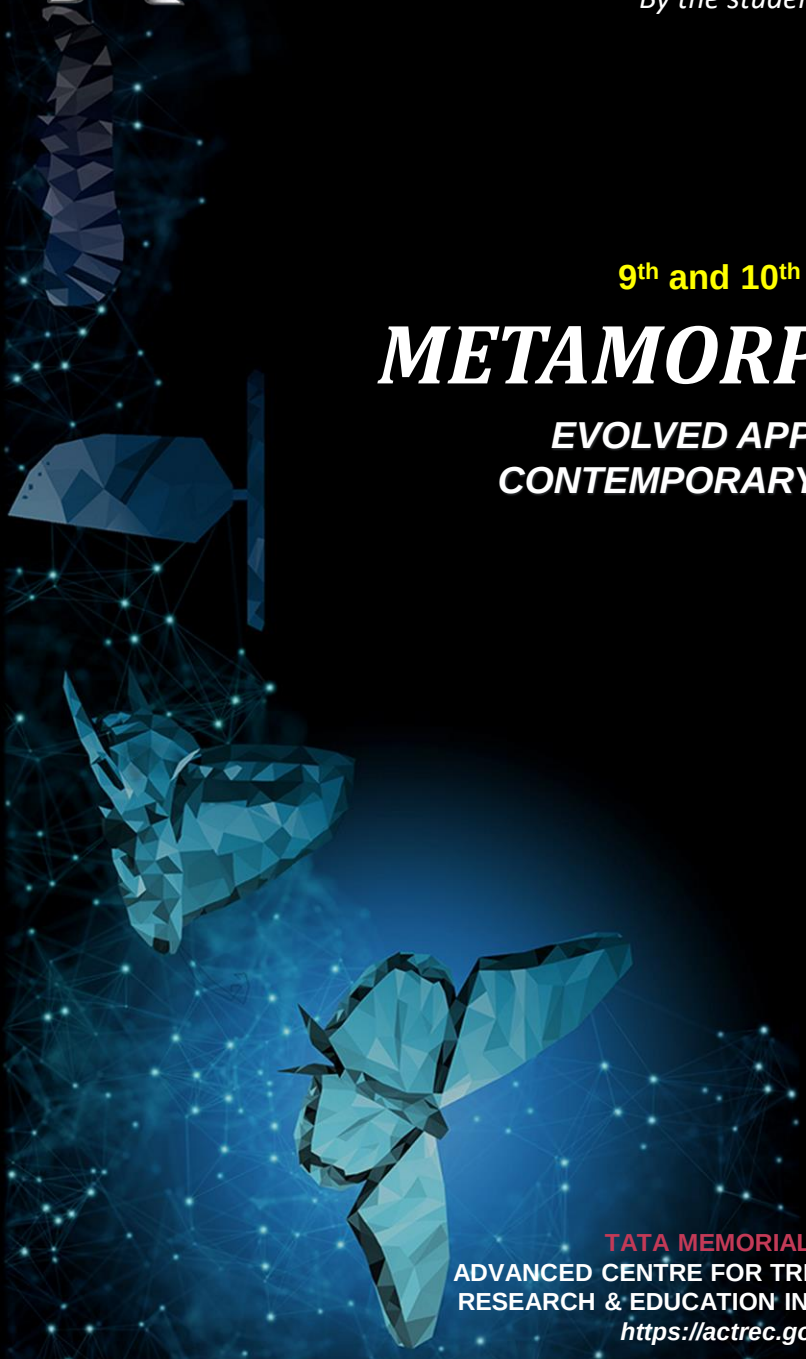
17th NATIONAL RESEARCH SCHOLARS MEET

By the students, for the students

9th and 10th December 2021

METAMORPHOSIS:

EVOLVED APPROACHES TO CONTEMPORARY BIOLOGICAL CHALLENGES



TATA MEMORIAL CENTRE
ADVANCED CENTRE FOR TREATMENT RESEARCH & EDUCATION IN CANCER
https://actrec.gov.in/nrsm

Preface

National **R**esearch **S**cholars' **M**eet (NRSM) is an annual event organized solely by the research scholar fraternity of ACTREC with the motto "By the students, for the students". The conference spanning over two days encourages young and enthusiastic minds across the country to utilize this platform for sharing their innovative ideas and research work as well as gain scientific insights. NRSM receives wide participation from masters and graduate students from both basic and clinical sciences.

This year, NRSM is stepping into its 17th year with the theme, **"Metamorphosis: Evolved approaches to contemporary biological challenges".** Through which, we aim to address the ever-changing outlook towards life sciences, research and medicine. The conference will touch upon the subjects like species evolution, evolution of basic and translational research and technological advances towards answering the contemporary challenges in biology through approaches like Artificial Intelligence. Taking in consideration that the upcoming decade is going to be detrimental for what path the life on Earth takes, this year's conference will also address the cardinal concern of "Climate Change" through a thought provoking panel discussion. Adapting with the changing times guided by the COVID19 pandemic, this year the conference undertakes a hybrid mode wherein participants across India as well as eminent speakers from various fields of biology will interact with each other virtually as well as in person.

Along with developing scientific aptitude, NRSM also promotes the creative side of researchers. The conference plays host to 'Scientifia', a scientific quiz and "Creative corner", an event for showcasing participants' talent in photography, painting, and poetry as well as a "Cultural Evening" where art coalesces with science.

NRSM 2021 Organizing Committee

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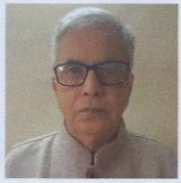
Prof. P.R. Vasudeva Rao, FNAE, FNASc
Vice-Chancellor

प्रो. पी.आर. वासुदेव राव, एफएनएई, एफएनएएससी
कुलपति



Message

I am glad to learn that the 17th National Research Scholars' Meet is being organized by the Research Scholars of ACTREC-TMC on 9th & 10th December 2021. Over the years, this has become an excellent forum for research scholars to share and discuss their results among themselves and with eminent experts in life sciences from across the Country and across the Globe. Having perused the technical program of the event, I am very confident that the present edition of NRSM will also turn out to be a great success.

The canvas of higher education has been changing continuously, and today's scenario demands that students need to learn not only the scientific and technical aspects, but also a variety of other skills in order to succeed in their career. Communication skills, organizational skills and network skills have become particularly important. Meetings such as NRSM will be important platforms for students to acquire such skills.

The Covid pandemic has taught us several lessons, and among those, the most important perhaps is the importance of scientific research and particularly collaborative research. I understand that the development of the COVID-19 vaccines was made possible by more than a decade of research into mRNA vaccines. The pandemic has also brought to center stage the importance of distance learning and online courses. More than ever, the students now have great opportunities to learn from experts far away from their own institutions. HBNI will strive to exploit the advantages offered by modern technology to place learning resources from across the world within reach for the students.

I convey my hearty congratulations to the students of HBNI at ACTREC for keeping up the tradition of organizing RSMs even under challenging circumstances. I wish the program all success.

(P.R. Vasudeva Rao)

Message from the Director

Dr Sudeep Gupta, MD, DM,

Professor of Medical Oncology

Director ACTREC

Yet another year draws to a close…but not just any other year. It is almost two years to the month when a new virus called SARS-CoV-2 was first announced to the world. Over the ensuing months it unleashed itself on an unsuspecting humanity in the form of a pandemic. And life has not been the same ever since. What were once unimaginable have come to pass – face masks as a way of life, children studying in online schools, every travel plan clouded by uncertainty, many lives cut short and loved ones passing away, reduction in human life span in many countries reversing the trend of many decades, and others.

The most depressing news now is the lack of certainty that this virus will ever 'completely' go away and that we will ever get back to 'pre-COVID' way of life. The virus has mutated and continues to mutate, finding new ways to evade whatever we throw at it, and has established reservoirs in other animal species.

On an optimistic note, it is a sign of the remarkable resilience of our species that we have quickly adapted to the new 'reality'. After some hiccoughs, life has gone on…including the National Research Scholars Meet. In its 17th avatar this year NRSM has introduced several 'firsts'. The submitted abstracts will be published in a peer reviewed journal. On the agenda, among others, is climate change, a theme that will make or break humanity in the decades ahead. This year was also the occasion when, rather unexpectedly, the Nobel Prize in physics was awarded to three scientists (Manabe, Hasselmann and Parisi) for their contributions to the theory and modelling of climate change in particular, and complex systems in general. Climate is about as complex as it gets, somewhat similar to the incredible complexity of many biological systems.

Humanity is only beginning to learn how to come to grips with complexity in many domains. Computing power, including the ability to digest and assimilate big data, is now an invaluable ally in this quest. However, 'intelligent' computers (if they will ever be possible in the 'real' sense of 'intelligence') are still some time away. There are tantalising glimpses into the future. Two papers which have impacted me most in the past few years belong to the domains of artificial intelligence and computing. The first paper demonstrated how a computer program, fed only with 'rules of the game', learnt three complex board games entirely through self-play, as perhaps a child would, and became so good at them that it beat 'world champion' programs that had been fed with enormous amounts of data and 'hand-crafted' human algorithms. [1] The second paper reported a breakthrough in quantum computing, establishing proof-of-concept of 'quantum supremacy'. [2] I am delighted that AI is also on the agenda of 17th NRSM.

Aldous Huxley's 'Brave New World', conceived in another era and another context, was perhaps never so imminent as it is now.

Dr Sudeep Gupta, MD, DM,

Professor of Medical Oncology

Director ACTREC

1. Silver D, Hubert T, Schrittwieser J, et al. A general reinforcement learning algorithm that masters chess, shogi, and Go through self-play. Science. 2018 Dec 7;362(6419):1140-1144.

2. Arute F, Arya K, Babbush R, et al. Quantum supremacy using a programmable superconducting processor. Nature. 2019 Oct;574(7779):505-510.

Message from the Deputy Director, CRI

Dr. Prasanna Venkatraman

Deputy Director, Cancer Research Institute
ACTREC-TMC

An Odyssey to the future by the NRSM

As the NRSM unfolds this winter, batch of 2018 has kept with the tradition to bring in a new flavor to the 'For the students, By the students' National meeting. They have been very bold and are touching upon topics that are of immense concern worldwide- go green, climate change and metamorphosis of all sorts!! It is befitting that they have invited the stalwart as their chief guest Shri Padma Bhushan Dr Thirumalachari Ramasami who revolutionized leather tanning industry by adopting biotechnological methods to avoid pollution and accumulation of toxic materials. With that he saved several thousand employment and made it possible for research to impact masses. As a secretary to DST, he envisioned some of the notable programs such as the INSPIRE that attracts some of the best young minds to research. I am honored that he has accepted their invitation and is coming in person to interact with the young minds.

NRSM also has a good admixture of the eminent young and experienced faculties to deliver talks on a variety of interesting topics. With e- posters, a change induced by the pandemic the flavor is complete. Oral presentations, creative corner and scientific trivia will have the students flocking to the web. I wish the NRSM as always, the very best and I hope all my students at ACTREC, and away as well as the faculty enjoy the delightful intellectually provocative event.

I am also particularly looking forward to the style statements of the gentlemen and ladies of this NRSM batch!

Wishing you the very best
Affectionately,
Prasanna Venkatraman
Deputy Director, Cancer Research Institute
ACTREC-TMC

Message from the Deputy Director, CRC

Dr. Navin Khattry
Deputy Director, Clinical Research Centre
Professor, Dept. of Medical Oncology
ACTREC, Tata Memorial Centre

The National Research Scholars' Meet organised by the PhD students of ACTREC is truly a conference "for the students, by the students." On its 17th annual meeting, I have witnessed its metamorphosis from a small annual meeting of students of ACTREC to a more pan India meet of students. The theme each year is thought provoking, addressing various opportunities and challenges in contemporary research. This year is no exception with the broad theme of evolution of both biological life and technology. If there is any one issue that needs urgent attention in the next few decades, it is how to tackle climate change so as to arrest changes that would be truly detrimental to all life on earth! I am happy that this year's meet will also touch upon this subject.

As a faculty of Tata Memorial Centre, I am extremely proud of our student fraternity which organises this annual meet with clockwise precision on topics that are relevant to both science and life in general! I wish them the best for this meeting and I am sure all participating students from various institutes of our country would benefit from the scientific deliberations.

