysis using R	CDSA	ICGEB, New Delhi	07	45	29
15	Dec 14, 2015	Good Clinical Practice (GCP) (An Awareness Program)	ESIC Dental College & Hospital, Rohini, Delhi	ESIC Dental College & Hospital, Rohini, Delhi	07	108	17
16	Dec 15, 2015	Current Ethical & Regulatory Requirements (An Awareness Program) for Clinical Trials	ESIC Dental College & Hospital, Rohini, Delhi	ESIC Dental College & Hospital, Rohini, Delhi	05	86	13
17	Jan 12, 2016	BIRAC - CDSA Regulatory Workshop on Emerging Needs & Regulations Phytopharmaceuticals	BIRAC	CDSCO, Mumbai	13	39	20
18	Jan 19-20, 2016	Current Regulatory Requirements for Members of Institutional Ethics Committees (Awareness Program)	CDSA	PSGIMSR, Coimbatore	19	53	18
19	Feb 16, 2016	Good Clinical Practice (GCP) (An Awareness Program)	CDSA	TMH, Mumbai	10	246	69

S.No.	Date	Program Name	Funding	City	No. of Faculty	No. of Partici-pants	No. of Attendee Institutions
20	Feb 17-18,2016	Current Regulatory Requirements for Members of Institutional Ethics Committees (Awareness Program)	CDSA	TMH, Mumbai	10	185	65
21	Feb 24, 2016	BIRAC CDSA Regulatory Workshop on Current Regulation on Medical Devices & in vitro Diagnostic Kits”	BIRAC	CDSCO, Chennai	13	62	38
Total				9	233	1906	536

Outreach

We have mapped all the cities from where our participants come and a database of 5200+ professionals has been created at CDSA in order to reach out to all for any new events or upcoming training programs.

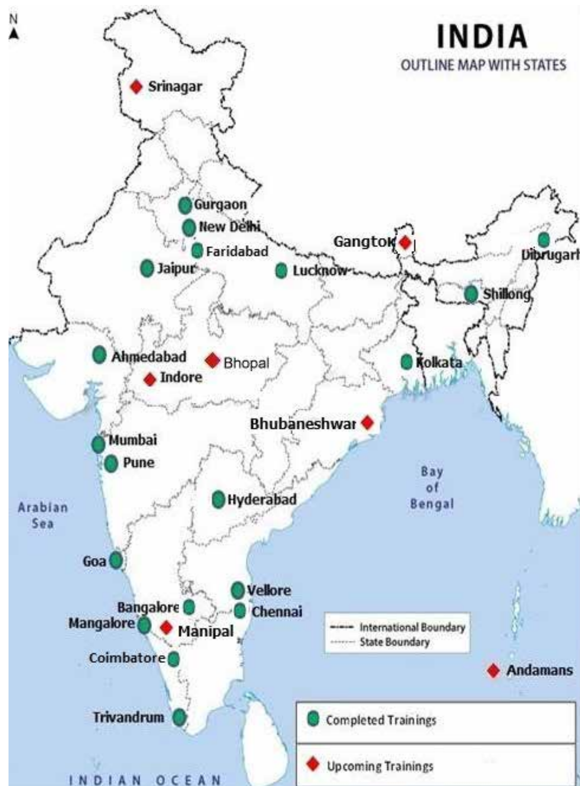

Figure-1: Cities where CDSA Programs had were held and to be held

Figure-2: Cities from where Participants have come to attend CDSA Programs

BIRAC CDSA Regulatory Workshop on Current Regulation on Medical Devices & *in vitro* Diagnostic Kits [CDSCO, Chennai; February 24, 2016]

Current Regulatory Requirements for Members of Institutional Ethics Committees [Tata Memorial Hospital, Mumbai; February 17-18, 2016]

Good Clinical Practice [Tata Memorial Hospital, Mumbai; February 16, 2016]

Current Regulatory Requirements for Members of Institutional Ethics Committees [PSGIMSR, Coimbatore; January 19-20, 2016]

Online Program with Manipal University

For the third year, CDSA has conducted the Bioethics Certificate Course in collaboration with Manipal University, taking responsibility for the online exam for the terminal assessment of all the participants.

Dedicated Portal

CDSA has developed a website portal dedicated for training activities (www.cidp.in). This website has all audio-video recordings, presentations, case studies, resource materials, faculty and participant details, etc. It has 'online registration' facility for all training programs. All training activities will be migrated to this portal from 2016 making this a live and dedicated portal facilitating our learning initiatives.

Miles to go .....

At CDSA, every month we get flooded with training requests. We take efforts to address many of them. India is a big nation, our efforts are immense but a drop in the ocean. The future is going to be demanding and challenging but the team is geared up and ready as it realises that it has miles to go .....

Clinical study support services

Clinical research is an essential activity for the development of new pharmacological treatments, diagnostic tests and devices. For interventional studies or clinical trials, there is a technical and regulatory system which is still evolving, but has many mechanisms in place to ensure quality of data. For non-interventional studies which provide an effective way of gathering real-world evidence, there are few mechanisms to ensure the integrity of the collected data.

Department of Clinical Portfolio Management

The department (CPM) has been established to undertake and implement cost effective clinical research services in product development for government, semi-government, government-funded academic and non-academic institutions, non-profit, and small and medium sized enterprises. The main objective of Clinical Portfolio Management department is to direct, supervise and coordinate a network of clinical studies in India. Specific objectives include:

- To provide monitoring support for clinical and community based studies
- To facilitate allied activities as and when required (Study start up activities, Site Assessment(s) and gap analysis, Project planning and Budgeting, SOP support)
- Identify the need and train human resources on quality practices for suitable conduct of both regulated clinical trials and observational studies that meets the demands of the country
- Collate evidences from research projects for review to aid formulation of a national policy
- Commission a consultative peer review process

CDSA-CPM department supports investigators/ academic institutes/ SMEs for clinical study planning, management and ensures quality and integrity of data generated in compliance with project requirements and applicable guidance. CDSA provides advisory support or assist researchers /academicians for the project planning and effective implementation of the research projects. This includes writing grants as co-applicant or reviewing the project proposal with special emphasis on operational aspect, project planning, tracking for milestones, risk identification and management for efficient implementation of research proposal. Services also envisage protecting the safety, confidentiality and well-being of the participants.

Through active participation for policy and advocacy we engage in health programs to improve or protect health policies. For example, CDSA as per the directions from inter-ministerial committee for severe acute malnutrition (SAM) alliance program has convened national consultative meeting for projects implemented and completed under SAM program.

Based on the experience gained while working as independent monitoring agency we plan to establish operational guidelines, educational module and study management tools customized to the need of the research in public health domain. These will be a value addition for the public health research for benchmarking the quality standards and CDSA will be extending advisory support for these as requested by the stakeholders.

We will establish a network of experts to support and mentor the department for improvisation of systems and processes, algorithm for the project selection and operational support mechanism for the research in public health domain.

Ongoing Clinical Projects/Programs

CDSA is providing clinical study support services to the following projects and programs listed in the table

Project Title	Principal Investigator/ Sites	Funding Agency	CDSA Role
Severe Acute Malnutrition (SAM) Programs			
To evaluate the impact of three feeding regimens on the recovery of children from uncomplicated severe acute malnutrition in India and to use the evidence to inform national policy	Dr. Nita Bhandari (03 sites: Delhi Vellore, Udaipur)	BMGF	● Co-monitoring with WHO ● DSMB constitution & coordination with WHO
Undiluted animal milk with added sugar and micronutrients versus WHO feeding protocol for management of severe acute malnutrition in non-breastfed infants aged 2-6 months of age: a randomized controlled trial.	Dr. Satinder Aneja (03 sites: Delhi)	DBT	● Site start-up support ● Clinical Monitoring ● DSMB constitution and coordination ● Data Management
Validation of Mid Upper Arm Circumference (MUAC) for Detecting Severe Acute Malnutrition against Weight for Height Below -3 SD Using as Gold Standard in Children Aged 6-59 Months	Dr. Umesh Kapil (single site: Meerut)	ICMR	● Study start-up support ● Clinical monitoring ● Coordinate monitoring by domain experts

Project Title	Principal Investigator/ Sites	Funding Agency	CDSA Role
Other Projects/Programs			
Inter-Institutional Program for Maternal, Neonatal and Infant Sciences: A translational approach to studying preterm birth	Multi-Institutional Program – THSTI, RCB, NII, NIBMG, AIIMS & SJH (single site: General Hospital Gurgaon)	DBT	• Study Start Up Support • Clinical monitoring • Quality Management • Lab Monitoring
A Phase IV, Interventional, Open label, multicentric, Single arm clinical trial to assess the Safety, Tolerability & Immunogenicity of Bivalent Oral Polio Vaccine (bOPV) in healthy Indian infants.	8 sites across India	BIBCOL/ DBT	• Medical Writing • Regulatory advisory • Project management • Clinical monitoring • Data management • Statistical services
Evaluating the Efficacy and Safety of Indigenous Goat Lung Surfactant Extract (GLSE) in a pilot sample of preterm infants with Respiratory Distress Syndrome (Phase II study)	Dr. Ramesh Agarwal (AIIMS) (12 sites across India)	Wellcome Trust	• Project Management • Clinical Monitoring • Data Management • Medical Monitoring
Determination of efficacy and safety of reflexology therapy for the Patients with intractable epilepsy: a multicenter randomized clinical trial	Dr. Krishna Dalal (AIIMS) (02 sites: Dibrugarh, Imphal)	DBT	• Site Start Up Support • Clinical Monitoring
Inhibition of host-induced mycobacterial efflux pumps as a novel strategy to counter drug tolerance and virulence of pulmonary tuberculosis	Dr. Soumya Swaminathan (NIRT). Clinical site: LRS Delhi	DBT	• Regulatory Advisory • Clinical Monitoring
Study to evaluate the anti-bacterial activity, safety, tolerability and pharmacokinetics of a combination of PA-824, Moxifloxacin and Pyrazinamide, or PA-824 patients with newly diagnosed MDR-Pulmonary Tuberculosis	Dr. Sarla Balachandran (CSIR) Clinical site: LRS Delhi	CSIR	• Project Management • Clinical Monitoring • Data Management • Safety Monitoring

Medical Affairs and Medical Writing

CDSA supports the investigators in medical writing and medical affairs and contribute to the development and evaluation of new products. We support investigators/ innovators through their studies along the product development journey. The department is providing the services as mentioned below:

Medical Support

- Support for GLSE Project:
  - Data Safety Monitoring Board constituted
  - Introductory meeting of DSMB conducted
  - Presented "Safety Monitoring and Causality Assessment" for

Investigator's Meeting

- Medical Monitoring services for OSDD Project
  - OSDD project transfer from GVK-Bio
  - Presentation of Safety Data for IDMC meeting at ICMR
- Supported CPM department for following Projects
  - UNICEF project
  - Thalassemia & SCD Control Program in Gujarat
- Supported CDM and Biostatistics for DNDi Project
- Drafted Protocol Synopsis for ISCR-MRCT Causality Assessment
- Presented CDSA at Pre-Meeting Workshop by Uppsala Monitoring Centre on latest developments in Pharmacovigilance, WHO Programme for International Drug Monitoring
- Co-ordinated and conducted Knowledge Sharing Sessions (5 sessions)

Medical Writing Support

- Medical Writing services provided to Malaria Vaccine Development Program (MVDP). The following documents had been reviewed:
  - Protocol
  - Patient Information Sheet & Informed Consent Form
  - Draft of CRF
  - DSMB Charter
  - Safety Management Plan
  - Protocol Presentation
- GLSE Project:
  - Surfactant therapeutic Training & protocol Training to Study Team at CDSA
  - Prepared DSMB Charter
  - Safety Management Plan drafted
  - Reviewed draft CRF
  - Reviewed Guidance document on trial related injury
  - Updated PIS & informed Consent form
- Verapamil Protocol amended as per regulatory authority recommendations
- Reviewed Informed Consent Document for Zinc Sepsis Study.
- A Repository of study related documents for the benefit of scientific fraternity. Following documents are uploaded on website under tab of "Resources"
  - Guidance document for Institutional Ethics committee
  - 5 generic IEC SOP templates
  - Medical Glossary Language (English)

- Quarterly Newsletter (4 issues of Newsletters has been released)
- Faculty for the CDSA workshops in medical affairs and medical writing domain
- Drafted following department SOPs
  - Medical Monitoring
  - Management of SAE
  - Protocol Development, Review, Approval and Amendment
  - Clinical Study Report Preparation, Review and Amendment
  - Investigator's Brochure
  - Preparation and Submission of Manuscript in a Biomedical Journal

Clinical data Management (CDM)

CDSA provides specialized Clinical Data Management services to, government and non-governmental innovators and SMEs and academic institutions to ensure consistent data quality and quicker turnaround.

CDSA offers CDM through Promasys v7.2 that has been installed in a secure and validated IT environment, ensuring quick and smooth transition of database build to database lock with operational cost advantage.

CDSA provides the following Data Management Services:

- Database Development and Maintenance
- eCRF designing
- Data Management Plan
- Data Validation Plan
- Edit Checks Programming
- Coding of AE (MedDRA)
- Coding of Concomitant Medications (WHO-DD)
- SAE data reconciliation
- Audit Trail
- Third Party Vendor data import set-up (Central Lab, ECG etc.)
- Design and Program Data Listings (for Sponsor Review)
- Create and Provide Patient Profiles (for Sponsor review)
- Database lock (final analysis)
- Data backup
- Data Export
- Electronic Signatures functionality
- Customized reports

No	Project Titlet	Funding Agency	PI/Site	CDSA Role
1	Evaluating the efficacy and safety of an innovative and affordable Goat Lung Surfactant for the treatment of respiratory distress syndrome in preterm neonates: a multi-site randomized clinical trial	WT	Dr. Ramesh Agarwal (AIIMS) (12 sites across India)	● Tendering, bidding & vendor selection (M/s Trianz) ● Completed service agreement formalities ● CRF designing ● eCRF filling guidelines ● Database functional design specification document ● Training on CRF completion (To research nurses)
2	A Phase II, Open Label, Randomised, Clinical Trial to Evaluate the Anti-Bacterial Activity, Safety, Tolerability and Pharmacokinetics of a Combination of PA-824, Moxifloxacin and Pyrazinamide, or PA-824 When Administered with the Category IV Regimen of RNTCP in Adult Patients with Newly Diagnosed Multi-Drug Resistant Pulmonary Tuberculosis: An 8Weeks Study	CSIR-OSDD	Dr. Rohit Sarin (NITRD) (1 site, NITRD)	● Study handover (GVK) ● Data retrieved from site ● Database updated ● Generated & closed DCFs ● Attended ICMR meeting ● Archival of study documents
3	Promasys v7.2	N/A	N/A	● Reinstallation & Upgradation of Promasys v6.2 to Promasys v7.2 ● AMC & Service agreement ● Installation Qualification & Operational Qualification completed ● Performance Qualification initiated ● Attended administration & user creation training

No	Project Titlet	Funding Agency	PI/Site	CDSA Role
4	Support to Training department	N/A	N/A	● Done Training Feedback analysis for the following: ▪ EC (Pune) ▪ EC (Bangalore) ▪ GCP (Chennai) ▪ EC (Chennai) ▪ BIRAC CDSA workshop Demystifying Indian drug Regulations for New Drugs Approval (Bangalore) ▪ C-Camp Regulatory requirements for biopharmaceuticals (Bangalore) ▪ Birac (Hyderabad) ▪ EC (Lucknow) ▪ Ethics (Rohini) ▪ ESIC GCP (Rohini) ▪ EC (Coimbatore) ● India mapping for training locations ● Formatting of BIRAC regulatory handbook
5	CDSA contact database	N/A	N/A	● Total 5179 records ● Removal of duplicate records ● Export of email ids for newsletter circulation & Training program awareness ● Removal of bounced email ids from the database ● Adddition of new contacts on regular basis
6	Support to Biostatistician for the training in 'R' software	N/A	N/A	● Printing of training documents ● Assistance for technical issues during training
7	Job Descriptions	N/A	N/A	● Prepared for Data Manager, Quality analyst, Clinical Data Coordinator, Database Designer, Database Administrator, SAS Programmer and Director CDM

No	Project Titlet	Funding Agency	PI/Site	CDSA Role
8	SOP			● Draft SOP for Clinical Study data backup

Bio-Statistics

This division aims to provide bio-statistical support to various clinical projects. CDSA uses mainly Statistical Analysis System® (SAS) Version 9.4 for statistical analysis. A dedicated IT-infrastructure (data servers, systems, access-controls, etc.) has been deployed to run the activities as per the standards of data security and safety.