Navin Khattry

Deputy Director, Clinical Research Centre

Professor, Dept. of Medical Oncology

ACTREC, Tata Memorial Centre

17th NRSM- Event Schedule

Day 1	9th December 2021 Venue - Auditorium, Khanolkar Shodhika, ACTREC
8:00 am – 9:00 am	Registration and Breakfast
9:00 am – 9:10 am	Introduction to the National Research Scholars Meet in Life Sciences
9:10 am – 9:15 am	Inauguration of the 17th National Research Scholars Meet in Life Sciences
9:15 am – 9:20 am	Welcome address by Dr. Sudeep Gupta, Director, ACTREC
9:20 am – 9:25 am	Welcome address by Dr. Prasanna Venkatraman, Deputy Director, CRI, ACTREC
9:25 am – 9:30 am	Welcome address by Dr. Navin Khattry, Deputy Director, CRC, ACTREC
9:30 am – 9:40 am	Address by Guest of Honor, Prof P R Vasudeva Rao, Vice-Chancellor, HBNI
9:40 am – 10:20 am	**Chief guest address: Dr. T Ramasami**
10:20 am – 11:00 am	**Keynote session 1: Dr. L. S. Shashidhara**
11:00 am – 11:15 am	Tea break
11:15 am – 11:55 am	**Keynote session 2: Dr. Uma Ramakrishnan**
12:00 pm -01:15 pm	**Oral presentations**
01:15 pm – 02:00 pm	Lunch and Interaction with delegates
02:00 pm – 03:15 pm	**Oral presentations**
03:20 pm – 04:00 pm	**Keynote session 3: Dr. Debojyoti Chakraborty**
04:00 pm – 04:15 pm	Tea Break
04:20 pm – 05:50 pm	**Poster presentation Session**
05:50 pm – 06:20 pm	Scientifia
6:30 pm – 7:30 pm	Cultural Evening
7:30 pm onwards	Dinner

17th NRSM- Event Schedule

Day 2	10th December 2021 Venue - Auditorium, Khanolkar Shodhika, ACTREC
08:15 am – 09:15 am	Breakfast
09:30 am – 10:10 am	**Keynote session 4: Dr. Collins Assisi**
10:10 am – 10:50 am	**Keynote session 5: Dr. Karishma Kaushik**
10.50 am – 11:10 am	Tea Break
11:10 am – 01:00 pm	**Poster Session + Creative corner**
01:00 pm – 02:00 pm	Lunch
02:00 pm – 03:15 pm	**Panel Discussion on Climate change**
03:15 am – 03:30 am	Tea Break
03:30 pm – 04:00 pm	**Company talk + Poster Session**
04:00 pm – 05:00 pm	Award Ceremony and Valedictory
05:00 pm – 05.30 pm	High Tea

Speaker Abstracts

Chief Guest

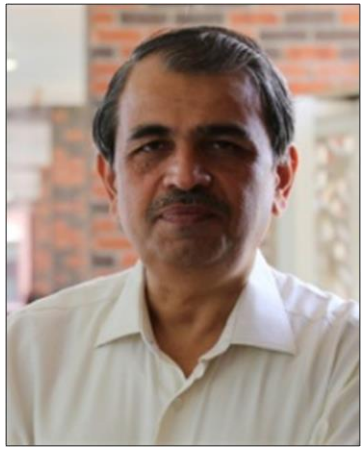
Dr. T. Ramasami
Former Secretary, Ministry of Science and Technology &
Distinguished Professor of Eminence, Anna University, Chennai

Metamorphosis

Metamorphosis is a transformative process adopted by Nature inspires mankind for addressing several challenges faced by the society. It is proposed to enlist ten contemporary biological challenges that merit close scrutiny by the research community for designing and developing a solution science approach. The enlisted challenges are infectious diseases, life style diseases, Infectious diseases and pandemic break out, challenged biodiversity and conservation, protection & restoration, carbon footprint of lifestyle and carbon neutral growth, agriculture and farmers' livelihood opportunities, nutritional imbalance and maternal health, Water borne diseases and infantile mortality among the poor, threat perception from bioweapons, degenerative diseases and regenerative medicine and regulation science for a contemporary world order. Some paradigm changes and approaches needed for designing R&D based solutions to the ten enlisted problems will be highlighted. Metamorphosis in nature is a self-regulated process. A case will be made for embracing self-regulation in development of R&D solutions for the enlisted challenges. A case will be made for the establishment of a national centre for regenerative medicine and positioning of robust regulatory framework based on rigorous principles of science. An advocacy will be made for responsible innovations as the next best step in addressing cotemporary biological challenges.

Keynote Speaker

Dr. L. S. Shashidhara
Dean, Ashoka University



Evolution of "*endless forms, most beautiful*"

Molecular mechanism of evolution involves simple nucleotide changes in DNA during replication, resulting in changes in protein structure and possible emergence of new functions. This talk will walk you through how the same ubiquitous mechanism results in changes in tissue/organ morphology. If the emergent properties of such new anatomical features add to the fitness of the organisms, those would be subjected to evolutionary selection. The breath-taking biodiversity that the earth has borne for the past 3.5 billion years is the result of this simple, but reiterative evolutionary mechanism.

Keynote Speaker

Dr. Uma Ramakrishnan
NCBS, Bangalore

Conservation genomics to save endangered species

We are in the midst of a biodiversity loss crisis. While we know that biodiversity is critical to human wellbeing, we are unsure how to conserve species. On the other hand, genomic technologies and the ability to gather genomic data have grown exponentially. I present work from my lab where we have used next generation sequencing and genomic approaches to better understand endangered species and set priorities for their conservation. I will show case studies from tigers, dholes and critically endangered vultures, and how genomic tools have helped better understand isolated populations, species biology and population estimation.

Keynote Speaker

Dr. Debojyoti Chakraborty
CSIR-IGIB, Delhi

Reading and writing the genome with precision: implications in cancer therapy

The advent of CRISPR-Cas (Clustered regularly interspaced short palindromic repeats-CRISPR associated) makes it feasible to target/edit the genome with precision. FnCas9 (Cas9 derived from *Francisella novicida)* shows a high specificity along with an extremely high mismatch sensitivity making it possible to target and edit genes with precision. The proof-of-concept for which has been shown by the correction of the Sickle Cell Disease (SCD) point mutation (in patient derived iPSCs) and the detection of SCD/COVID-19 by FELUDA (FnCas9 linked uniform detection assay is a robust, rapid, and inexpensive point-of-care test). The applications can extend beyond standard gene correction to more extensive therapeutic strategies relevant in cancer.

Keynote Speaker

Dr. Karishma Kaushik
Savitribai Phule Pune University

Of men, mice and microbes: How are we building human-relevant infection models to replace animal studies?

Infection biology, including the study of bacterial infections and biofilms, has typically relied on standard laboratory approaches or animal model studies to understand disease pathogenesis and for the evaluation of therapeutics. It is well-recognized that laboratory studies based on microbial growth and susceptibility testing are far too reductionist to recapitulate the complexity of the infection state. On the other hand, animal testing presents a 'host' of challenges, including lack of scientific relevance to humans, and ethical and cost considerations. Bridging this gap prompts the need to develop human-relevant models that faithfully recapitulate the infection microenvironment, as well as enable selective and precise control, and overcome ethical and cost limitations. Towards this, there has been a concerted expansion of *in vitro* technologies such as advanced models with human-relevant components, organoid and organ-on-chip systems. These approaches have been leveraged to build human-relevant models of a range of infection states such as Tuberculosis (single organism, immune pathology) and bacterial pneumonia in the Cystic Fibrosis lung (multiple organisms, host defect). While these model systems have provided notable host-pathogen insights and potentially reduced the use of animals, bringing them into the preclinical screening and drug discovery pipeline has presented challenges. This session will provide an overview of approaches and technologies in the field, with a special focus of current and ongoing advances in building human-relevant models of polymicrobial infections and biofilms.

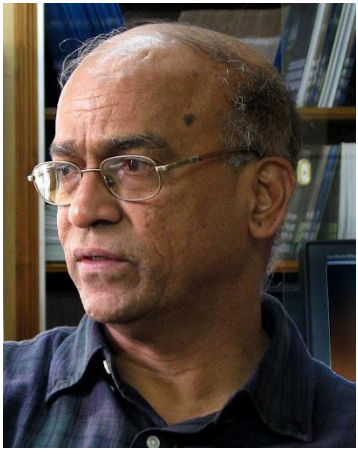
Keynote Speaker

Dr. Collins Assisi
IISER, Pune

Neural invariants of odor percepts – An algorithm in wetware

Olfaction, with other chemical senses, is phylogenetically the oldest sense. Therefore, understanding the mechanisms underlying olfactory perception will provide insights into a successful, and perhaps optimal, biological algorithm for processing complex information. Olfaction, like other sensory systems, uses the timing of spikes to encode incoming sensory inputs. The identity of an odorant that arrives in a steady stream can be reliably ascertained by examining the spatiotemporal sequence of spikes that are generated by neural networks involved in olfaction. However, odor inputs come, not as a steady stream, but riding upon chaotically pulsed plumes of air. Each time an input arrives, it follows a unique temporal pattern. As a consequence, the spatiotemporal spike sequences that presumably encode the odor must also be different every time an animal encounters temporally varying odor input. It really is a wonder then that animals are capable of decoding sensory cues and navigating highly complex and turbulent 'odorscapes'. Why, despite varying inputs, are odor percepts invariant (alternatively, why does a rose always smell like a rose)? I will address this question using a computational model of the insect olfactory system.

Climate Change Panelists



Prof. N.H. Ravindranath
IISC, Bangalore

Dr. Samir Damare
NIO, Goa

Dr. Binish Desai
Recycle Man of India

Oral Presentation Abstracts

OP1: Expression pattern of CD244, a novel SLAM protein and its clinical utility in the diagnosis of Acute leukemia.

Anilkumar A[1,2], Rajpal S[1,2], Chatterjee G[1,2], Shah N[1,2], Girase K[1,2], Ghogale S[1,2], Deshpande N[1,2], Badrinath Y[1,2], Sriram H[1,2], Patkar N[1,2], Gujral S[1,2,3], PG Subramanian[1,2], Tembhare P[1,2*]

[1]Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Tata Memorial Centre, Kharghar, Navi Mumbai, Maharashtra- 410210. [2]Homi Bhabha National Institute (HBNI), BARC Training School Complex, Anushaktinagar, Mumbai, Maharashtra- 400094. [3]Tata Memorial Hospital, Parel East, Mumbai, Maharashtra- 400012, India.

Background: CD244, a member of the signaling lymphocyte-activation molecule (SLAM), is expressed on all NK cells, a subpopulation of T cells, monocytes, and basophils as well as leukemic stem cells. With the advent of immunotherapy, we aimed to assess the expression of CD244 in patients with acute leukemias. **Materials and Methods:** Flow Cytometric Immunophenotyping was performed to study the expression pattern of CD244 on normal cells and leukemic blasts using 10-13 colour panel. The study included 50-B-ALL, 50- TALL, 20- MDS and 91- AML cases, along with 7 normal bone marrow controls. Analysis was done on Kaluza v-2.1 Software. **Results:** NK- cells and T- Cells were taken as positive controls with a median nMFI of 8 (0.049- 9.99) and 2 (0.32- 7.75), respectively. Comparison of AML and MDS blasts vs granulocytes and monocytes revealed that CD244 can distinguish between AML blasts and normal granulocytes (p-value<0.0001), monocytes (p-value<0.0001), MDS blasts from granulocytes (p-value<0.0001) and monocytes (p-value<0.0002) respectively. NK cells express CD244 and can be distinguished from T-blasts which are negative for CD244 (p value<0.0001). Note the nMFI of hematogones is of normal bone marrow. The calculated median nMFI and range is given in Table 1. **Conclusion:** This is the first study to demonstrate CD244-expression in acute leukemia & can be used as a potential marker for MRD monitoring in AML as it distinguishes MRD from granulocytes, monocytes and T-lymphoblasts from NK cells in T-ALL which may serve as a potential therapeutic target in patients with AML.

OP2: An efficient diagnosis of Diffuse Parenchymal Lung Disease (DPLD) from spirometry exploring the novel role of FEF_{25-75}

Debkanya Dey[1], Mintu Paul[2], Gourab Saha[2], Sayoni Sengupta[2], Dipanjan Saha[2], Rajat Banerjee[1], Parthasarathi Bhattacharyya[2]

[1]Department of Biotechnology, University of Calcutta, Kolkata, West Bengal. [2] Institute of Pulmocare and Research, Kolkata, West Bengal.

Background: The peripheral interstitial involvement in DPLD can affect the small airways and, thus, influence the FEF_{25-75} (forced expiratory flow), a marker of small airway function in spirometry. Here we evaluate the role of FEF_{25-75} for the diagnosis of DPLD that is otherwise diagnosed by high-resolution computerised tomography of chest. **Materials and Method:** FVC (forced vital capacity), FEF_{25-75}, FEV_1(forced expiratory volume) and derived variables (FVC/ FEF_{25-75}) are used to differentiate DPLD from normal, obstructive lung disease (OLD) populations. Using different combinations of variables, receiver operating characteristic curve and regression analysis are done to identify the best possible combination to diagnose DPLD from spirometry. **Result:** We included 639 adult subjects' [normal- 64, DPLD- 268, OLD- 307 including asthma- 153 & COPD- 154] spirometry data. FEF_{25-75} alone could differentiate unmixed DPLD from OLD with sensitivity and specificity of 86.9% and 85.1%. The derived variables also showed promising diagnostic accuracies. A regression equation using age, FVC, FEV_1/FVC, FVC/FEF_{25-75}(%) is found to have 79% diagnostic accuracy of DPLD on test population (n=639). The same equation showed 90% and 87% diagnostic accuracy of DPLD in validation population of 427 mixed patients from same centre and 100 mixed patients from different centre. **Conclusion:** The inclusion of derived variables from spirometry and the regression equation using age, FVC, FEV_1/FVC, FVC/FEF_{25-75} (%) appears rewarding in the diagnostic exercise for DPLD. Hence, this spirometric approach needs further validation from multiple centres to be included in clinical practice.

OP3: Identification of Profilin 2 in the testis and its interaction with HDAC6

[1]Pratibha Verma, [1]Smita Yevate and [1]Priyanka Parte*

[1]Department of Gamete Immunobiology, ICMR-National Institute for Research in Reproductive Health, Mumbai 400012, India.