CDSA provides the following services through its data management & biostatistics group:

- Statistical inputs to study design and clinical trial protocol
- Sample Size Calculation
- Randomization
- Statistical Analysis Plan
- Statistical Analysis of Data
- Reporting of Statistical Analysis (e.g. Listing, Figures and Tables)
- Training and Education
- Preparation of Technical Documents (Reports & Publications)

Delivery of Statistical Services

No	Project Title	Month	Funding Agency	PI
1.	Evaluating the efficacy and safety of an innovative and affordable Goat Lung Surfactant for the treatment of respiratory distress syndrome in preterm neonates: a multi-site randomized clinical trial	March 16 – Till Date	All India Institute of Medical Sciences, New Delhi	Dr. Ramesh Agarwal Additional Professor Department of Pediatrics ICMR Advanced Centre for Newborn Health Research All India Institute of Medical Sciences, New Delhi - 110029, India
2.	Study of Breast Cancer Subtypes based on Estrogen Receptor, Progesterone Receptor, Her2neu Expression with Special Emphasis on Triple Negatives by Immuno Histochemistry	Feb 16 – May 16	Assam Medical College, Dibrugarh	Dr. Gayatri Gogoi, Associate Professor, Department of Pathology, Assam Medical College, Dibrugarh

Dissemination of awareness of Good Statistical Practice

No.	Training Title	Number of Days	Date	Venue	No. of Participants
1.	Essentials of Statistics and Clinical Data Analysis Using R	Four	December 1 – 4, 2015	ICGEB, New Delhi	46

Resource Person

1 Annual Faculty/Teachers' Convention – 2015 at Jamia Hamdard University, New Delhi on Nov 3 – 4, 2015.

2 Workshop on "Methodology in Biosocial Research" at Amity University Uttar Pradesh on March 29, 2016.

3 Annual Event (ANACoD 2016) of National Brain Research Institute, Gurgaon on May 3, 2016

Regulatory Affairs

CDSA provides regulatory advisory services for development and registration of new drugs, medical devices, diagnostics and biopharmaceuticals/biosimilars including vaccines to SMEs and public funded pre-clinical and clinical stage research projects. The advisory cell provides:

- Advice on regulatory processes encompassing product development & registration
- Consultation on regulatory dossier preparation, e.g. New drugs; IND; CTD, etc.
- Regulatory input to ongoing clinical trials in CDSA (e.g. surfactant study, Study with TB drugs and Malaria vaccine study)
- Advice on Registration and re-Registration of Ethics Committees with CDSCO,
- Advice and review proposals coming from BIRAC (especially clinical trial protocols and regulatory training)

CDSA Team

High-end Instruments at THSTI

Academia

CORE 1975
V.I.P.

Ph.D. Program

Translational Health Science and Technology Institute (THSTI) is a recognized R&D institute of the Jawaharlal Nehru University (JNU), New Delhi to offer doctoral programs in biomedical and clinical research tracks for candidates with medical, life sciences (including biomedical, health, pharmaceutical, nutritional science, public health, and nursing), veterinary science, engineering, or mathematics background.

The broad domains of ongoing research at THSTI are:

- Biology of infectious diseases such as dengue, Japanese encephalitis, hepatitis E and tuberculosis, vaccine and anti-viral development
- Physiology of nutrition and the developing immune system, immune responses in pregnancy and childhood
- Clinical research and epidemiology focused on maternal and child health
- Auto-immune diseases, infection and inflammation
- Understanding disease through the human microbiome
- Diagnostics and therapeutics
- Medical devices and implants
- Mathematical modelling to understand biological problems

Students selected for the program are required to undergo a pre-PhD course work followed by the submission of a research thesis. The PhD program at THSTI is governed by the JNU rules.

The students admitted to THSTI-JNU PhD program will be required to undertake courses to earn 14 credits to be eligible to undertake thesis research work. THS-1, THS-2, and THS-3 are core courses of 8 credits and compulsory for all students. All other courses are optional and the student has the choice to earn 6 credits by choosing any combination. The courses offered over the two semesters are:

Semester-I

- Biomedical Research : Concepts and Methods
- Clinical Research Methodology
- Research Internship

Semester-II

- Health Policy and Decision Analysis in Health
- Infectious Disease Biology
- Infectious Disease Epidemiology
- Immunology and Immunotechnology
- Special Topics in Epidemiology
- Essentials of Clinical Trials
- Essentials of Regulatory Trials
- Introduction to Biodesign

Biomedical research : concepts and methods

This course has been designed to introduce the students to the practical world of life science research. The curriculum covers fundamental concepts of basic and translational research as well as educates them in identifying and executing innovative research ideas. The course also allows students to gain theoretical and practical understanding of techniques relevant to modern day life science research.

Clinical research methods

Students across life science disciplines require a sound knowledge of the fundamentals of research methods to understand, design, analyze and communicate their research. Students will be exposed to the basics of the components of a good research proposal and the essential epidemiological and statistical concepts that form the framework of sound research. Students will learn to use simple designs and statistical methods to formulate questions and analyze data. In addition to lectures and seminars the course will involve multi-faculty workshops, exercises in biostatistics with STATA statistical software and group tasks. Students across diverse fields are expected to understand the basics of clinical research methods and the language of clinical research by the end of the course.

Research internship

Students are required to work in the lab/clinic of the assigned supervisor in the afternoon and undergo training in various research methodologies and participate in laboratory/clinical discussions. At the end of the semester, students are required to write a report (8-10 pages) on various tasks assigned and make a presentation on their accomplishments to the assessment committee. The supervisor will provide his/her assessment of the student's performance during the internship, participation in laboratory/clinical activities and discussions, and the quality of report. Students are also expected to fine tune their analytical and scientific communication skills by presenting a seminar on a relevant topic (in consultation with their supervisor), detailing the existing knowledge and their opinion about future perspectives on that topic.

Health policy and decision analysis in health

Application of solutions for public health in the real world requires robust decision-making and analysis of risks. Economic analyses of policy decisions become even more critical in contexts of constrained resources. Public health professionals will be trained in techniques of decision and risk analyses. Many of these techniques can be used at the bedside as well for population & community health decisions. These methods can also be adapted to decision making for research priorities, outcomes and funding. Bayesian probability, evaluation of diagnostic tests, decision trees, QALYs, disease burden quantification, utilities and cost effectives analysis are some of the topics that will be covered. The course will involve lecture sessions with active class participation, in-class and take home exercises.

Infectious disease biology

This course aims at educating students about important human infectious diseases prevalent in India and worldwide. Introductory lectures by clinical

experts on few important infectious diseases will provide a practical overview of those diseases from a doctor's point of view. Further emphasis will be given to understanding the molecular basis of various bacterial and viral infections. This course will also cover classical as well as modern approaches to developing prophylactic and therapeutic strategies against those pathogens.

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Immunology and immunotechnology

This course covers both theory and techniques relevant for immunological research. The first part of the course aims at explaining the fundamental concepts of immune system and its components and illustrate the importance of these under various conditions such as infections, cancer or transplantation therapy. The course also covers the importance of human microbiome in mediating immune functions. The second part of the course will cover theoretical and practical aspects of techniques important for immunological research in the laboratory as well as discuss the concepts of important diagnostic techniques.

Special topics in epidemiology

This course covers advanced topics in epidemiology providing detailed understanding of the design and implementation of cohorts and case control studies as well as other epidemiologic studies. The course will consist of lectures, seminars, and reading material. In addition, a number of in-class and take home exercises will form a strong component of the course. Students will also spend substantial time in group-activity across disciplines to learn to work collaboratively to formulate translational research questions and to design studies to answer them. Students are expected to participate in discussions in classes.

Essentials of clinical trials

This course addresses critical methodological aspects of clinical trials. At the end of this course the student will be able to demonstrate understanding of the principles of clinical trials, assess and select relevant research designs for clinical trials, conduct random allocation, blinding and sample size estimations, critically interpret published results from clinical trials.

Regulatory trials

This course is designed to create awareness about the regulations & guidelines related to clinical research in India as well as globally. The students will understand how to manage a clinical study effectively by good documentation and clinical data management practices. The course prepares the participants to face audit and regulatory inspections from regulatory agencies.

Post-doctoral Program

THSTI provides post-doctoral training under the mentorship of its senior faculty. Young scientists who have recently completed their doctoral training are encouraged to correspond with faculty members whose research area may be of interest to them. THSTI will provide sponsorship for DBT post-doctoral fellowship to deserving candidates based on the recommendation of a faculty member. THSTI offers post-doctoral training to young researchers through specific options for post-doctoral program at various niche centers of THSTI. These schemes usually span for a period of five years and are widely advertised in national newspapers and on THSTI website.

The various post-doctoral options are

- ‘Vaccine Research Innovation (VRI) Award Scheme’ in the Vaccine and Infectious Disease Research Center.
- ‘Innovation Award’ scheme in Biodesign in the Centre for Biodesign and Diagnostics, focusing on diagnostics, implants and medical devices.
- ‘Microbiome Innovation Award’ scheme in the Centre for Human Microbial Ecology.

Also there is a sandwiched post-doctoral program, ‘Indo-Finnish Post-doctoral fellowship in Diagnostics’ funded by DBT and administered by THSTI. This program is for young researchers who have inclination for research in areas in diagnostic product development and platform technology development. The fellows get training at THSTI, India and University of Turku, Finnland.

PHD Students selected during 2015-16

Sapna Jain, Ms. Aarti Tripathi, Indu Bisht, Saimah Raza, Shailendra Chauhan, Jyoti Verma and Bugga Paramesha

Administration

THSTI Administration

Mr. M V Santo

The THSTI Administration performs relentlessly to provide unstinted support for the smooth scientific functioning of the institute. The personnel in the administration comply with the Government of India Rules and related financial norms in their functioning. The THSTI Administration comprises of several functional sections namely General Administration, Human Resource (HR) Management, Finance & Accounts, Stores & Purchase, Information Technology (IT), Engineering & Estate Management, Intellectual Property Management, Interim Bio-incubator, and legal. Information on some of the important activities performed by various sections under THSTI Administration is provided below.

General Administration

THSTI Governing Body/ THSTI Finance Committee / THSTI Society Meetings

On the governance front, THSTI conducted two meetings of the Finance Committee and Governing Body and one meeting of the Society and all the decisions taken were implemented.

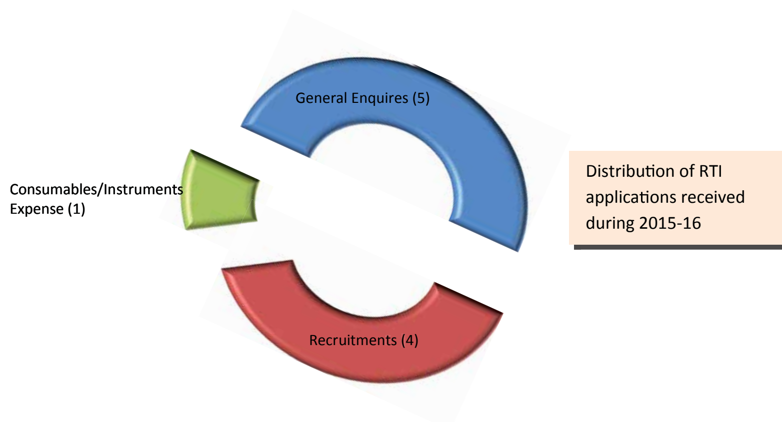
THSTI Internal Committees

In order to provide support to the Executive Director in decision making various internal committees have been constituted. THSTI also has various statutory committees like Human Ethics Committee, Animal Ethics Committee, Biosafety Committee, Internal Complaints Committee dealing with Sexual Harassment complaints. The constitution and roles of these Committees are given in the later part of this report.

Joint RCB-THSTI committee

To facilitate making the campus operational, the existing 2 partners of the NCR Biotech Science Cluster namely THSTI and RCB jointly constituted a “Committee of Operational Protocols” to make recommendations regarding common requirements in the new campus. The continuous efforts of this committee resulted in finalizing good contractors for electromechanical services, house-keeping services, security services, horticultural services, catering services, environmental management services, transport services and signage.

Right to Information



On the communication front, it has been the endeavor of THSTI to maintain high degree of transparency with regard to the entire official processes through the website especially about recruitments and tenders. Strict compliance to the requirement of Right to Information (RTI) Act 2005 is practiced in the Institute with regard to suo-motu disclosures. During the period 2015-16, we received 10 applications under the RTI act. Among these applications, five were with respect to THSTI related activities and the information was disseminated under the provisions of RTI Act. The rest were applications transferred from DBT seeking general information. The Parliament questions, references from DBT and other organizations were responded within the stipulated deadlines.

Events at THSTI

THSTI observed all the important occasions as directed by the Govt. of India along with the Foundation day of THSTI. A brief on these activities is provided below.

Independence Day and Republic Day

THSTI celebrated 69th Independence Day and 67th Republic Day with lots of activities like tree plantation, dance and drama, rangoli competition, poem recitation competition, Children fashion show. THSTI faculty participated warmly in the events with their family members.

Hindi Saptah Samaroh

Hindi Week was celebrated from 16th September 2015 to 21st September 2015. As a part of the celebration, various competitions were organized in Hindi such as poem recital, essay competition, extempore speech and quiz. Professor Hari Mohan Sharma, Head, Department of Hindi, Delhi University was the chief guest for the valedictory session.

Sadbhavana Diwas

Sadbhavana Diwas was observed on 20th August 2015. Sadbhavana Pledge was administered by the Executive Director and all students, staff, officers and faculty/scientists of the Institute took the pledge.

World Environment Day 2015

Amaltash plants were planted in NCR Bio-cluster campus by THSTI employees and faculty on the occasion of World Environment Day 2015.

Swachhta Divas

THSTI conducted cleanliness drive on 2nd October, 2015 on the occasion of the national campaign by the Government of India to clean the streets, roads and infrastructure under Swatch Bharat Mission.

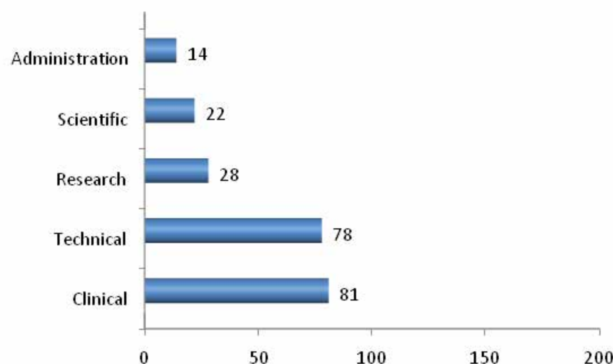
Vigilance Awareness week

THSTI observed vigilance awareness week from 26th October 2015 to 31st October 2015. The week commenced with a pledge administered by Dr. G. R. Medigeshi, CVO. During the week, various competitions were organized on the topic of anti-corruption drive.

6th Foundation Day

The occasion was celebrated with enthusiasm on 15th July 2015 by the THSTI community along with officials from the Department of Biotechnology, collaborators and well-wishers. The chief guest was Dr. Satyajit Rath.