Background: Previous reports have demonstrated the involvement of HDAC6 in sperm motility. Presence of HDAC6 has been reported in germ cells, to get a snapshot on role the of HDAC6 at specific stage of spermatogenesis, GC-1spg cells were employed which represent cell type between type B spermatogonia and preleptotene spermatocytes. **Materials Methods**: Mouse HDAC6 was transfected in GC-1spg cells, confirmed by Realtime, Western blot analysis and Indirect Immunofluorescence (IIF), its effect on germ cell transcriptome was investigated by Microarray gene expression array. IIF and Coimmunoprecipitation experiments were performed for colocalization and protein interaction. **Results:** Many transcripts that were differentially regulated, Profilin2 reported previously as a neuronal specific isoform, was observed as one of the genes highly upregulated at the transcript level, which was further confirmed by real-time PCR, protein confirmed by IIF. Profilin2 colocalized with HDAC6 both in GC-1 and sperm. On the sperm, presence of profilin2 was detected throughout the flagella, its colocalization with HDAC6 seen conspicuously in the mid piece region of the flagella. Coimmunoprecipitation confirmed Profilin2 interaction with HDAC6. Docking studies using Z dock suggested the interaction of 8 residues of HDAC6 forming hydrogen bonds with 6 residues of Profilin2. **Conclusion:** Present study identified Profilin2 as a novel interacting partner of HDAC6 and its presence in testis, GC-1cells and sperm for the first time. Based on our observations and literature, we propose that their interaction may be integrated to regulate the actin cytoskeleton and possibly involved in migration during spermatogenesis.

OP4: Modulation of cellular aging in the *Drosophila* hematopoietic organ alters the cell lineage trajectories and blood cell homeostasis

Kishalay Ghosh and Rohan Jayant Khadilkar*

Stem cell and Tissue Homeostasis laboratory, CRI, TMC-ACTREC

Background: Organismal aging is accompanied by a progressive decline in cellular and molecular functioning affecting organismal health and longevity. Stem cell aging is a hallmark of aging that results in disruption of normal functioning of organs. Our current study investigates the effect of aging on the hematopoietic system using *Drosophila*. **Materials and methods**: Using Gal4-UAS technology, we have overexpressed Atg8 or FOXO transgenes to stall/induce reverse aging in various cell populations of the Lymph Gland (LG), larval hematopoietic organ. These genetic models have been subjected to *in-vivo* analysis using organ dissections and immunofluorescence analysis. Post-acquisition analysis has been done using ImageJ. **Results:** Stalling aging by spatially modulating expression of autophagy protein, Atg8 and transcription factor, FOXO in different cell compartments of lymph gland has differential effects on the niche cells, hematopoietic progenitors, and the differentiated blood cells. Genetic manipulation of molecular circuitry of aging in the fat-body systemically alters dynamics of hematopoiesis in LG at many levels. Genetic modulation of aging alters cell lineage trajectories leading to either an increase or decrease in a given differentiated blood cell type. **Conclusion**: Our data show that modulating cellular aging leads to changes in cellular differentiation potential thereby altering tissue homeostasis. Further studies will help in understanding the mechanistic framework and signaling that regulates cellular aging to maintain blood cell homeostasis.

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OP5: Inter-subunit Crosstalk via PDZ Synergistically Governs Allosteric Activation of Proapoptotic HtrA2

Aasna L. Parui,[1, 2] Vandana Mishra,[3] Shubhankar Dutta,[1] Prasenjit Bhaumik,[3] Kakoli Bose[1, 2,*]

[1]Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Centre, Kharghar, Navi Mumbai – 410210, Maharashtra, India. [2]Homi Bhabha National Institute, BARC Training School Complex, Anushaktinagar, Mumbai – 400094, Maharashtra, India. [3]Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Powai, Mumbai – 400076, Maharashtra, India.

Background: Mitochondrial serine protease - HtrA2 is associated with various diseases including neurodegenerative disorders and cancer. Despite availability of structural details, the reports on HtrA2's mechanistic regulation still remain non-concordant. The purpose of this study is to identify the allosteric modes of communication adopted by HtrA2 under different activation scenarios. **Materials and Methods:** We generated heterotrimeric HtrA2 variants differing in the number of protein-protein interaction domain - PDZ and/or active-site mutations via a protomer-mixing strategy. Various structure-guided biophysical studies such as enzyme kinetics, MD simulations, single-molecule photobleaching and X-ray diffraction studies were adopted to demonstrate differences in various conformational modes of these ensembles. **Results:** Sequential deletion of PDZs from the trimeric ensemble significantly affected the total residual activity of each HtrA2 variant in a way that proffered a hypothesis advocating *intermolecular* allosteric crosstalk via PDZ domains in trimeric HtrA2. The single-molecule photobleaching studies established the existence of step-wise binding of substrate molecule to each subunit of the trimer, while enzymatic studies with active-site variants affirmed the role of *trans*-mediated allosteric communication within the pyramidal protease ensemble. Furthermore, structural and computational snapshots affirmed the role of PDZs in secondary structural element formation and coordinated reorganization of the N-terminal region and regulatory loops of the protease. **Conclusions:** This study unequivocally describes different activation mechanisms of HtrA2 at the atomic level using a unique retrospective approach through dissecting and rebuilding protein structure. Apart from providing cues for devising structure-guided therapeutic strategies, it establishes a working model of complex allosteric regulation through a multifaceted *trans*-mediated cooperatively-shared energy landscape

OP6: The differential interplay of the Notch-3/Jag-1 axis modulates disease progression in epithelial ovarian cancer (EOC)

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Background: A classical way of cell-to-cell communication is through receptor-ligand interaction, forming the basis of Notch signaling. EOC displays a heterotypic/homotypic-interplay between tumor-mesothelial/tumor-tumor cells, conniving its propensity of peritoneal metastasis. These warrant investigating differential Notch-3 activation in the tumor through heterogenous mesothelial/cancer cells-mediated jagged-1 induction affecting cellular characteristics. **Materials and methods**: We developed a co-culture-based model, NIH3T3 differentially-overexpressing *jagged1* and SKOV3-expressing Notch-3-sensor [10XCSL-driven firefly-luciferase] (SCFT) and employed bioluminescence-imaging for monitoring Notch-3 activity. We performed assays like CFSE, MTT, Matrigel-invasion to study the cellular functions and transcriptional profiling of SCFT post different co-cultures by RT-PCR-array to identify Notch-3 effector candidates. **Results**: We showed that incremental membranous-jagged-1 expression in NIH3T3 leads to proportional Notch-3 activation in SCFT upon co-culturing as reflected by the luciferase signal. We corroborated these observations in SCFT by co-cultures with HGSOC-patients' ascitic-mesothelial cell and jagged-1-expressing EOC cell lines (A2780, OVCAR-3, and OAW42). NIH3T3$^{J1\text{-highest}}$ and OVCAR3-cocultured SCFT showed proliferation index (48 h) 10.56 and 3.03 compared to SCFT. The resistance index (cisplatin) of SCFT co-cultured with NIH3T3$^{J1\text{-highest}}$ and OVCAR-3 were 2.71 and 2.53, respectively. SCFT invaded 2-fold higher after NIH3T3$^{J1\text{-highest}}$ co-incubation. Five genes (CDKN1A, VEGFA, TNFSF10, SERPINA3, FOXC1) showed significant fold-regulation across co-culture conditions, and we assessed two genes with fold-change^{max} (CDKN1A and VEGFA) for their roles. **Conclusion**: We had developed a robust, sensitive real-time co-culture-based Notch-3 reporter sensor. Further, we found that differential Notch-3 activation affected several critical phenotypes like proliferation, invasiveness, and chemoresistance proportionately. Two putative critical regulators of Notch-3-mediated effects, CDKN1A and VEGFA, were identified, impacting the overall disease progression.

OP7: Novel CDK-7 inhibitor suppresses transcription of oncogenes and enhances Venetoclax mediated apoptosis in Acute Myeloid Leukemia

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Background: Acute myeloid Leukemia remains a highly fatal disease. Based on the available evidence of deregulated pathways of proliferation & apoptosis in AML, we hypothesize that pharmacological modulation of AML blasts by inhibitors of BCL-2 (Venetoclax) and novel CDK7 inhibitor (CRI-256) will be potently synergistic for the treatment of AML. **Materials and methods:** AML cell lines & primary blasts from 50 AML patients were used for antiproliferation assays. Cell proliferation & apoptosis was assessed using flow cytometry and western blotting. CDK7 CRISPR knockout was performed using pSp-Cas9-PURO-CDK7 vector. NOD/SCID mouse models of AML were used to evaluate antileukemic activity of CRI-256. **Results:** Novel CDK7 inhibitor (CRI-256) downregulated proliferation in leukemic cell lines and in primary AML blast. CRI-256 inhibited the phosphorylation of RNA pol-II leading to decrease in transcription of oncogenes that plays major role in AML proliferation. RNA sequencing data provided further evidence that expression of genes involved in high cell proliferation was downregulated after treatment. Cell cycle arrest as well as induction of apoptosis was detected in dose dependant manner. Significant tumor reduction was observed in AML xenografts after 2 weeks of CRI-256. The anti-proliferative data derived from AML cell lines treated with CRI-256 and Venetoclax has shown significant synergism. **Conclusion:** The data indicated that targeted therapy by novel CDK7 inhibitor induce apoptosis, decrease cell proliferation and has greater anti-leukemic activity compared to standard of care in AML.

OP8: Multimodal approach to characterize missense mutation identified in h-BRCA2

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Background: BRCA2 protein plays an essential role in homologous recombination. Germ-line mutations in BRCA2 confer an increased risk of breast cancer. Many missense mutations have been identified in BRCA2; however, most of them are classified as variants of 'Uncertain Significance' due to a lack of structural, functional, and clinical studies. **Materials and Methods:** Here, multi-disciplinary *in-silico*, *in-vitro* and biophysical approaches have been explored to characterize an unclassified missense mutation, BRCA2 Arg2502Cys, identified from a case-control study. *Wild-type* and mutant proteins were overexpressed and purified using bacterial expression system. Comparative secondary and tertiary studies were performed using circular-dichroism and fluorescence spectroscopy. **Results:** Circular-dichroism and Fluorescence spectroscopy showed that the Arg2502Cys mutation in hBRCA2 (residues 2350-2545) decreases the α-helical/β-sheet propensity of the *wild-type* protein and perturb the tertiary structure conformation. In molecular dynamics simulation studies, the mutation perturbs the structural integrity and conformational dynamics by altering the intramolecular H-bonds, overall compactness and stability of the hydrophobic core. Principle component analysis indicated that Arg2502Cys mutant exhibits comparatively large conformational transitions and periodic fluctuation conformers throughout the 250ns trajectories. **Conclusion:** Therefore, to our conclusion, BRCA2 Arg2502Cys mutation perturbed the structural integrity and conformational dynamics of BRCA2, which in turn may affect the function of the protein and could result in cancer predisposition.

OP9: Localized delivery of Gemcitabine using Chitosan-Poly Vinyl Alcohol film inhibits pancreatic tumor growth in pre-clinical settings

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Background- Gemcitabine (GEM) is used for treatment of Pancreatic cancer (PC) patients. However, the limiting step in GEM dosage is its low plasma half-life, non-specificity and high cytotoxicity. To overcome these limitations we have developed GEM loaded Chitosan-Poly Vinyl Alcohol (CP-GEM) based biodegradable film for localized and sustained release of GEM for PC management. **Material and Methods-** The CP-GEM was synthesized using solvent-casting method. The cell viability assays was carried in both 2D and 3D PC cell culture with different dosages of GEM for 6hours(mimicking the clinical scenario), 5days and equivalent amount CP-GEM for 5days . Pre-Clinical antitumor efficacy study was carried out in subcutaneous PC model. **Results-**The GEM gets released in small dose over a period of 2 weeks. It was observed that CP-GEM has enhanced cell killing capability in both 2D and 3D cell culture. Intriguingly, GEM resistant PC cells developed, on treatment of free GEM for 6hours and 5days, whereas no such development was observed on treatment with CP-GEM even after 5days from end of the treatment. CP-GEM also inhibited cell proliferation and lead to DNA double strand break. Further, we observed that CP-GEM inhibited tumor growth in mice indicating the anti-tumor capability of the GEM when loaded in the film. **Conclusion-** Thus, continuous and sustained release of GEM not only killed PC cells but also inhibited development of chemoresistance. The CP-GEM further inhibited tumor growth in mice. In future CP-GEM should be investigated for anti-tumor efficacy in orthotopic PC model.

OP10: Reoxygenation post-acute hypoxia imparts survival advantage to parental and therapy-resistant breast cancer cells

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Background: In solid tumors, hypoxia due to insufficient blood supply associates with failure of anticancer treatment strategies. Intermittent reoxygenation of hypoxic cells is a clinically relevant condition potentially affecting biological outcomes. This study highlights the biological effect of hypoxia-reoxygenation on parental and therapy-resistant breast cancer cells for better therapeutic targeting. **Materials and methods:** Parental and radio-resistant breast cancer cells were exposed to hypoxia and/or reoxygenation for deciphering the cellular and molecular alteration compared to untreated cells. The comparative studies were carried out in cancer cells using Raman spectroscopy, electronmicroscopy, western blotting, confocal microscopy, and other functional assays. **Results:** In response to hypoxia and reoxygenation, the parental and radio-resistant cells showed mitochondrial fusion, fission, and swelling with distinct spectral peaks of cytochrome-c at 750 and 1585cm^{-1} compared to untreated cells. Incoherence, the increased levels of total and mitochondrial ROS with enhanced γH2AX foci formation and decreased pATR levels were also observed in hypoxia-re-oxygenation exposed parental and radio-resistant cells compared to the untreated cell population. The mitochondrial structural and functional proteins also showed altered levels in parallel with changes in mitochondrial structural changes. These cells showed increased survival and migratory potential, possibly due to increased pAKT and pERK1/2 levels. **Conclusion:** Differential mitochondrial alterations, ROS signaling, and increased levels of antioxidant enzymes play an essential role in cell proliferation and survival of parental and radio-resistant cells under hypoxia and reoxygenation. Therefore, the targeted interruption of the mitochondria-to-cell redox pathway is potentially a promising target for future therapy.