Human Resource Management



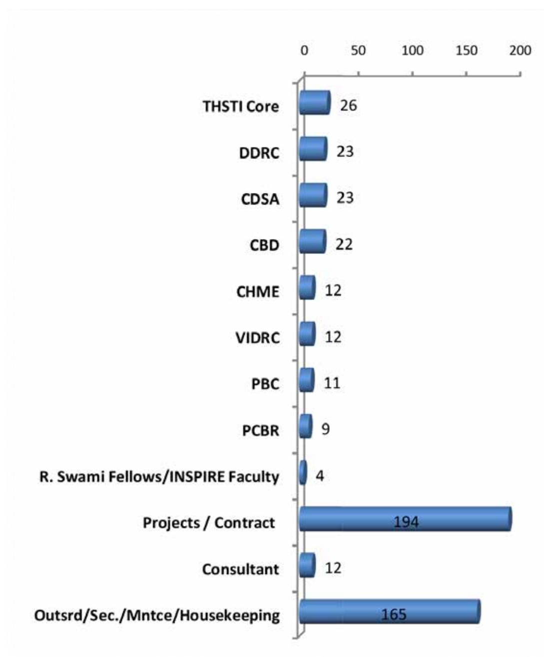
Distribution of recruitments during 2015-16

During this financial year, THSTI posted 32 recruitment notices for filling 223 positions. THSTI started posting notices in the Faridabad Employment Exchange and National Career Service Portal to comply with appropriate rules of Central and Haryana government, in addition to recruitment notices on THSTI website, national dallies and Employment News. The rolling advertisement in the case of JRF/SRF/RA positions, which was introduced during the year 2013-14, to cater to the frequent requirements of the projects, was continued with new vacancies being advertised and filled up successfully every month. For all positions, the selection process included a skill test followed by the interview.

In order to enhance the capabilities, competencies and effectiveness of employees, THSTI encourages employees to attend workshops, seminars etc. to widen their scientific knowledge for better output.

As per DoPT guidelines in modified FCS, three faculty members were promoted from assistant professors to associate professors during this financial year. All the

scientific/ technical/administrative personnel belonging to VIDRC and PBC were rigorously assessed for their performance and granted new contract for five years.



Distribution of Employees at THSTI as on 31st March, 2016

24 Hours ICICI ATM

Finance & Accounts

The Institute gets grant-in-aid from the Department of Biotechnology (DBT) and extramural funds from DBT and various other external funding agencies like Wellcome Trust, Bill and Melinda Gate Foundation, ICMR, DST, WHO etc. for various research projects. Finance & Accounts section of the Institute monitors and controls the expenditure against the above funds and also attends to the day-to-day financial matters, payments to contractors/suppliers, payment of salaries to staff, payment of personal claims in respect of the employees of the Institute etc. Since the numbers of employees have increased, salary software was purchased during the year 2015-16 which has made the process of preparation of salary more efficient with better MIS outputs for grant-wise details and automatic dissemination of salary slips to employees through email. The Section is also responsible for preparing the annual statement of accounts.

Audit for the period between 2009-10 and 2014-15 was conducted by the CAG team during December, 2015. The audit paras raised by the team of Audit Officers were promptly responded by THSTI through DBT.

The audited annual statements of accounts for the FY 2015-16 as presented before the Finance Committee, Governing Body and the Society has been provided in the later part of this report.

Stores and Purchase

The Stores and Purchase section is responsible for the purchase of scientific equipment, perishable and non-perishable chemicals and reagents, other consumables and services from overseas and local markets. In order to minimize the procurement cost, the section has started warehousing the plastic-ware and the requirements are taken as a whole for all the labs. All

perishable and non-perishable shipments were being cleared promptly from the port in order to avoid demurrage. THSTI has invested Rs. 463.83 Lakhs and Rs. 155.48 Lakhs respectively in consumables and equipment during this financial year. In consonance with the directives received from the Department of Biotechnology (DBT), THSTI has started publishing their tenders through e-Procurement module of CPP portal (Central Public Procurement Portal). E-procurement not only makes the system transparent and efficient, it also instils confidence among the vendors in the tendering system. The section procures equipment and non-consumable materials costing more than 10 lakhs through e-procurement.

Information Technology

THSTI understands that connectivity with national and international scientific community is essential for developing healthcare technologies and translation of lab scale technologies into marketable products. During this financial year, THSTI IT section which takes care of the hardware, networking, website and software requirements for the institute, helped in installation of M/s Reliance lease line over RF for getting fast and un-interrupted 30 Mbps RF. National Knowledge Network (NKN) lease line of 100 Mbps over fiber connectivity was installed during this financial year which made VC and Skype meetings possible at THSTI. Now, THSTI employees can fill and submit most of the administrative forms like medical reimbursement, leave request etc. online, which has reduced request disposal time and enhanced uniformity.

Engineering and Estate Management

The Engineering section develops and maintains the Institute's physical infrastructure and developed various new facilities this financial year such Radio activity lab, DDRC lab, PCBR office etc. The primary responsibility of this section is to ensure that all the scientific equipment is kept functional. The section takes full responsibility for the electricity supply system, HVAC system, fire system etc. The section has started in house preventive maintenance of the equipment for minor repairs/service of i/c HVAC, electrical, fire etc. due to which the dependence on expensive maintenance (Contracts)has been considerably reduced.

The engineering section successfully overcame the biggest challenge to make HVAC system and other facilities in Small Animal facility (SAF) functional. The process of handing and taking over of the new hostel buildings including electromagnetic equipment in the electrical substation, Installation of 2x500kva DG sets, lifts, fire-fighting system etc. was carried out methodically on the civil engineering side, challenges with regard to addition, modification, and maintenance was carried out efficiently.

Small Animal Facility

Balance Sheet

TRANSLATIONAL HEALTH SCIENCE AND TECHNOLOGY INSTITUTE,FARIDABAD

BALANCE SHEET AS AT 31ST MARCH, 2016

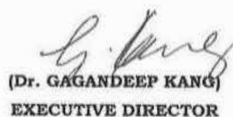
Amount (In Rs.)

LIABILITIES	Schedule	31.03.2016	31.03.2015
Corpus / Capital Fund	1	1,40,29,24,129	1,14,03,33,546
Reserves and Surplus	2	7,77,66,161	9,79,14,323
Earmarked/Endowment Funds	3	-	-
Secured Loans and Borrowings	4	-	-
Unsecured Loans and Borrowings	5	-	-
Deferred Credit Liabilities	6	-	-
Current Liabilities and Provisions	7	23,59,69,984	9,63,97,847
TOTAL		**1,71,66,60,274**	**1,33,46,45,716**
ASSETS			
Fixed Assets	8	1,31,12,02,875	1,06,95,59,605
Investment From Earmarked/Endowment Funds	9	-	-
Investment-Others	10	-	-
Current Assets, Loans, Advances etc.	11	40,54,57,399	26,50,86,111
Miscellaneous Expenditure		-	-
(to the extent not written off or adjusted)			
TOTAL		**1,71,66,60,274**	**1,33,46,45,716**
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 S.M.Saini & Associates
Chartered Accountants

(C. B. YADAV)
FINANCE & ACCOUNTS OFFICER



(Dr. GAGANDEEP KANG)
EXECUTIVE DIRECTOR

(LAXMIKANT SAINI)
PARTNER
M No.512056

Place: Faridabad
Date: 28/09/2016

Income and Expenditure

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31st MARCH, 2016

Amount (in Rs.)

INCOME	Schedule	31.03.2016	31.03.2015
Income from Sales/ Services	12	1,28,656	1,23,080.00
Grants/Subsides	13	16,99,67,000	17,50,00,000
Fees/Subscriptions	14	2,41,768	-
Income from Investments	15	-	-
Income from Royalty, Publication etc.	16	-	-
Interest Earned	17	1,08,81,328	1,57,93,029
Other Income	18	26,94,875	26,53,954
Increase/(Decrease) in stock of Finished goods and works in progress	19	-	-
Deferred Income-Fixed Assets		6,80,28,866	5,32,97,156
TOTAL (A)		**25,19,42,493**	**24,68,67,219**
EXPENDITURE			
Establishment Expenses	20	7,76,17,344	5,49,59,696
Other Administrative Expenses etc.	21	12,64,44,445	11,28,78,329
Expenditure on Grants , Subsidies etc.	22	-	-
Interest	23	-	-
Depreciation (Net Total at the year-end-corresponding to Schedule 8)		6,80,28,866	5,32,97,156
Prior period Adjustment A/c (ANN-A)		-	-
TOTAL(B)		**27,20,90,655**	**22,11,35,181**
Balance being excess of Expenditure Over Income (A-B)		**(2,01,48,162)**	**2,57,32,038**
Transfer to special Reserve(Specify each)		-	-
Transfer to /from General Reserve		(2,01,48,162)	2,57,32,038
BALANCE BEING SURPLUS /DEFICIT CARRIED TO CORPUS/CAPITAL FUND		-	-
SIGNIFICANT ACCOUNTING POLICIES AND NOTES ON ACCOUNTS	24		
CONTINGENT LIABILITIES	-		

Schedules 1 to 24 form an integral parts of Accounts

As per our separate Report of even date attached
For S.M.Saini & Associates
Chartered Accountants

(C. B. YADAV)
FINANCE & ACCOUNTS OFFICER

(Dr. GAGANDEEP KANG)
EXECUTIVE DIRECTOR

(LAXMIKANT SAINI)
PARTNER

Place: Faridabad
Date: 28/09/2016

Consolidated Receipts and Payments

TRANSLATIONAL HEALTH SCIENCE & TECHNOLOGY INSTITUTE (THSTI)
Faridabad

CONSOLIDATED RECEIPTS AND PAYMENTS ACCOUNT FOR THSTI,PROJECTS & FELLOWSHIP FOR THE YEAR ENDED 31ST MARCH, 2016

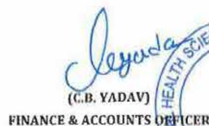
AMOUNT-IN-RUPEES

RECEIPTS Particulars	31.03.2016		31.03.2015	
OPENING BALANCE:-				
THSTI	2,68,40,781		1,28,08,651	
Projects	21,18,62,939		8,86,38,457	
Fellowship	2,35,931		1,11,15,286	
Grant-in Aid Received:-				
THSTI	28,99,67,000		22,43,37,000	
Projects	58,28,17,222		34,02,01,712	
Fellowship	3,11,79,830		2,00,22,515	
Other Receipts -THSTI				
Miscellaneous Receipts	750		10,010	
Other Receipts	21,24,504		-	
Sales of Scrap	1,28,656		1,23,080	
Overhead THSTI	85,000		22,95,324	
RTI Receipt	150		20	
Guest House Receipt	75,500		66,300	
Admission Fees	-		74,500	
Recruitment Fee	59,400		66,300	
Tender Fee	1,66,000		1,41,500	
Security / Hostel Deposit Received	13,86,121		4,02,014	
Earnest Money Deposit	4,82,466		-	
Interest Received	1,08,81,328		84,29,095	
Accrued Interest Received	4,35,443		73,53,934	
Refund of Security Deposit	2,50,000		-	
Application fees	61,500		-	
Penalty Receipt	66,904		-	
Rental/License fees/Usage charges	1,16,667		-	
Receipt from short term training program	1,80,268		-	
Govt. Dues Payable	33,18,797		4,31,672	
Other Liabilities/Payable	46,52,051		4,41,436	
Decrease in advances	1,41,33,655		9,43,669	
TOTAL		**1,18,15,08,863**		**71,79,02,475**

AMOUNT-IN-RUPEES

PAYMENTS Particulars	31.03.2016		31.03.2015	
THSTI				
Fixed Assets	2,45,73,948		1,47,37,575	
Work -in- Process- Building	8,00,00,000		2,70,00,000	
Manpower	5,91,60,860		5,49,88,958	
Consumables	4,63,83,355		3,88,14,656	
Administrative Expenses	8,24,75,762		7,01,61,735	
Advances , Receivables & Liabilities	2,97,49,800		2,53,80,800	
Projects	46,00,39,500		21,69,77,230	
Fellowship	3,15,81,241		3,09,01,870	
Closing Cash & Bank Balance				
THSTI	**3,30,69,216**		**2,68,40,781**	
Projects	**33,46,40,661**		**21,18,62,939**	
Fellowship	**(1,65,480)**		**2,35,931**	
TOTAL		**1,18,15,08,863**		**71,79,02,475**

AS PER OUR SEPARATE REPORT
OF EVEN DATE ATTACHED.
FOR S. M. SAINI AND ASSOCIATES
CHARTERED ACCOUNTANTS



(C.B. YADAV)
FINANCE & ACCOUNTS OFFICER

(DR. GAGANDEEP KANG)
EXECUTIVE DIRECTOR

(LAXMIKANT SAINI)
PARTNER

Place: Faridabad
Date: 28/09/2016

Page 1 of 106

Auditors Report

S.M.SAINI &ASSOCIATES
Chartered Accountants

120, Mohyal Colony, B/H MMISchool,
Sector-40, Gurgaon-122001
Ph.09310832563, 09868275687, 0124-2381062
laxmikant_saini84@yahoo.co.in

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 2016 and the relative Income &Expenditure Account& Receipt & Payment Accountfor the period ended on that date, annexed thereto. 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 the Income & Expenditure account and Receipt & Payment Account dealt 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, the said account a true and fair view in conformity with the accounting principles generally accepted in India

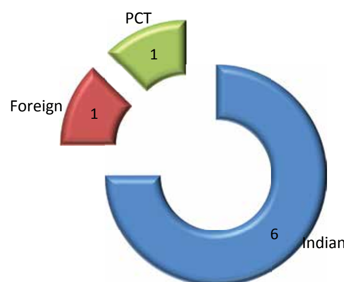
      i. In the case of the balance sheet, of the state of affairs of the Institute as at 31st March 2016; and
      ii. In the case of Receipt & Payment Account, of the receipt and payment for the period ended on that date
      iii. In the case of Income and expenditure Account, excess of expenditure over income for the period ended on that date.

ForS.M.Saini & Associates
Chartered Accountants

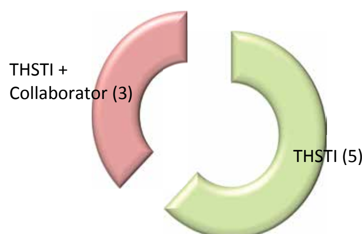
Laxmikant Saini
Partner
Membership No.512056
Place: Gurgaon
Date:28/09/2016
FRN: 014267N

Branches: - Delhi, Rewari and Jaipur

Intellectual Property Protection



Distribution of patent filings during 2015-16 [Geography]



Collaborative Patent Filings during 2015-16

THSTI understands the importance of intellectual property and it screens outcomes from projects on routine basis to identify Intellectual Property vested in it. During this financial year, THSTI as an applicant filed 6 Indian Patent applications, one PCT application and one Korean patent application. THSTI has also filed two copyright applications strengthening its Intellectual Property assets. THSTI also understands that collaborative research involving sharing of expertise and infrastructure has very positive impact on development and commercialization of technologies. This reflects from the fact that 3 out of 8 patent filings during this financial year are outcome of collaborative research.

Technology Commercialization

THSTI understands that commercialization of Intellectual Property assets is as important as creating Intellectual Property assets. Hence, Intellectual Property assets owned by THSTI are regularly advertised on its website to seek industry partners having capacity to commercialize the laboratory scale technologies. THSTI has adopted provisions provided in lines with IP Policy of National Centre for Cell Science, Pune and developed a SOP for commercialization of technologies developed by THSTI either alone or in collaboration with other parties. THSTI has also adopted Rules and Regulations provided in DBT OM: BT/NBDB/13/01/2014 dated 25th November, 2014 and DSIR OM: 3/3/2009-TU/V/Knowledge-to-Equity dated 25th May, 2009 which allow THSTI to take equity stake in Scientific Enterprise in exchange of technologies and support services made available to Scientific Enterprise.