Flash Presentation Abstracts

FP1: Withaferin-A prevents the onset of acute Graft versus Host Disease by inhibiting JAK2-STAT3 signaling pathway

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Background: Acute Graft versus Host Disease (aGvHD) is a major complication of allogenic Bone Marrow Transplantation (alloBMT). Withaferin-A (WA) as an anti-inflammatory and immunomodulatory agent may have clinical utility either as a single agent or in combination for the prevention of aGvHD. **Material and methods:** Recipient BALB/c (H-2K^d) mice were given 6.5 Gy of total body radiation. Irradiated BALB/c mice were transplanted with 5x10^6 bone marrow cells and 15x10^6 spleen cells from donor C57BL/6 (H-2K^b) mice. Further, efficacy of WA were evaluated and compared with standard prophylactic regimen of cyclosporine and Methotrexate (CSA+MTX). **Results:** Oral administration of WA significantly decreased the clinical severity score of aGvHD by 7.5 points with respect to control (9.5 vs 2.0). Mortality associated with aGvHD was also significantly reduced compared to the control (HR=0.07 (0.01-0.35); p=0.013). Furthermore, WA arm had better overall survival compared with standard prophylactic regimen of CSA+MTX [HR=0.19 (0.03-1.13), p=0.09], although the effect was not statistically significant. *In-vitro* WA treatment to splenic lymphocytes resulted in marked decrease in pJAK2 and pSTAT3 levels. **Conclusion:** Oral administration of WA prevents aGvHD by inhibiting JAK2-STAT3 signalling. WA as a single agent demonstrated comparable efficacy as CSA+MTX. The efficacy of WA for the prophylaxis of aGvHD should be tested in prospective clinical trials either alone or in combination with standard regimen

FP2: Serum Raman Spectroscopy study in DMBA induced oral carcinogenesis – Hamster buccal pouch model

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Background: Oral cancer presents with poor 5-year survival-rates owing to delayed clinical detection. Raman spectroscopy has demonstrated stratification of healthy, inflammatory, premalignant and oral cancer conditions, proving potential in early diagnosis. This study aims to evaluate if Serum Raman Spectroscopy (SRS) can probe the sequential biochemical changes occurring during oral carcinogenesis. **Materials and methods:** The serum collected across 14 weeks from DMBA induced oral carcinogenesis in hamster buccal-pouch model was used to identify the sequential biochemical changes. Sequential spectral variations were observed in controls and carcinogen (DMBA) treated hamster sera. Serum Raman spectra were subjected to PCA and PC-LDA analysis, both week-wise and group-wise. **Results:** The PCA scatter plots show distinct clustering of spectra as the DMBA treatment progresses. In week-wise analysis, it is evident that serum spectra from the DMBA treated and physical injury group do not get misclassified as untreated group spectra. However, the serum spectra of untreated and physical injury controls showed misclassification with spectra from the DMBA treated group at later weeks. In group-wise analysis, the serum spectra from the untreated control and DMBA treated groups showed classification in their respective weeks, suggesting SRS can detect the age-related changes. **Conclusion:** Detection of age related changes demonstrates sensitivity of the technique. The misclassifications between the injury and DMBA group indicate mechanical irritations/injuries as an etiological factor. While no spectral classification of both above groups with untreated or vehicle controls, suggests SRS as a rapid and objective screening adjunct in oral cancer diagnosis.

FP3: Understanding the histone alteration during drug tolerance leading to survival of the cancer cells and development of drug resistance

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Background: Recent studies have shown heterogeneity within cancer results in the generation of reversible early drug-tolerant persister (DTP) cells due to altered transcription. Further, these cells lead to stable drug-resistant cells (DRC). The present study focuses on differential histone alteration in DTP and DRC for their potential therapeutic importance. **Materials and Methods:** The gastrointestinal cell lines treated with cisplatin were used to study sequential events in DTP and DRC. The characterization was performed based on phenotypic and molecular changes such as cell cycle, cellular plasticity, apoptosis, and histone modifications in DTP, DRC in comparison to parental cell lines. **Results:** The sequential treatment of AGS and Hep3B cells with cisplatin leads to survival of 5-8% of the cell population in the early-stage, defined as DTP cells. The long-term treatment leads to the development of DRC from the DTP population. The surviving DTP cells were in the quiescent state with significantly less cell death. Preliminary data have shown increased expression of stem cell markers CD44 and CD133. Moreover, DTP cells showed an increased level of heterochromatin markers, H3K9me3, and H3K27me3, with increased incorporation of histone isoform, H3.2. The methylase, EHMT2, and demethylase, KDM3 of H3K9me3, showed increased and decreased expression respectively. **Conclusion:** The G0/G1 arrest and increased heterochromatin marks in DTP cells provide an advantage for cell survival. Therefore, potentially targeting the altered histone modifiers required for histone modifications for the survival of DTP cells with specific inhibitors will help to prevent the development of drug resistance cells.

FP4: The role of Lipocalin 2 (LCN2) in regulating ferroptosis and therapy resistance

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Background: Increased expression of LCN2 leads to chemo-resistance and inhibiting LCN2 function results in chemo-sensitivity, due to LCN2-mediated inhibition of iron-dependent programmed cell death process called ferroptosis and induction of autophagy. We aim to assess how LCN2 stimulates ETS1 expression to inhibit ferroptosis and how LCN2 promotes autophagy leading to chemo-resistance. **Materials and Methods:** All the protocols have been described previously in Chaudhary N et. al., Int. J. Cancer. 2021;149(7):1495–1511. shRNA was cloned into pLKO.1-TRC cloning vector (a gift from David Root) to generate stable LCN2 knockdown in DLD1 cells to confirm the observation obtained with the LCN2 antibody (3D12B2). **Results:** LCN2 overexpressing cells showed increased levels of ETS1, xCT and GPX4 while LCN2 knockdown cells showed decreased levels, confirming the results obtained by treatment with LCN2 antibody and consistent with the observation that LCN2 promotes resistance to 5-FU by inhibiting ferroptosis. Further, LCN2 overexpressing cells showed increased LC3II levels and increased LC3 foci suggesting LCN2 promotes autophagy in response to therapy. Also, activated EGFR expression is decreased in LCN2 knockdown cells suggesting that the increase in ETS1 levels observed upon LCN2 expression is probably due to increased activity of EGFR. **Conclusion:** LCN2 inhibits ferroptosis by increasing ETS1 expression and promotes autophagy. By inducing autophagy, it probably increases the recycling of EGFR, such that activation of EGFR signalling increases expression of ETS1 which inhibits ferroptosis to promote chemoresistance.

FP5: Autophagy inhibition invoke resistance to arsenic trioxide induced apoptosis in acute promyelocytic leukemia

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Background: Despite significant advances in the management of acute promyelocytic leukemia(APL),10-20% of *de novo* and 30-50% of relapsed patients show resistance towards arsenic trioxide (ATO) therapy. To investigate factors contributing ATO resistance using ATO sensitive and resistant APL cell model system, we examined the role of autophagy in ATO resistance. **Materials and methods:** *PML-RARA* positive ATO sensitive and resistant NB4 cell lines ($NB4^S$ & $NB4^R$), ATO (INTAS Pharmaceuticals). IC_{50} by CTG assay and synergy calculation using Calcusyn. Apoptosis assays by flow cytometry. qRT-PCR & Western blots for transcripts and protein levels studies. **Results:** The IC_{50} of ATO was found to be 3.8 times higher in $NB4^R$ compared to $NB4^S$ cells (0.72µMvs2.77µM). Flow cytometry data showed higher rate of apoptosis in sensitive than resistant cells (50%vs18% at 4µMATO). The protein levels of autophagy markers (p62, LC3-I/II) in $NB4^R$ remained unchanged at higher ATO concentrations. Levels of p62 were decreased 90% in dose dependent manner in $NB4^S$ cells. Conversion of LC3 I to II on ATO treatment was found abysmal in $NB4^R$ cells suggesting autophagy inhibition. Interestingly, 3µMATO eradicated PML-RARA protein in $NB4^S$ cells but failed to conduct in $NB4^R$ cells suggesting that degradation of PML-RARA in ATO sensitive cells is mediated through autophagy. **Conclusion:** Preliminary data highlight the role of autophagy inhibition in resistance to ATO induced apoptosis leading to reduced degradation of PML-RARA.

FP6: Structural and molecular dynamics studies of mutations identified in functional domains of Ribosomal Protein S6 Kinase A1 (RSK1)

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Background: Ribosomal Protein S6 kinase (RSK) is one of the effector kinases of RAS-MAPK pathway. Cancer associated mutations identified in the different domains of RSK1 have not been well-studied for their structure and dynamics. Current work focuses on structure and functional studies of the mutations in RSK1-CTKD. **Methods:** Mutations were retrieved from cBioPortal and characterised for pathogenicity by *in-silico* prediction tools (PROVEAN, PhD-SNP, pMut, PANTHER). Evolutionary conservation was assessed by the ConSurf. Effect of mutation on protein stability was then determined with iStable tool. Molecular dynamics simulation was used to investigate the structural changes induced by the mutations. **Results:** 140 retrieved nsSNPs were screened for pathogenicity and 10 nsSNPs were identified in highly conserved regions and were categorised as deleterious. These mutations were located at functionally important region and ERK1/2 interaction interface. The molecular dynamics simulation analysis revealed major structural alterations in 6 nsSNPs (R434P, T701M, A704T, S720C, R725W, and S732F). Principal component analysis uncovered few eigenvectors deciphered to be principal components and that can be co-related with their collective motion. Gibbs free energy landscape of PC1 and PC2 of R725W, S732F, and S720C were observed to have higher conformational stability than wild type. **Conclusion:** This study unveil that highly pathogenic mutation affects the stability and dynamics of RSK1-CTKD.

FP7: Multiplexed STAT3-phosphoBRET sensor for ready distinction between canonical vs. non-canonical PTM mediated pathway activation in cancer cell

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Background: STAT3 is an intracellular signaling protein, which get activated via phosphor-PTMs marks at Y705 (canonical) and S727 (non-canonical activation). Considering the complexity of STAT3 activation and signaling in various cancer contexts, we aim to develop a multiplexed, color-coded STAT3-phosphoBRET sensor to detect canonical or non-canonical PTM within a cancer cell population in live culture. **Methods:** Fusion constructs of Nluc-STAT3, Turbo-STAT3, and mOrange-STAT3 were synthesized in N-terminus of STAT3 by PCR based cloning method. Mutant STAT3 (Y705F and S727A) constructs were developed using SDM. Plasmid DNAs were co-transfected in HT1080 and MCF7 celllines using Lipofectamine2000 protocol. Protein expression was measured by western blot and immunofluorescence. BRET imaging was done using IVIS spectrum. **Results:** Expression of Nluc-STAT3, Turbo-STAT3, and mOr-STAT3 was detected in transiently transfected cells. STAT3 activation and dimerization was observed with an increase in BRET signal using multicolor based STAT3-BRET pairs, Nluc-STAT3-Turbo-STAT3 and Nluc-STAT3-mOr-STAT3 with different WT and mutant constructs post EGF stimulation and drug treatment, including sttatic and niclosamide. Time-dependent STAT3 phosphorylation with EGF or drug treatment was also confirmed with western blotting and survival assays. **Conclusion:** While BRET has proved to be a useful live cell assay for measuring protein dimerization, this study for the first time shows spectral multiplexing-based distinction and screening possibility of STAT3 activation in live cell formats. Notably, this multiplexed STAT3 sensor can be used to screen potential drug molecules against complex STAT3 signaling.

FP8: Non-invasive imaging-guided *In vivo* estimation of photothermal therapy using cancer targeted gold-solid-lipid nanomaterial

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Background: Photothermal therapy (PTT) is a promising cancer treatment modality which employs near infrared laser on gold-nanomaterials for localised heat-mediated tumour ablation. We estimate PTT effect of a novel CSC targeted photothermal agent, i.e., hyaluronic acid (HA)-tagged gold-solid-lipid nanoparticle (Au-SLN) on breast cancer (BC) cells and xenograft tumors. **Methods:** Au-SLN was surface-functionalised with HA and characterised. *In vitro* uptake and biocompatibility of HA-Au-SLN over Au-SLN were done. To evaluate PTT efficacy *in vivo*, 4T1-FL2 BC orthotopic NOD-SCID mice were given PTT (750nm NIR-laser, 650mW, 4minutes) after intratumoral Au-SLN injection. Tumour regression was quantitated by 2D-BLI and DLIT of IVIS-Spectrum. **Results:** HA-Au-SLN showed no shift in NIR absorbance efficiency. HA coating was confirmed by FTIR and TEM. *In vitro* studies showed significant cell death (>75%, $p<0.0001$) within 2 minutes of PTT across BC cell lines. Enhanced uptake of HA-Au-SLN over Au-SLN was observed in MDA-MB-231 cell line. HA-Au-SLN showed higher biocompatibility over Au-SLN at concentrations beyond 100µg/ml in L929 cell line. In concordance to planar imaging estimation, volumetric assessment by DLIT simulation signified a 10^4-fold dip in source signal upon photothermal insult, leading to complete regression in primary tumour. **Conclusion:** This is the first report of an actively targeted gold nanomaterial for PTT showing excellent anti-tumor efficacy bringing this therapy closer for clinical application.