Faculty Entrepreneurship

Although entrepreneurship is included in the mission of THSTI, faculty entrepreneurship has come to realty only after adopting provisions provided in DBT OM: BT/NBDB/13/01/2014 dated 25th November, 2014 and DSIR OM: 3/3/2009-TU/V/Knowledge-to-Equity dated 25th May, 2009. During the current financial year, THSTI received three applications from scientific staff seeking permission to take equity stake in scientific enterprise. As all three applicants fulfilled eligibility criteria provided in DBT OM, permission was granted and in consequence THSTI's first faculty start-up Tritek Innovation Private Limited came into existence.

Interim Bio-incubator

Biotech Science Custer (NCR-BSC) has got approval to set up a bio-incubator from DBT and NCR-BSC is recognized by funding agencies like BIRAC for various funding programs. During the current year after financial, an Interim Bio-incubator was set-up in the 2nd floor of THSTI building and came into operation when Dr. Jonathan Pillai and Dr. Pawan Mehrotra got Biotechnology Ignition Grant under individual category with prerequisite condition that they must be associated with a bio-incubator for the grant. Incubates at interim Bio-incubator have been given access to instruments at THSTI on sharing basis under various payment modules. Apart with from this, infrastructure and furniture with from also provided to incubatees to facilitate research.

Publication, Patenting, and Technology Commercialization Metrics

THSTI proactively maintains in-house metrics on Publication, Patenting and Technology Commercialization in order to provide information for various studies conducted by DBT, BIRAC, DST, DSIR, NIC, and other government agencies to measure patenting, publication, technology commercialization activities on national scale.

Inter-institutional Agreements

THSTI collaborates with many national and international organizations for research and development which is well reflected in collaborative publications and IP sharing data. At THSTI, we maintain a well-defined database to store information about agreements executed and stamp duty paid to the government. All agreements are duly vetted before execution and terms and conditions accepted are duly complied during the entire term of the project.

THSTI Housing and Hostel

THSTI Committees (2015-16)

S. No.	Committee	Members
01.	**THSTI Management Committee**	a. Dr. G. B. Nair b. Dr. Sudhanshu Vrati c. Dr. Shinjini Bhatnagar 4. Dr. Kanury Rao d. Dr. Sudhakar Bangera e. Mr. M.V. Santo **Chairperson-Dr. G.B. Nair**
02.	**Academic Committee**	a. Dr. Sudhanshu Vrati b. Dr. Shinjini Bhatnagar c. Dr. Kanury Rao d. All convenors of the sub-committees formed by the Chairperson e. Mr. M. V. Santo f. Mr. J. N. Mishra **Chairperson - Dr. Sudhanshu Vrati**
03.	**Maintenance Committee**	a. Dr. Guruprasad Medigeshi b. Dr. Nisheeth Agarwal c. Dr. Savit Prabhu d. Dr. Ashutosh Tiwari e. Dr. Shilpa Jamwal f. Mr. G R Agarwal g. Mr. Vishal Gupta h. Mr. Chandrabhan Yadav **Chairperson – Dr. Guruprasad Medigeshi / Dr. Nisheeth Agarwal**

S. No.	Committee	Members
04.	**Purchase Committee**	a. Dr. Ranjith Kumar b. Dr. Sanjay Banerjee c. Dr. Gaurav Batra d. Dr. Shailaja Sopory e. Dr. Niraj Kumar/Dr. Jonathan Pillai f. Mr. Prashant Bhujbal g. Mr. Mohd. Shahid **Chairperson- Dr. Ranjith Kumar / Dr. Sanjay Banerjee**
05.	**IT & Communication Committee**	a. Dr. Jonathan Pillai b. Dr. Arup Banerjee c. Dr. Sushmita Chaudhuri d. Mr. Irudayaraj e. Mr. G. R. Agarwal f. Ms. Taruna **Chairperson – Dr. Jonathan Pillai / Dr. Arup Banerjee**
07.	**Human ethics committee (Human Research)**	a. Prof. Satinder Aneja b. Ms. Vidhya Krishnamoorthy c. Prof. Subir Kumar Maulik d. Dr. Suvasini Sharma e. Mr. Munawwar Naseem f. Dr. Ujjayini Ray g. Ms. Jasmine Singh h. Mr. D. Raghunandan i. Dr. Ashutosh Tiwari j. Dr. Sarmila Mazumder k. Dr. Tarun Batra l. Prof. Rajiv Janardhanan m. Dr. Sivaram Mylavarapu **Chairman - Prof. Satinder Aneja**

S. No.	Committee	Members
08.	**Animal ethics committee**	a. Dr. Sudhanshu Vrati b. Dr. S. Jayaraman c. Dr. Deepak Sharma d. Major General Dhillon e. Dr. Nagarajan f. Dr. Krishnamohan Atmakuri g. Dr. Amit Kumar Pandey h. Dr. Niraj Kumar i. Dr. Amit Awasthi **Chairperson – Dr. Sudhanshu Vrati** **Dr. Amit Awasthi (Co-ordinator)**
09.	**Biosafety Committee**	a. Dr. Sudhanshu Vrati b. Dr. Susmita Chaudhuri c. Dr. Nisheeth Agarwal d. Dr. Shailaja Sopory e. Dr. Vinay Kumar Nandicoori f. Dr. Uma Chandra Mouli Natchu g. Dr. Anirban Basu **Chairperson – Dr. Sudhanshu Vrati**
10.	**RTI Act**	a. Dr. Amit Pandey – PIO b. Dr. Shinjini Bhatnagar – Appellate Authority c. Dr. Sudhanshu Vrati – Transparency Officer d. Mr M. V. Santo – Nodal Officer e. Dr. G. B. Nair – Public Authority
11.	**Complaints Committee to Internal Complaints Committee (to enquire into complaints of sexual harassment)**	a. Dr. Shinjini Bhatnagar b. Dr. Shobha Broor (external member) c. Dr. Nita Bhandari d. Dr. Manjula Kalia e. Dr. Monika Bahl f. Mr. M. V. Santo **Chairperson – Dr. Shinjini Bhatnagar**

S. No.	Committee	Members
13.	**Student Welfare and Hostel Committee**	a. Dr. Amit Awasthi (Hostel Warden) b. Dr. Pallavi (Hostel Warden) c. Dr. Mohan Babu Appaighari d. Dr. Sucheta Kurundkar e. Mr. J. N. Mishra f. Two students' representatives **Chairperson – Dr. Amit Awasthi / Dr. Pallavi**
14.	**Tender Opening Committee**	a. Mr. Deepak Baghele b. Mr. Manoj Kumar c. Mr Eklavya Srivastava
15.	**Vigilance Officer**	Dr. Guruprasad R. Medigeshi

Our Augmented Strength

Chair & Honorary Faculty

Biotechnology Chair
Prof. John David Clemens
Executive Director
International Centre for
Diarrhoeal Disease Research Dhaka, Bangladesh

National Chair
Dr. T. Ramamurthy
Dr. Kanury Venkata Subba Rao

Visiting Professor of Eminence
Prof. N. K. Ganguly

Honorary International Visiting Faculty
Dr. Madhukar Pai, MD, PhD
Associate Professor, McGill University
Associate Director, McGill International TB Centre.