FP9: BEND4, a novel prognostic marker for adverse AML

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Background: Cytogenetic and molecular abnormalities-based prognostication in acute myeloid leukemia (AML) is useful for managing the heterogeneous disease. Most of these abnormalities are either mutation or gene fusion-based. Unfortunately, gene expression-based biomarkers, which are easy to assess in clinical settings, are understudied due to lack of availability of paired normal tissue. **Materials and Methods**: Raw transcriptome data of n=1775 patient's (6 independent datasets) available in public domain were processed and independently analysed for Z-score-based statistic, differential gene expression, bivariate, multivariate, and survival analyses using R software. qRT-PCR and IHC-IF were used to validate the identified gene in two independent cohorts of patient samples. **Results:** In an independent analysis of each transcriptome dataset, BEND4 was identified as the only common gene with high expression in adverse cytogenetic and in relapsed AML patients, leading to poor survival. These findings were further validated in our sample cohort of 31 de novo adult AML samples and 6 paired AML relapse patients. Bivariate analysis shows association of BEND4 expression with known AML cytogenetic risk factors like blast count, FLT3-ITD, and NPM1 mutation. Importantly, multivariable analyses, adjusted for those cytogenetic risk factors, as a time-dependent variable, confirmed the independent association of high BEND4 expression with a higher hazard ratio. **Conclusion:** We identified significant association of BEND4 high expression in AML patients of adverse prognosis with 81% sensitivity and 91% specificity. Thus, we demonstrate that BEND4 can be a novel poor prognostic marker for AML and provide rationale for further investigation of its biological function.

FP10: Sfrp1 in regulation of skin cancer and cancer stem cells associated through non-canonical WNT signaling

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Background: Wnt-signaling involved in regulation of different cancer and cancer stem cells (CSCs). SFRP1, a Wnt-inhibitor, is downregulated in various human cancers; earlier study observed inverse correlation between SFRP1 and SOX2 in human oral and breast cancers. However, role of SFRP1 and molecular-mechanism involved in skin tumor and CSC regulation remains unexplored. **Methods:** We used the cutaneous skin squamous cell carcinoma cell line (A-3886). Flow cytometry was performed to isolate CSCs by using $CD133^+$, while $CD133^-$ as non-CSCs control. Expression profiles was performed using microarray. Further, the differentially expressed genes were validated by RT-PCR and protein expression was checked by western blot. **Results:** We observed 6-8% of CSCs population from A-3886 cells. CSCs population was checked by IFA using the CD133 marker, sphere formation and *in vivo* tumorigenesis assay. Expression profiling of CSCs showed upregulation of genes involved in EMT (Vimentin, TWIST1), stemness marker SOX2 and non-canonical Wnt signaling that suggest an enhanced tumorigenic potential of CSCs. We observed the upregulation in protein expression of non-canonical Wnt signaling cascade (Rac1-Dvl2-JNK-c-JUN-SOX2). We observed the interaction of c-JUN and SOX2 by co-immunoprecipitation and ChIP-qPCR in the absence of SFRP1. Further, we found that knockdown of JNK1 decreases the SOX2 levels and tumorigenic potential. **Conclusion:** Our data revealed the role of SFRP1 and molecular mechanism behind the skin CSCs regulation through non canonical Wnt signaling cascade (Rac1-Dvl2-JNK), and interaction between c-JUN and SOX2 thereby resulting in an increased tumorigenic potential of CSCs.

FP11: Investigating the molecular association between p53 and HER2 expression in Gastric Cancer

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Background: Gastric Cancer (GC), a 5th most common cancer worldwide shows frequent p53 mutations and around 20% cases exhibit HER2 overexpression. In multiple cancers, mutant p53 regulates HER2 signaling. We aim to investigate the association and molecular relation between p53 and HER2 in GC cell lines and Indian patients. **Materials and Methods:** Immunohistochemistry is performed for p53 and HER2 in paired (Chemonaïve and NACT) patient tumor blocks. Role of wild-type p53 in regulating HER2 expression was studied by ChiP assay in AGS cells. Isogenic cellular model systems expressing mutant & wild-type p53 in p53 null KATO-III cell line are under construction. **Results:** In a small cohort of GC patients (n=19), 8 among 19 patients possess mutant p53 and the rest harbor wild type p53. Intriguingly, 3/19 cases showed intense HER2 membrane staining and all of them harbor mutant p53. In AGS cell line, platinum treatment led to enhanced p53 and decreased HER2 expression at protein as well as mRNA level. Using JASPAR software, three p53 binding sites were identified on HER2 promoter (Score >80%). ChiP assay revealed increased p53 binding to at site 2 and 3 on the HER2 promoter upon platinum treatment in AGS cell line. **Conclusion:** This preliminary study suggests that wild type/mutant p53 might transcriptionally regulate HER2 expression in GC cell lines and tumor tissues. Detail molecular mechanism and identification of specific p53 mutations are currently under investigation.

FP12: Mitochondrial targeted curcumin inhibits glutathione reductase and modulates mitochondrial redox: A potential novel strategy for the treatment of resistant NSCLC

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Background: The dedicated thioredoxin and glutathione redox systems are the central antioxidant defense mechanisms by which mitochondria neutralize the excess ROS. In cancer these anti-oxidant systems get upregulated to cope with oxidative stress insult caused due to dysfunctional mitochondria. These upregulated antioxidant systems lead to drug resistance in lung cancer. **Materials and Methods:** The cytotoxicity of mitocurcuminin A549 cells at different time points and its IC_{50} was calculated by MTT assay. Cell free and cell based glutathione reductase (GR) inhibition was studied by DTNB reduction assay. Autodockvina and LigPlot tools were used for molecular docking and ligand interaction studies. **Results:** Mitocurcumin exhibited cytotoxicity on A549 cells with IC_{50} of 7.91, 5.37, and 3.37 µM at 24, 48 and 72h respectively.It inhibited recombinant GR in cell free system with IC_{50} of 1.27 µM and mitochondrial GR in A549 cells. GR inhibition was independent of NADPH, which serves as the cofactor in enzyme catalysis, and showed mixed-II type inhibition where Km and Vmax both decreased. The inhibition of GR affected mitochondrial and cellular GSH pool by increasing both mitochondrial and cellular ROS in a dose and time dependent manner. *In silico* docking studies revealed that mitocurcumin binds to the site other than active site of GR. **Conclusion:** Mitocurcumin affects proliferation of A549 cells in dose and time dependent manner. It inhibits GR in cell free system and in A549 cells which in turn modulates mitochondrial redox leading to ROS dependent apoptosis in A549 cells.

Poster Presentation Abstracts

PP1: Isolation and characterisation of circulating PLAP$^+$ serum exosomes across pregnancy

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Background: Exosomes are cellular vesicles (30-100nm) of endosomal origin released in extracellular space and body fluids. During pregnancy, exosomes are produced by the syncytiotrophoblast and released into the maternal blood circulation. The characterisation of these exosomes in maternal blood during pregnancy may represent a clinically useful, non-invasive test for placental physiology. **Materials & Methods:** The isolated exosomes were characterized in terms of their shape using TEM, exosomal size using DLS method and expression of placental enriched markers, PLAP and Cullin-7 by western blot. The DNA methylation status of LINE 1 promoter by pyrosequencing and miRNA expression including C19MC cluster were assessed. **Results:** The ratio of PLAP-specific exosomes to serum exosomes in pregnant women (3rd trimester) was 74% (± 8.37) and 39% (± 26.84) in non-pregnant women. The C19 miRNA cluster miRNAs (miR 515-5p-519e and miR-520f) along with miR-133-3p, miR210-3p and miR-223-3p were found to be present in PLAP-specific exosomes. LINE1 promoter methylation pattern in DNA derived from PLAP-specific exosomes corroborated with previous studies on LINE 1 methylation in placental tissue. Exosomal marker CD63, and placental markers PLAP and Cullin-7 were present in the total protein lysate of PLAP-specific exosomes. **Conclusion:** These findings suggest that the circulating PLAP$^+$ exosomes may be a non-invasive tool to determine placental health during pregnancy.

PP2: Role of GATA3-expression in the T-cell Lineage Assignment for the diagnosis and classification of Acute Leukemia

Ranade J[1,2],Rajpal S[1,2], Chatterjee G[1,2], Sriram H[1,2], Girase K[1,2], Ghogale S[1,2], Deshpande N[1,2], Badrinath Y[1,2], Patkar NV[1,2], Gujral S[1,2,3], PG Subramanian[1,2], Tembhare P[1,2*]

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Background: GATA-binding protein 3 (GATA3) is the transcription factor that regulates early T-cell differentiation. Other markers of T-cells including CD7, CD5, CD2 are frequently aberrant and not lineage specific. Hence, we evaluated the utility of GATA3 expression and studied its utility as a T-lineage marker in the lineage-assignment of acute leukemia. **Materials and Methods**: Anti-GATA3 (PECF-594, clone-L50-823) antibody staining was standardized and studied in the leukemic blasts along with CD7, CD5, sCD3, CD34, CD45 and TdT using MFC. We also evaluated four permeabilization reagents: FACS Lyse (BD Biosciences), Fix-&-Perm (Invitrogen), Foxp3-fixation-kit (eBiosciences) and True-nuclear transcription-factor staining buffer-set (Biolegend). **Results**: GATA3 expression was studied in 60 T-ALL, 11 AML and 7 MPAL with T/myeloid patients. B-cells and normal-T-cells were taken as negative and positive controls with a median nMFI of 0.13 (0.0 -5.44) and 0.80 (0.0 - 25.0), respectively. Median nMFI of GATA3 in T-ALL, AML and MPAL patients were 2.57 (0.0 - 8.04), 0.17 (0.00 - 5.23) and 2.02 (0.00 - 6.92) respectively. Taking a cut-off of 8.33%, 71.66%, 71.42% and 36.66% of T-ALL, MPAL and AML respectively were positive for GATA3. The sensitivity and specificity of GATA3 for T-cell lineage is 71.67% and 72.73% respectively. **Conclusion:** We first-time standardized flow cytometric assessment of GATA3 expression in the clinical setting. FACS Lyse (BD Biosciences) was the best permeabilization reagent. Flow cytometric GATA3 expression can give confidence in assignment of T-lineage in the presence of dim cCD3 in difficult cases of AL.

PP3: Genetic modulation of molecular circuitry governing cellular ageing in the *Drosophila* intestinal stem cells alters tissue homeostasis

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Background: Ageing is the progressive physiological decline of cellular and molecular functions. On the cellular level; ageing drives genomic instability, chronic inflammation, altered protein turnover, and mitochondrial dysfunction. These result in the accumulation of derogatory effects, causing age-related disorders. Here we have investigated the effects of ageing on intestinal tissue homeostasis. **Materials & Methods:** To elucidate the cellular ageing phenomenon and to understand their effects on tissue homeostasis using thorough in-vivo analysis, we genetically either accelerated or stalled ageing in the *Drosophila* larval midgut using the Gal4 -UAS based technology. The guts were dissected, immunostained and analysed using Confocal microscopy and Image J Software. **Results:** Acceleration of ageing specifically in the intestinal stem cells using inflammageing approach or via production of reactive oxygen species results in an increased DNA damage, apoptosis and differential cellular proliferation. On the contrary, when ageing is stalled by over-expression of a key autophagy regulator, Atg8 in the intestinal stem cells, there is decrease in DNA damage, cell differentiation and shows increased intestinal stem cell proliferation. **Conclusion:** Given, the considerable conservation between *Drosophila* and mammalian intestinal pathophysiology and signalling pathways, we aim to gain mechanistic insights into the cellular and molecular circuitry that governs cellular ageing. Here we have shown that modulating ageing in either direction will have differential impact on various cellular parameters and organismal function.

PP4: Utility of flow cytometry in cytokine profiling from human plasma samples.

Dalvi K[1,2], Sriram H[1,2], Khanka T[1,2], Gujarathi S[1,2], Girase K[1,2], Ghogale S[1,2], Deshpande N[1,2], Yajamanam B[1,2], Chatterjee G[1,2], Rajpal S[1,2], Patkar N[1,2], Gujral [1,2,3], PG Subramanian[1,2], Tembhare P[1,2*]

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Background: Cytokines, biological molecules pivotal in induction and regulation of immune responses and emerged as focal point of biomarker research. Detection of cytokines by flowcytometric multiplex bead-based assays increases the clinical utility. In this study, we established the normal-range, evaluated changes in cytokine levels under different pathological conditions in cancer setting. **Material and methods**: Whole blood collected in K_2-EDTA vacutainer tubes. The tubes centrifuged at 1000xg for 10mins for plasma separation. BD CBA-FLEX set assays were procured for human selected cytokines. Cytokine assay was performed using 50µL plasma as per manufacturer's protocol. Samples were acquired on BD LSRFortessa™ Data analysis was performed on FCAPArray™v3.0.1. **Results**: Normal range of cytokines was evaluated in 33 healthy samples. Cytokine profiling was performed in 323 plasma samples including, but not limited to, suspected GVHD, sepsis, HLH diagnosis and immunotherapy monitoring. Lower limit of detection for IL-6 was 0.49, IL-8 1.04, IL-10 0.57, IFN-γ 0.79, MIP-1α 0.11 and GM-CSF 0.08pg/mL. We noticed elevation in levels of inflammatory cytokines under the conditions evaluated ($p<0.05$). Our results indicate a stark increase in IL-8 during sepsis ($p<0.0001$). Concordant with literature, we observe a significant increase in IL-8 in HLH ($p=0.02$). Additionally, we have used these assays monitoring response to novel-therapeutic agents like Blinatumomab. **Conclusion**: Our study indicates that cytokines are increased in disease-specific manner and can be used as diagnostic/prognostic markers. Although our cohort included only plasma samples, similar large-scale studies on other clinical specimens might prove useful.