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

Adjunct Faculty/Honorary Visiting Professor

Dr. Satyajit Rath
Sr. Scientist, National Institute of Immunology

Dr. Vineeta Bal
Sr. Scientist, National Institute of Immunology

Prof. Anil K. Tyagi
Vice Chancellor, Indraprastha University

Dr. Navin Khanna
Group Leader, ICGEB

Dr. Nita Bhandari
Jt. Director, CHRD-SAS

Dr. Amit Sharma
Group Leader, ICGEB

Dr. Jaya SivaswamiTyagi
Professor, Department of Biotechnology
All India Institute of Medical Sciences

Seminars at THSTI (2015-16)

Date [dd/mm/yyyy]	Topic	Speaker
23/02/2016	Role of α4β7 integrin in mucosal transmission of SIV/HIV	Dr. Siddappa Byra Reddy, Nebraska Center for Substance Abuse Research, Durham Research Center, USA
18/02/2016	Targeting Mast Cells to Boost Immunity to Vaccines	Dr. Soman Ahraham, Duke University, USA
23/12/2015	Single cell genomics identifies novel regulators of autoimmunity and tolerance	Prof. Vijay K. Kuchroo, Harvard Medical School, Boston, USA
8/12/2015	Genome/epigenome dynamics in bacterial evolution	Prof. Ichizo Kobayashi, University of Tokyo, Japan
27/11/2015	An Epidemiological Approach to Find Important Bacteria Associated with Diarrhea and Prevention: 16S analyses and quantitative PCR	Prof. O. Colin Stine, University of Maryland, School of Medicine, Baltimore, USA
26/06/2015	Membrane biology of leishmania infection: therapeutic role of liposomal cholesterol	Syamal Roy CSIR-Indian Institute of Chemical Biology
20/08/2015	Impact Sans Impact Factors	Prof. P.N. Tandon National Research Professor & President, National Brain Research Centre
14/07/2015	Dr.ug delivery strategies to engineer T cell responses	Dr. Siddharth Jhunjhunwala, Indian Institute of Sciences, Bengaluru
22/04/2015	Genome-based paths to epidemiology and vaccines in bacteria	Prof. Dr. Trinad Chakraborty Dean, Faculty of Medicine Justus-Liebig University
6/4/2015	Deciphering the key paths of preterm labor and its therapeutic opportunities	Dr. Mukesh K Jaiswal, Research Assistant Professor at Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA

Industrial Collaborations

Understanding gut and vaginal microbiome in Indian population

Dr. Bhabatosh Das and his team at CHME, THSTI are working in collaboration with Tata Consultancy Services for understanding the diversity, dynamics and biology of vaginal microbiome of pregnant Indian women. The team is also working on gut microbiome and gut inflammation in severe acute malnourished Indian children with TCS. In addition to this, Dr. Bhabatosh and his team is also working on MicrobDiab which is study of interactions between the gut microbiome and the human host biology to elucidate novel aspects of the pathophysiology and pathogenesis of Type 2 Diabetes in collaboration with TCS and NNFCBMR, Denmark

Developing metabolome-based models for prognosis and diagnosis of metabolic syndrome (MetS)

Dr. Samrat Chatterjee and Dr. KV Subba Rao at DDRC, THSTI are working in collaboration with Hyderabad based Revelations Biotech Pvt. Ltd. for developing metabolome-based models for prognosis and diagnosis of metabolic Syndrome.

Developing metabolome-based models for prognosis and diagnosis of metabolic syndrome (MetS)

Dr. Sagarika Haldar and her team at CBD, THSTI are working in collaboration with Ambala based Advanced Microdevices Private Ltd. to make a novel, simple and rapid diagnostic device for TB, MDR-TB and XDR-TB.

High Sensitivity Multiplex point-of-care assay systems for the detection of blood borne infections in emergency setting

Dr. Gaurav Batra and his team at CBD, THSTI are working in collaboration with Arrow Weighing Systems Pvt. Ltd. and Finland based Kaivogen OY to develop high sensitivity multiplex point-of-care assay systems for the detection of blood borne infections in emergency setting.

International Research Collaborations

HIV Vaccine Design

HVTR Laboratory in VIDRC, THSTI is constantly working on HIV Vaccine engineering since its establishment as joint research laboratory between VIDRC, THSTI and International AIDS Vaccine Initiative. A group of scientists in this laboratory is working on screening and identification of broadly neutralizing antibodies in Indian donors chronically infected with HIV-1 clade C and purification and characterization of antigenic properties of HIV-1 trimeric envelope proteins obtained from broadly neutralizing plasma of Indian donors. The team is also working on identification of neutralizing antibody epitopes on Indian and South African HIV-1 subtype C viruses for HIV vaccine design. Another group of scientists in this laboratory is working on stabilization of 4-2.J41 ENV in a native-like trimeric structure and role of C-terminal domain in restoration of wild type conformation for 4.2.J41 ENV with deleted cytoplasmic tail at protein level. This group is also doing a comparative immunogenicity study in rabbits of cleavage competent Indian clade-C ENV (4-2.J41) or with clade-B JR-FL ENV or in combination of these two by using DNA priming followed by soluble trimeric protein boost immunization format. This laboratory works in collaboration with USA based Scripps Research Institute, New York based Weill Cornell Medical College, Amsterdam based Academic Medical Center, and Johannesburg based National Institute of Communicable Diseases.

Identification of correlates of severe dengue disease

Dr. Guruprasad R. Medigeshi and his team at VIDRC are working in collaboration with New Delhi based ICGEB-Emory Vaccine Center to identify correlates of severe Dengue disease.

Antiviral signaling during Japanese encephalitis virus infection

Dr. Sudhanshu Vrati and his team at VIDRC are working in collaboration with Tokyo Medical and Dental University to study antiviral signaling during Japanese encephalitis virus infection of neuronal cells. The team is also working in collaboration with University of Adelaide, Australia on identification of the Japanese encephalitis virus attachment and receptor system. In collaboration with Paris based Centre de Recherche des Cordeliers, the team is studying interactions between Japanese encephalitis virus and host cellular pathways.

Identification of novel scaffolds and drug targets to combat tuberculosis

Dr. Ramandeep and his team at VIDRC are working in collaboration with KRICT, Korea for identifying novel scaffolds and drug targets to combat tuberculosis. KRICT and THSTI have filed a patent application at KIPO which will be used as basic application for filing convention patent application in India.

Functional characterization of mycobacterial aminopeptidase

Dr. Krishnamohan Atmakuri and his team at VIDRC are working on characterization of mycobacterial aminopeptidase in collaboration with Emory University, USA

Integrative genomics of host-pathogen interaction to identify new drug targets against persistent Mycobacterium tuberculosis

Dr. Amit Kumar Pandey and his team at VIDRC are working in collaboration with ASTAR, Singapore on identifying new drug targets against persistent Mycobacterium tuberculosis using integrative genomics of host-pathogen interaction.

The Effects of Human Intestinal Microbiota on Immune Responses

Dr. Bhabatosh Das and his team at CHME are working with Osaka University to study effects of microbiota in the human intestine on immune responses.

Interplay between effector and regulatory T cells in the pathogenesis of intestinal inflammation

Dr. Amit Awasthi and his team at CHME are working in collaboration with Harvard Medical Schoool, USA on interplay between effector and regulatory T cells in the pathogenesis of intestinal inflammation.

Assessment for Global Strategy and Plan of Action on Public Health, Innovation and Intellectual Property for India

Dr. Bratati Mukhopadhayay and her team at PCBR are working in collaboration with WHO SEARO and Delhi based WCO on the assessment ofglobal strategy and plan of action on public health, innovation and intellectual property for India.

Enteric fever in India- a retrospective review of existing data on surveillance for enteric fever in Asia project (SEAP Phase I)

Dr. N. K. Ganguly and his team at PCBR are working in collaboration with Albert Sabin Vaccine Institute, USA on retrospective review of existing data on surveillance for enteric fever in Asia project.

Identification of gaps to strengthen health R & D in India

Dr. N. K. Ganguly and his team at PCBR are also working on Identification of gaps to strengthen health R & D in India in collaboration with WHO SEARO.

Zinc as an adjunct for the treatment of clinical severe infection in infants younger than 2 months

Dr. Nitya Wadhwa and her team under the leadership of Dr. Shinjini Bhatnagar are working in collaboration with Center for International Health, University of Bergen; Norway, Institute of Medicine; Tribhuvan University; Nepal, Patan Hospital; Nepal, Kanti Children's Hospital; Nepal, Center for Health Policy/ Primary Care and Outcomes Research Stanford University; on Zinc as an adjunct for the treatment of clinical severe infection in infants younger than 2 months.

ट्रांसलेशनल स्वास्थ्य विज्ञान
एवं प्रौद्योगिकी संस्थान

TRANSLATIONAL HEALTH SCIENCE
AND TECHNOLOGY INSTITUTE

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