PP5: Gcn5 regulates blood cell lineage differentiation and maintains tissue homeostasis in the *Drosophila* hematopoietic organ, the lymph gland

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Background: General Controlled Non-repressed 5 (GCN5) is the Histone Acetyl Transferase (HAT) involved in leukemic stem cell maintenance and overexpressed in acute myeloid leukemia (AML). Here, we are employing the *Drosophila* larval lymph gland (LG) to understand the physiological function and regulation of Gcn5 during developmental hematopoiesis which remains unexplored. **Materials and methods**: A multi-pronged genetic approach consisting of whole animal *gcn5* mutants, modulation of expression using knockdown or over-expression of Gcn5 in various cell populations of the LG will be used. The genotypes are then subjected to *in-vivo* analysis using organ micro-dissections and immunofluorescence analysis using confocal microscopy. **Results:** Our results indicate that abrogation of Gcn5 expression by knockdown or over-expression in niche cells, stem cells or differentiated blood cells leads to de-regulation of hematopoiesis resulting in a leukemia-like scenario. Specifically, there is an alteration in the number of niche cells and the number of differentiated blood cell types. **Conclusion**: Gcn5 regulates blood cell homeostasis in the *Drosophila* LG. Our study will help in understanding the mechanistic role of Gcn5 during hematopoiesis thereby gaining insights into the biology of AML.

PP6: AMPing up the search: A structural and functional repository of antimicrobial peptides (AMPs) for biofilm studies (Biofilm-AMP), and a case study of its application to *Corynebacterium striatum*, an emerging pathogen

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Background: Antimicrobial peptides (AMPs) have been recognised for their ability to target crucial biofilm formation processes. Identifying potential AMP candidates remains a significant challenge, necessitating preliminary *in silico* studies prior to *in vitro* and *in vivo* studies. **Materials and Methods:** We have developed Biofilm-AMP (B-AMP), a curated 3D structural repository of AMPs, focusing on AMPs relevant to biofilm studies. To demonstrate the user applicability of B-AMP, we use the sortase C biofilm target of the emerging pathogen *Corynebacterium striatum* as a case study. **Results:** B-AMP contains predicted 3D structural models of 5544 AMPs (from the DRAMP database) generated with a suite of molecular modelling tools, with user-friendly, multi-option search capabilities. AMPs are annotated to existing biofilm literature, consisting of over 10,000 publications, enhancing the functional capabilities of B-AMP. For the case study, 100 structural AMP models from B-AMP were subject to *in silico* protein-peptide molecular docking against the catalytic site residues of the *C. striatum* sortase C protein. We propose a preference scale based on docking scores and interacting residues to select candidate AMPs for further evaluation. **Conclusion:** B-AMP (https://b-amp.karishmakaushiklab.com/) is a comprehensive structural and functional repository of AMPs, serving as a starting point for future studies on AMPs in biofilms. B-AMP will be regularly updated with AMP structures, interaction models with potential biofilm targets, and annotations to biofilm literature.

PP7: FGFR-3 Overexpression by Flow Cytometry is Highly Sensitive and Specific For Prediction Of t(4;14) In Multiple Myeloma.

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Background: Multiple Myeloma (MM) is a genetically heterogenous plasma cell malignancy. Translocation t(4;14) with IgH-FGFR3 fusion is found in 6% cases and is known for poor prognosis. Flow cytometry can be an easy and reliable method for detection of FGFR3 expression, predicting presence of t(4;14) and help in rapid R-ISS staging. **Methods:** We included MM cases with more than 10% plasma cells (PC) on bone marrow aspirate for studying FGFR3 expression by FC using 13-color antibody panel on CytoFlex (Beckman Coulter). Data was analyzed on Kaluza version 2.1 software. Cytogenetic analysis was performed using FISH. **Results:** We studied 140 cases of myeloma. Median age of patients was 57 years (range:17-79). Cytogenetic results were available in 115 patients. FGFR3-positivity was defined with cut-off of ≥15.8% FGFR3-positive plasma cells using ROC curve analysis. FGFR3 was found positive in 23/135 (17.03%). Cytogenetic results were available in 115 and t (4; 14) was positive in (10%) patients. The sensitivity and specificity of FGFR3 expression by FC for the prediction of t (4; 14) in MM by FISH was 90.9% and 85.6% respectively. **Conclusions:** We standardized FGFR3 expression by FC which is highly sensitive and specific for rapid detection of cases of MM with t(4;14) and early risk stratification. Hence, it should be included in routine practice as an alternative screening method.

PP8: A biomimetic, 4D pre-clinical platform to evaluate novel wound biofilm treatments

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Background: Evaluation of novel anti-biofilm agents against wound infections has relied on *in vitro* models, which fail to recapitulate the infection state, or scientifically and ethically questionable animal models. Biomimetic pre-clinical platforms that represent the wound infection state, and enable the evaluation of novel and combination anti-biofilm therapeutics are urgently needed. **Materials and methods**: We built a 3D host-cell scaffold of fixed dermal fibroblasts and epidermal keratinocytes, on which biofilms of *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA) were grown in an *in vitro* wound milieu[2] (host matrix and biochemical factors). Biofilms were characterized using confocal microscopy and the XTT assay for antibiotic susceptibility. **Results**: The recapitulated 4D platform supports the development of mono- and mixed-species PA and SA biofilms over 24 h, with characteristic features of biofilm structure and interspecies interactions. Notably, the thickness and distribution of PA and SA biofilm aggregates are influenced by microenvironmental factors such as host cells and the IVWM. Further, antibiotic-tolerance of PA biofilms (to tobramycin) is higher in the composite 4D platform compared to standard laboratory media; SA biofilms (with vancomycin) did not show a difference. **Conclusion**: We have developed a 4D biomimetic platform that recapitulates key features of wound biofilm structure and antibiotic tolerance. It can serve as a high-throughput, selective and precise approach to characterize novel and combination anti-biofilm approaches, and thereby fill a gap in the development of biofilm treatments.

PP9: 'Wound infection on a chip': A macrofluidic platform to recapitulate the dynamic wound infection microenvironment

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Background: The wound infection state is a dynamic microenvironment with intricate interplay across host elements, biofilms, chemical cues, and biophysical factors. Biofluidic forces influence range of wound healing outcomes including cell proliferation, migration, and communication. However, the effects of biophysical forces in context of the composite microenvironment, remains to be explored. **Materials and Methods:** Using 3D printing, we have developed a palm-sized 'macrofluidic device' made of biocompatible polymer, polylactic acid, consisting of a transparent centrally placed chamber, with inlet and outlet channels connected to an *in-house* developed peristaltic pump. Human epidermal keratinocytes and dermal fibroblasts are seeded from the top portion of the chamber. **Results:** The central chamber of the reconstituted 'wound infection on a chip' supports the proliferation of HaCaT and HDFa cells, alone and in co-culture (Kadam, Vandana, & Kaushik, 2020). Using the scratch assay, to mimic wound injury, the co-cultured 'wound bed' demonstrates robust host cell migration and wound closure. These effects are currently being evaluated in presence of flow and shear stress applied at various time intervals. **Conclusions:** The next steps include leveraging the 'wound infection on a chip' system to study the effects of biophysical forces on the formation and development biofilms grown on host cells and dissect host-microbe interactions, with the inclusion of a relevant wound chemical milieu (Kadam et al., 2021).

PP10: An *In vitro* Breast Cancer Cellular Dormancy Model

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Background: Cancer recurrence at distant sites is common among breast cancer patients. Dormancy plays a pivotal role in mediating these late recurrences. The processes involved in the regulation of quiescence in disseminated tumor cells (DTCs) persist predominantly undiscovered. Isolation and analysis of dormant DTCs from clinically disease-free patients is exceptionally complicated. **Materials and Methods**: We developed an *in vitro* model that replicates dormant estrogen-positive (ER+) breast cancer cells in the bone marrow (BM), as they are likely to remain dormant for an extended period. The BM comprises basic fibroblast growth factor (FGF-2) and fibronectin, providing a favourable microenvironment for the DTCs to enter dormancy. **Results:** MCF-7 cells were incubated at clonogenic densities in the presence of FGF-2, on fibronectin-coated dishes. FGF-2 treatment was given for seven days, resulting in the formation of dormant clones on fibronectin-coated dishes. These treated cells were non-proliferating, had a large cytoplasm to nucleus ratio, and a distinctly flat appearance. In addition, a high p38/ ERK ratio and a negative senescence staining demonstrated that they were at a quiescent stage. Conversely, MCF-7 cells incubated without FGF-2 form much bigger colonies on fibronectin-coated and non-coated dishes. **Conclusion:** Both FGF-2 and fibronectin have to be present in the microenvironment of DTCs for them to enter a state of dormancy. This assay functions as a tool to glean information about the molecular mechanisms necessary for the establishment and survival of dormant tumor cells.

PP11: Prevalence and Susceptibility of Carbapenem Resistance Gram Negative Bacteria in an ICU setup.

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Background: With the increasing use of carbapenems in clinical practice has led to emergence of carbapenem-resistant Gram-negative bacilli which now poses a great threat to human health. Carbapenem resistant Gram-negative bacilli are priority pathogens with limited options available for their treatment. **Materials and Method:** This is a prospective study over a period of 5 months during which, of the samples received from ICU, 59 samples grew Gram negative bacilli which for which identification and susceptibility was performed by Vitek-2 method. Carbapenem resistant Gram-negative bacilli (CRGNB) were considered in the study. Intrinsic carbapenem resistant bacilli, Non-ICU samples were excluded from the study. **Result:** Of the 60 carbapenem resistant isolates, 23.7% belonged to 41-50 age group followed closely by 51-60 (22.03%) and 61-70 (18.6%) age groups; males outnumbering the females. Most of the isolates were cultured from Resp samples (sputum, Tracheal aspirate, BAL). *Klebsiella pneumoniae* constituted the highest proportion (36.7%) of CRGNB followed by *Acinetobacter spp.* (22%). Tigecycline, colistin, aminoglycosides were the most useful antibiotics for CRGNB. Klebsiella spp had the highest resistance (75%) to tigecycline while *Acinetobacter spp*. had the highest resistance to colistin. **Conclusion:** CRGNB is on the rise in ICU set up, Klebsiella spp dominating the scenario. The available options for these bacilli are limited to tigecycline, colistin and aminoglycosides. Tigecycline is the, most effective antibiotic for E,coli and Acinetobacter spp but colistin should be preferred for Klebsiella spp.

PP12: Scaffold based gene delivery for immunotherapeutic applications

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Background: Gene delivery is one of the major achievements in the field of immunotherapy, but the cost and complexity of manufacturing large numbers of genetically programmed cells remains a major hurdle. We thereby illustrate a method to deliver genes to cells in native microenvironment thus overcoming existing limitations. **Method:** Polyethylene glycol based scaffold implant was fabricated and carbamide chemistry was used to conjugate poly-L-lysine and immobilize gene carrying lentiviruses on the scaffold. This implant was characterized for physical and biological properties. Further, GFP and Luciferase expressing lentiviruses were loaded in the scaffold and efficiency of gene delivery was characterized. **Results:** Characterization of scaffold showed a highly interconnected macroporous structure in SEM thereby revealing a higher surface area for immobilization of lentiviruses. The scaffold was also biocompatible and hemocompatible. Further, conjugation of poly-L-lysine significantly improved the immobilization of lentiviruses on the scaffold. *In vitro* studies show efficient delivery of GFP gene into HEK293T cells via the scaffold loaded with GFP expressing lentiviruses. Mice implanted with luciferase expressing lentivirus loaded scaffold showed higher transduction efficiency when compared to bolus lentivirus delivery. **Conclusion:** Here, we demonstrate a practical, low cost, broadly applicable gene delivery strategy that can genetically program cells without the requirement for *ex vivo* manipulation of patient cells. Further, engineering techniques can be used to attract and modify specific cells for various disease settings.

PP13: Prevalence of ESBL positive gram-negative bacteria in community acquired UTI in a tertiary care centre in the eastern part of india

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Background: Urinary tract infections (UTI) are one of the most common infections in humans. The aim of the study was to find the incidence of gram-negative bacteria along with its antibiotic sensitivity pattern in community acquired UTI and to determine the prevalence of Extended Spectrum Beta Lactamase (ESBL) positivity among them. **Materials and methods:** A total of 515 urine samples were tested and the causative bacteria were identified. ESBL test was performed to identify the prevalence of ESBL producing isolates. Antibiotic susceptibility test was performed for all the isolated bacteria. **Results:** From a total of 515 samples, 65 (12.65%) were positive for UTI. The rate of infection was higher in females than males. *Eschereria coli* were the most frequently isolated pathogen followed by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Out of total positive, 43.08% ESBL producing strains. The antibiotic susceptibility pattern revealed that the isolates showed resistance to Ciprofloxacin and Cefixime and sensitivity to Gentamicin, Ertapenem and Nitrofurantoin. **Conclusion:** Females have higher prevalence of UTI than males. Mostly, females suffer from UTI in their reproductive years whereas males suffer in their old ages. UTI treatments should be done properly and antibiotic resistant and sensitivity patterns should be considered to prevent overuse and misuse of antibiotics.

PP14: Investigating the Molecular Basis of c-FLIP/Calmodulin Interaction for Modulating Apoptosis

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Background: Overexpression of anti-apoptotic protein c-FLIP in cancer and its interference with DISC leads to apoptosis evasion. Preventing c-FLIP recruitment to DISC is difficult due to its structural similarity with procaspase 8. c-FLIP needs to bind to calmodulin to access the DISC. Thus, targeting c-FLIP/CaM interaction can be a good strategy. **Materials and Methods:** The genes were cloned in expression vectors. Proteins were purified using Ni-NTA and amylose resins followed by Circular Dichroism and Fluorescence Spectroscopy. Interactions were checked using pull-down assays. Schrodinger software was used for in-silico experiments. Calmodulin Sepharose resin was used to check interactions with mutant and wildtype c-FLIP. **Results:** We confirmed the interaction between the two proteins and also the critical amino acid residues of the binding interface of CaM/c-FLIP using *in silico* methods, protein engineering and biochemical tools. The structural conformation and thermal stability of the purified mutant and wild type proteins was determined and they were found to be stable. Furthermore, using this information, we have designed peptides that interfere with this binding. Crystal trials for the c-FLIP DED domains showed microcrystal formation in few buffer systems. **Conclusion:** This array of information will be crucial toward devising small molecules for therapeutic benefit after appropriate testing and validation.

PP15: Identification and Characterization of FDA Approved Drugs as Novel Binders of CLIC1

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Background: CLIC1, a metamorphic ion channel, can exist as a soluble monomer and upon sensing oxidative stress can dimerize and further oligomerize to form a functional ion channel on the membrane. CLIC1 overexpression is associated with poor prognosis and metastasis in GBM, colorectal cancer, HNSCC and HCC. Ion channels are promising therapeutic targets, but most of them have a normal physiological function and targeting them have adverse side-effects. Since CLIC1 is known to preferentially localize on the membrane of cancer cells, taking advantage of the existing CLIC1 structure to repurpose FDA approved drugs reduces the risk of adverse side-effects. **Materials and Methods:** Potential inhibitors of CLIC1 were identified by docking of ~ 100 compound using two different algorithms- Glide and AutoDock Vina. Nano-differential scanning fluorimetry, limited proteolysis followed by SDS-PAGE and Microscale thermophoresis were used to test interaction of the best fit compounds to CLIC1 in vitro. **Results:** All the drugs bound to CLIC1 but at varying affinities as observed by their ability to protect CLIC1 from proteolysis, MST and nano DSF. One of the compounds (Ash1) bound with low □M affinity and was used to set up co-crystals with CLIC1. Ash1 reduced ERK1/2 activation by phosphorylation, decreasing the migratory potential. These results provide an excellent opportunity to improvise the leads obtained to find an alternate inhibitor of CLIC1 which is already FDA approved. **Conclusion:** Using a combination of in-silico and in-vitro methods, we have identified a promising first-generation inhibitor of CLIC1, which can be optimised into a potent inhibitor.

PP16: Characterization of VRK2A-BclxL interaction and implication in apoptosis

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Background: In intrinsic apoptotic pathway, there are many regulatory molecules that together form mitochondrial membrane protein complexes. Recently, VRK2A has been identified as one such regulator. It is a protein belonging to VRK (Vaccinia related kinase) family, a Ser-Thr kinase family that regulates several signal transduction pathways. Interestingly, VRK2A has been found to be a component of the well-established BAX -Bcl-xL complex, however, the exact role of the former is yet to be delineated. It has been hypothesized that at high VRK2A level, this ternary complex delays apoptosis induction possibly by stabilizing the ternary complex. **Materials and methods:** Cellular localization of VRK2A was analysed through live cell imaging. Interaction between VRK2A and Bcl-xL has been validated using biochemical and cell biology probes. To identify the binding interface as well as the critical residues involved, this interaction, *in silico* studies including molecular modelling and docking were done. **Result:** For better understanding of this complex formation and its significance in apoptotic pathway, characterization of these interactions become imperative. Therefore, we checked their expression levels in different cancer cell lines and mitochondrial localization. We identified crucial residues of both proteins involved in VRK2A and Bcl-xL interaction. **Conclusion:** With these leads, variants of both the interacting partners are being generated to validate the interacting residues and determine their biophysical parameters. These studies would further extended toward studying the role of VRK2A in stabilization of Bcl-xL-Bax complex so as to devise ways to modulate the interactions with desired characteristics for disease intervention.

Other Abstracts Accepted For Publication At IJMPO

AB1: Unravelling the epigenetics of murine testis specific histone variants, TH2A and TH2B in the mature caudal sperm

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Background: Sperm retained histones/-variants are one of the most significant epigenomic contribution of father to embryo. Two such histone variants, TH2A and TH2B escape spermiogenic nuclear remodelling and are retained in the mature sperm. Importance of TH2A /TH2B in histone eviction during spermiogenesis is acknowledged. However, their significance post spermatogenesis is unknown. **Materials and Methods:** Chromatin Immunoprecipitation sequencing (ChIP-seq) analysis was done to understand the epigenomics of the retained TH2B and TH2A, using murine caudal sperm. **Results:** Genomic distribution revealed 35% of TH2B peaks within ±5kb of TSS whereas TH2A was mainly intergenic. TH2B was enriched at spindle assembly and meiosis specific genes, and embryo development was the most significant term amongst TH2B associated genes (TBAGs). This is important as TH2A/TH2B DKO mice have defective cohesin release. Also, TH2A was enriched with mitochondrial function genes. Presumably, TH2A is associated with mitochondrial DNA inserted in nuclear DNA. Also, there was 26% evolutionary conservation between human and murine TBAGs including genes crucial for embryogenesis. Most importantly, heterogeneity in the epigenetic landscape of TH2A and TH2B was seen. **Conclusion:** The murine TH2A/TH2B ChIP-seq analysis has attributed novel functions to sperm retained TH2B regarding embryogenesis and spermatogenesis. Conservation of TH2B-DNA linkage across two mammalian species highlights the significance of programmed histone retention for embryogenesis. Also, TH2B/TH2A is relatively independent in their epigenetic landscape. The TH2A-MtDNA link needs to be explored.

AB2: Expression pattern of a new marker, Gl7 in different stages of B-Cell Maturation and its utility in B-Lymphoblastic Leukemia/Lymphoma Measurable Residual Disease Assessment

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Background and Aims: Increasing use of targeted therapies such as anti-CD19 Blinatumomab/CAR-T cells and anti-CD20 Rituximab obviates the need for additional B-cell maturation-associated markers to evaluate deviation-from-normal in bone marrow (BM) in patients with B-lymphoblastic leukemia/lymphoma (B-ALL) by multicolor flow cytometry (MFC). GL7, a novel marker has been studied for the same. **Methods:** Expression of GL7 was studied on leukemic blasts in 65 B-ALL patients at baseline, 15 end-of induction (EOI=D28-35) BMRD samples and on hematopoietic cells including B-cell precursors (BCP) from 5 uninvolved staging BM samples using 16-color MFC. Expression pattern of GL7 was determined as MFI and CV-IF on Kaluza-v2.1. **Results:** The proportion of various hematopoietic cells expressing GL7 (MFI and CV-IF) are provided in Table-1. GL7 appeared at the late BCP2 stage of BCP2 and in a subset of BCP1. The brightest level of GL7 was observed in mature B-cells. Among non-B cells, a distinct proportion of T-cells and neutrophils expressed GL7. Among B-ALL patients, a median of 58.7%(0.07-99.6%) of leukemic B-blasts expressed GL7, however the expression level was dim-to-heterogeneous. Among 15 BMRD EOI samples, six patients had detectable MRD (median MRD 0.024%(0.0006%-9.7%)) and 8%(6.1%-34.6%) of residual B-blasts expressed GL7. Asynchronous expression of GL7 was observed in 2/6 MRD+ patients. **Conclusions:** GL7 appears in B-cell maturation in BM at late BCP2 stage and is expressed in a proportion of B-ALL patients. GL7 provides a highly useful additional marker for the assessment of B-ALL MRD.

AB3: An improved bioavailability of total lutein oxidized products (LOPs) extracted from *Tagetes erecta* flower petals in C57BL/6 Mice

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Background: The lutein extracted from shade dried *Tagetes erecta* flower petal powder by simultaneous solvent extraction and saponification, was exposed to sunlight (31±2ºC) for 10 days. The resultant lutein oxidized products (LOPs) exhibited significant inhibition of pancreatic lipase in *in-vitro* assay with IC_{50} of 1.5953µg/ml. **Materials and methods:** Bioavailability and absorption kinetics of LOPs in comparison with lutein (parent molecule) were analysed on C57BL/6J mice. Time course plasma kinetics was studied by collecting blood plasma, liver, intestine and eyes after 1st, 2nd, 3rd, 6th and 9th hour of intubation. **Results:** Postprandial LOPs concentration in plasma was maximum at 2nd hour. The area under concentration (AUC) and area under moment concentration (AUMC) of LOPs were 1139.418 pg/h/ml and 17750.69pg/h^2/ml respectively with a half-life of 11.353h.The mean residence time (MRT) of LOPs was 15.573 h with a volume of distribution (Vd) of 2.874 and clearance (CL) of 0.175. The eyes had 59.80pg/ml at 9th hour after pooling due to less concentration of LOPs in single eye. Whereas, mean lutein concentration in plasma, liver, intestine and eye was significantly less in lutein in comparison with LOPs. **Conclusions:** Thus the above data suggest that LOPs reaches systemic circulation and target tissue unaltered and enhance the absorption rate in comparison to the parental compound lutein and with further in-vivo examinations, LOPs can be developed as a potent nutraceutical with anti-obesity action.

AB4: Flow cytometric standardization of pax5 for the B-cell lineage assignment in acute leukemia

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Background: PAX5 is a nuclear protein used in immunohistochemistry as B-lineage marker. Expression by MFC has not been studied in clinical settings. In complicated cases, confident lineage assignment as per WHO criteria requires multiple markers. We standardized PAX5 expression by MFC and studied its utility as a B-lineage marker in Acute-leukemia. **Materials and Methods:** Anti-PAX5 (PE, clone-REA140) antibody staining was standardized and studied in the leukemic blasts using MFC. We also evaluated four permeabilization reagents: FACS Lyse (BD), Fix-&-Perm (Invitrogen), Foxp3-fixation-kit (eBiosciences) and True-nuclear transcription-factor staining buffer-set (Biolegend). **Results:** Foxp3-fixation-kit showed best results for anti-PAX5-antibody expression (titre=5µl). PAX5 expression was studied in 21 AML, 25 B-ALL and 10 (MPAL, B/myeloid) patients. Normal T-cells and B-cells were taken as negative and positive controls with their respective median (Table-1). Similarly, median nMFI of PAX5 in B-ALL and AML patients were calculated (Table-1). AML patients expressed negative PAX5 (nMFI<3) except one t(8;21) positive AML, with aberrant CD19 expression. B-ALL showed strong PAX5 (nMFI>7) expression. PAX5 was homogenous in B-ALLs with median;(SD) 58.9;(41.7) as compared to 101.5;(131.8) in AML. PAX5 was also expressed in two CD10-negative pro-B-ALLs and four B/Myeloid MPAL patients. **Conclusion:** We standardized flow cytometric evaluation of PAX5 expression in the clinical setting. Foxp3-fixation-kit (eBioscience) was the best permeabilization reagent. Flow cytometric PAX5 expression was highly specific for B-lineage and could be used in determining B-cell lineage in challenging cases of acute leukemias.

AB5: Analysis of circulating tumor DNA to detect EGFR mutations as a diagnostic tool for NSCLC patients

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Background: Somatic DNA mutations are highly tumor specific which can be used as optimum diagnostic markers. Non-invasive mutation detection by assessing circulating tumor DNA (ctDNA) offers many advantages over tumor biopsy. **Material and methods:** ctDNA from plasma of 96 non-small cells lung cancer (NSCLC) patients were analyzed for the presence of EGFR activating mutations using highly sensitive BEAM*ing* PCR. We could identify mutations in ctDNA from 31 of 96 patients (32%). *McNemar* test was done to determine the concordance between BEAM*ing* and qPCR done on original tumor tissues. **Results:** Our results showed high concordance with *p*-value of 1.00 for all the exons (Exon 19, 20 and 21) between BEAM*ing* and qPCR-EMR. Similarly, BEAM*ing* data when compared with qPCR-EasyLine data showed *p*- value of 1.00 for exon 21 and *p*=0.625 for exon 19. Exon 20 BEAM*ing* vs qPCR-EMR/ qPCR- EasyLine were not estimable as all cases were negative in one of the comparative methods. Since the *McNemartest p*-value is larger than the significance level (α=0.05), here the null hypothesis could not be rejected, which signifies our data were highly concordant between BEAM*ing* vs qPCR-EMR and BEAMing vs qPCR-EasyLine kit. **Conclusion:** EGFR mutation detection by BEAM*ing* PCR showed high overall agreement and good correlation with that in tumor biopsy with a detection sensitivity of 0.1%. Thus, feasibility and practicality of ctDNA analysis is a reliable technique and may translate into an alternative tool for anti-EGFR treatment selection.

AB6: Detrimental impacts of amygdalin compound against honeybees present in pollen and nectar of (clove) Syzygium aromaticum (L.) Merr. & L.M. Perry & Pliny the Elder

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Background: Some plants containing cyanogenic glucoside. Honeybees may be effected negatively when they collect the nectar of CNglc-containing plants such as a clove. This study aimed to understand the response of honey bees against cyanogenic glucoside. **Materials and methods:** 40-70 Bees were introduced to Hording cages. Cages were kept in laboratory in open conditions at temperature ranged from 17.5 to 25C. RH ranged from about 18 to 37%. Mortality rate of died bees was measured by counting on 1-8 days following initial dosing on day 0. *ad libitum* was used for bees feeding. Data were analyzed to providing the estimation of the dose of CNglc. Analysis of controls to determine an LD50 provided a significant model, but had 95% fiducial limits of one to infinity. **Results**: Table.-1 shows the estimated duration of exposure. At each experimental dose of amygdalin required to kill 50% of the bees (LD 50) in days. **Conclusion**: According to the results, it can be concluded that doses which are similar to those found in pollen and nectar of *S. aromaticum* is not having much toxic effects to kill honeybees, but extra doses of this toxic compounds may be harmful for honey bees.

AB7: Phosphodiesterase 4 D gene polymorphisms and Risk of Ischemic Stroke: a systematic review and meta-analysis

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Background: Studies on the relationship between Phosphodiesterase 4 D (PDE4D) gene polymorphism with the risk of ischemic stroke (IS) have shown discordant results. The present meta-analysis was aimed to clarify the relationship between PDE4D gene polymorphism with the risk of IS by estimating pooled analysis of published epidemiological studies. **Methods:** A comprehensive literature search was performed in various electronic databases up to 31st October 2021. Pooled Odds ratios (ORs) with 95% Confidence Intervals (CIs) under dominant, recessive, and allelic models were calculated. Sensitivity analysis was used to detect the heterogeneity and Begg's funnel plot for publication bias. **Results:** In our meta-analysis, we identified a total of 47 case-control studies with 20644 ischemic stroke (IS) cases and 23201 control subjects, including 17 studies of Caucasian descent and 30 studies of Asian descent. There was a significant relationship between SNP45 gene polymorphism and risk of IS (Recessive model: OR=2.06, 95% CI=1.31-3.23), SNP83 overall (allelic model: OR=1.22, 95% CI=1.04- 1.42), Asian (allelic model: OR=1.20, 95% CI= 1.05-1.37), and SNP89 Asian (Dominant model: OR=1.43, 95% CI=1.29-1.59, recessive model: OR=1.42, 95% CI=1.28-1.58) respectively. However, no significant relationship was found between SNP32, SNP41, SNP26, SNP56, and SNP87 gene polymorphisms and risk of IS. **Conclusion:** Findings of this meta-analysis conclude that SNP45, SNP83, and SNP89 polymorphism could be capable of increasing stroke susceptibility in Asians but not in the Caucasian population. Genotyping of SNP 45, 83, 89 polymorphisms may be used as a predictor for the occurrence of IS.

AB8: Bilateral Tubular Adenoma-Breast, A Case Report

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Background: Fibroadenoma is one of the most common benign neoplasm of breast in a young female however tubular adenoma is a much rare benign breast neoplasm occurring in young women. These are clinically indistinguishable from other benign breast neoplasms and can be diagnosed only on the basis of histopathological examination. **Clinical Presentation**: 20 Years old female presented to our hospital with complaints of lump in both breasts. On clinical examination lumps were felt in upper-outer quadrants of both breasts below the nipple and areola. USG findings were suggestive of bilateral fibroadenoma of the breast.Patient underwent lump excision under the same provisional diagnosis of bilateral fibroadenoma. Resected lumps were sent to our department for histopathological examination. **Results (Histopathological evaluation):** Grossly the mass consisted of multiple irregular tissue bits which were pinkish-white in color. Microscopic examination of H and E stained sections revealed tightly packed proliferated tubules lined by cuboidal epithelium having normochromatic nuclei and scanty intervening stroma. Hence from the histopathological examination the final diagnosis was established as Bilateral Tubular Adenoma-breast, in a 20-year-old female. **Conclusion (and Why this case was chosen)**: Tubular adenoma is a rare benign epithelial neoplasm of breast seen in young females, and it cannot be diagnosed on the basis of clinical, cytological or radiological examination and is often labelled as fibroadenoma. Histopathology remains the gold standard method to diagnose this entity which is only seen in handful of cases and bilateral involvement being even rare.

AB9: Clinical Utility Cytoplasmic TRBC1 Expression in the Diagnosis and Minimal Residual Disease Monitoring in T - cell Acute Lymphoblastic Leukaemia

DasGupta N[12], Girase K[12], Sriram H[12], Rajpal S[12], Chatterjee G[12], Varma S[12], Ghogale S[12], Deshpande N[12], Badrinath Y[12], Patkar NV[12], Gujral S[123], PG Subramanian[12], Tembhare P[12*]

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Background: TRBC1 is a novel flow cytometry- based T - cell colonialist marker. Recently, it is being studied in T-cell NHL. However, studies evaluating its utility in the diagnosis, lineage-identification and MRD studies in T-cell acute lymphoblastic leukemia (T-ALL) are extremely rare. **Materials and methods:** Surface and cytoplasmic TRBC1 (Clone-Jovi1, PE) expression was studied 20 TALLs patients at diagnosis and 4 T-ALL MRD samples, using 13c antibody panel on DxFLEX Beckman Coulter flow cytometry. Data analysis was carried out on Kaluza Analysis 2.0 software. **Results:** We studied cytoplasmic TRBC1 expression in 8 childhood TALLs (median age= 6), 12 adult TALLs (median age= 25), and 4 T-ALL MRD samples. There was clonal restriction of TRBC1 in the form of negative expression in T- ALL (n=7) and TMRD (n=2). There was clonal restriction of TRBC1 in the form of positive cytoplasmic expression in TALL (pediatric n=3 and adult n=2). Clonal TRBC1 expression was positive in > 90% blast population. **Conclusion:** Our study demonstrates that flow cytometric immunophenotyping of surface and cytoplasmic TRBC1 can aid in detection of abnormal blasts in T-ALL cases and has a high potential in T-ALL MRD monitoring.

AB10: Synthesis of Zinc Oxide Nanoparticles using *Aspergillus species* stain RR-1 and RR-2

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Background: Myconanotechnology is the interface between mycology and nanotechnology, new applied interdisciplinary science that may have considerable potential.Purpose of this study is to synthesise Zinc Oxide Nanoparticles using *Aspergillus nidulans.* **Material and Methods:** Coriander seeds were taken (pre-sterilized) and sampled on PDA(Potato Dextrose Agar). The desired *A.nidulans* strains were isolated followed by LCB(Lactophenol Cotton Blue) staining. Fungal strains were grown aerobically in PDB and after processing with $ZnSO_4$ Salt, ZnO nanoparticles were synthesized. **Results:** Zinc Oxide Nanoparticles producing fungal strains RR-1 and RR-2 were isolated from the species. The fungal strain is grown in the Potato Dextrose Broth containing 1mM of $ZnSO_4$ salt to determine their metal resistance. The Zinc Oxide Nanoparticles were characterized by means of the UV-Vis Spectroscopy, Scanning electron microscopy. The synthesised Zinc Oxide Nanoparticles showed an absorption maxima at 340nm and 520nm respectively. **Conclusion:** ZnO nanoparticles which are synthesized using *A.nidulans* strains, can be produced at large scale, and used against many food-borne pathogens due to strong antibacterial activity.
This work have future implication in the form of such as activity against cancer cells, Gene induction to produce large quantity of Nanoparticles.

AB11: Investigating the role of herbal compounds in chemo-sensitive and chemo-resistant gastric cancer (GC) cell lines

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Background: GC is the third leading cause of cancer-related deaths worldwide. The treatment regimen consists of daily administration of Capecitabine increasing the likelihood of developing chemoresistance. However the prognosis remains poor (five-year survival rate of <20%). So, there is an unmet need to develop alternative therapeutic strategies. This study aims to elucidate the role of herbal extracts of *Withania somnifera* and *Punica granatum* as potential therapeutics for their minimal side effects in chemo-sensitive and chemo-resistant GC. **Materials and Methods:** We are developing 5-FU resistance model via pulse-treatment method in AGS cell line. *Punica* was dissolved in 30% DMSO. *Withania* was dissolved in 100% methanol. Cell viability assays and immunoblotting were performed to determine the IC50 values for 5-FU and herbal compounds to characterise autophagic flux and apoptosis in AGS. **Results:** The IC50 values for *Withania*, *Punica* and 5-FU are 2.5 µg/ml, 40 µg/ml and 10µM respectively. Immunoblotting analysis revealed that both *Punica* and *Withania* showed a blockade in the autophagic flux in AGS-sensitive cells. *Punica* increased PARP cleavage in a concentration-dependent manner indicating induction of apoptosis. In the chemoresistance model which is in progress, cells after 6 and 9 cycles of 5-FU treatment showed a 2 and 3 fold increase in their resistance indices. **Conclusions:** We show that *Withania* and *Punica* imparts cell death in chemo-sensitive cells primarily via an apoptotic pathway. The mechanism of cell death in chemo-resistant cells by these herbal compounds still remains elusive, which will be explored further in the study.

AB12: Investigating the effect of silencing of ATG5 gene in differentiation, chemo-resistance and stemness properties of Cancer Stem Cells of Epithelial Ovarian Cancer

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Background: Cancer Stem Cells (CSCs) are implicated to be the prime cause for tumor relapse and chemo-resistance in Ovarian Cancer as they are quiescent and intrinsically resistant to various chemotherapeutic drugs. Autophagy, a vesicular lysosome-mediated degradation mechanism maintains the homeostasis, stemness and chemo-resistance properties of Cancer Stem Cells. A key protein called ATG5 initiates the formation of the autophagosome membrane and is responsible for the fusion of autophagosomes with lysosome that marks the completion of autophagy flux. **Materials and Methods**: In our study, we are knocking down ATG5 in an indigenously developed late stage chemo-resistant A2780 cells. Two shRNA cassettes with one targeting 3' UTR of ATG5 gene and the other targeting both the 3'UTR and the coding region of the gene were designed. **Results**: Two shRNA constructs against ATG5 gene were successfully cloned into pLL3.7-EGFP plasmid. Positive clones were selected using the colony PCR technique and further validated by Sanger sequencing. FACS-sorted GFP positive cells from transiently transfected HEK293FT cells showed a knock-down efficiency of 20% and 60% for shRNA1 and shRNA2 respectively. HEK293FT cells were transfected with plasmid carrying shRNA2, PΔ and VSVG for production of lenti-viral particles. Virus particles concentrated by ultracentrifugation will be transduced in $A2780^{LR}$ cells to generate stable ATG5 knockdown cells. **Conclusion**: Successful cloning of shRNA1 and shRNA2 was achieved in pLL3.7 plasmid and 50-60% knock-down efficiency was achieved in HEK293FT cells. Virus particles have been constructed to transduce $A2780^{LR}$ cells to understand the consequences of autophagy blockade on the CSC phenotype with respect to stemness, proliferation and chemo-resistance.

AB13: "An in-silico analysis to elucidate the role of miRNAs in Hydroxyurea (HU) induced foetal haemoglobin (HbF) in sickle cell disease patients"

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Background: The most adaptive therapeutic form of sickle cell disease (SCD) treatment has been the induction of foetal haemoglobin (HbF) using Hydroxyurea (HU). Since the role of miRNAs in HU-induced HbF production in SCD patients remains elusive, our study highlights the differentially expressed miRNAs and their regulated mechanisms through computational studies. **Materials and methods:** The miRNA expression profile data set GEOD32025 and GEOD11060 were obtained from the GEO database. The data was statistically analysed with R software packages. The miRNA targets were identified, followed by the revelation of gene ontology and pathway enrichment analysis to highlight the involved cellular functions and pathways. **Results:** Out of the 50 DE miRNAs filtered, 32 were downregulated while the remaining ones were upregulated. A total of around 29000 genes were identified as targets for the DE miRNAs. The gene set enrichment analysis was performed upon the identified targets, which revealed 125 enriched biological functions. Subsequently, pathway enrichment analysis divulged B cell receptor signalling pathway, T cell receptor signaling pathway, B-cell receptor signaling pathway, and MAPK Signalling pathway. In GSE11060 dataset 4 enriched pathways were upregulated notably viz. axon guidance, renal cell carcinoma, pancreatic cancer and endocytosis. **Conclusions:** miRNAs might be plausibly involved in the HbF induction process following HU treatment. This information enlightens about the hydroxyurea efficacy mechanism while simultaneously pointing out novel targets for effective treatment of sickle cell anameia. The latter can be accomplished using recently developed genome editing and therapy tools.

AB14: Application of Zinc Oxide Nanoparticles as a catalyst in dissipation kinetics of Azoxystrobin 23 % SC in Different Soils under Photocatalytic

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Background: The Dissipation kinetics of Azoxystrobin 23 % SC under direct sunlight using Zinc oxide nanoparticles as a catalyst. The nanoparticles are synthesized and characterized by SEM, UV and FT-IR. The experiment was conducted by spiking into four different soils. The spiked sample were kept under sunlight. The sampling occasions were collected for four soils. The residues of azoxystrobin in different soils was dissipation to below the detectable level by 72 hour. DT50 values calculated using the following formula DT50 = ln 2/ (k). **Materials and methods**: Reference standard from Sigma-Aldrich. The compound was purchased from local market. The UHPLC with PDA system used, the Xterra column was used and column temperature at 30 °C. The injected volume was 10 µL. Mobile phase Acetonitrile : water (60 :40 %) the flow rate was 1.0 mL/min and wavelength 240 nm. **Results**: The initial concentration of Azoxystrobin 23 % SC in four soil samples were collected on 0^{th} hr showed initial concentration 0.499 µg/g and 0.998 µg/g, 0.324 µg/g, 0.714 µg/g, 0.265 µg/g, 0.611 µg/g, 0.156 µg/g, 0.486 µg/g, 0.056 µg/g and 0.186 µg/g respectively and complete dissipation of residues of compound to below detectable limit was observed in 72^{nd} hr. **Conclusion :** The photocatalytic degradation of residues of Azoxystrobin 23 % SC was clearly indicates that the sunlight photolysis was influenced by the addition of zinc oxide nanoparticles as a catalyst. In the presence of catalyst the compound persists very fast. The catalysis reaction proceeded rapidly degrading the molecules very fast in different soil samples.

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