PhD Projects for HBNI Healthscience 2023 admissions at TMH & ACTREC

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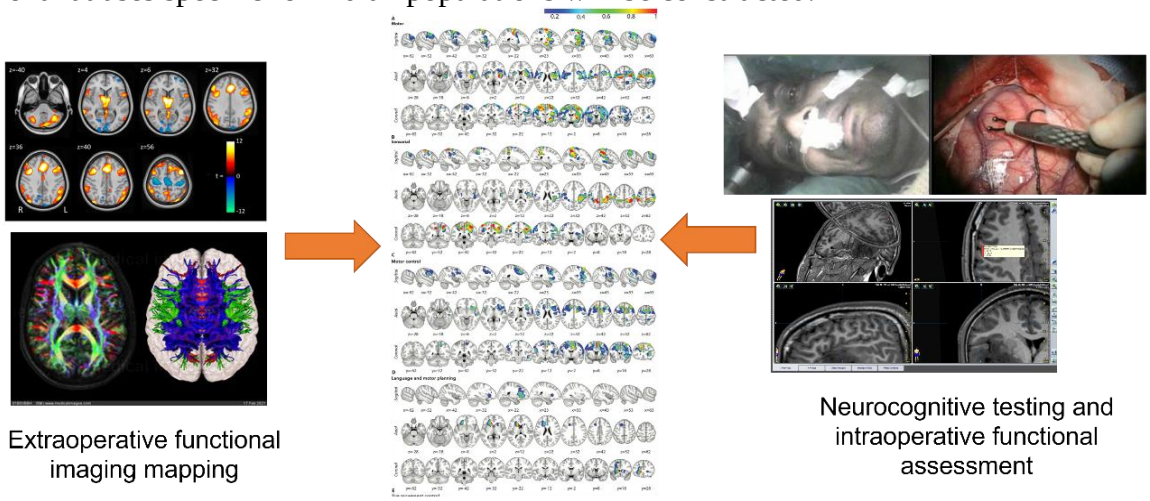
Project 1A: Development of functional brain maps using multimodal (extra & intra operative) functional characterization of brain cortical function in Indian subjects with brain tumors

PhD Guide: Dr Aliasgar V Moiyadi, MCh (Neurosurgery); DNB (Neurosurgery),
Professor, Neurosurgery; Tata Memorial Centre, Parel, Mumbai
Site of PhD work: TMH & ACTREC plus IIT-Bombay
Preferred education background of students: Neurosciences / Neuropsychology / Medical

The research project aims at exploring and documenting various complex brain functions using multimodal brain mapping techniques. Functional MR imaging (both task based and resting state fMRI) allow generation of images linking various brain regions involved in shared functions (networks). Intraoperative direct electrical stimulation mapping using customized tasks which are performed during awake craniotomies for brain tumor removal provides an unparalleled and unique opportunity to identify brain regions functionally and serves as the Gold standard for confirming various complex cognitive functions. Using advanced medical imaging analysis and machine learning tools, all this information can be collated and tumor maps and functional atlases specific for Indian populations will be constructed.



Tumor structural and metabolic maps will allow understanding brain tumor pathophysiology and functional maps will contribute to our understanding of complex brain function in the setting of brain tumors, allowing customization of therapies and rehabilitative strategies.

The PIs department is dedicated neurosurgical oncology setup at Tata Memorial Centre, Mumbai. It is a centre of excellence for brain tumor surgery and a pioneering centre, especially for the application of advanced brain mapping techniques in surgery for brain tumors. The PI has over 15 years of experience in this field and has been involved in many collaborative studies with expert groups from medical imaging at IIT-Bombay.

The candidate will work in the Department of Neurosurgical oncology at the Tata Memorial Centre. A significant proportion of the work will include collaboration with the clinical team (comprising of neurosurgeons, neuroradiologists, neuropathologists and neuropsychologists) for clinical evaluation of patients with brain tumors. This will comprise of pre and postoperative detailed neuropsychological testing, developing new testing paradigms, maintenance of a comprehensive database and correlating with patient outcomes. A major component of the project will include developing and administering a customized testing battery for intraoperative assessment of cognitive functions. In parallel, image analysis (MRI) using standard image-analysis software and image registration tools will be done. The student will be expected to have/acquire basic knowledge of medical imaging techniques like MRI and basic computer coding skills to work on image processing softwares (like iTK SNAP, Slicer, ANts etc). Image processing, computational and data analysis skills are desirable.

The project entails skill sets across neurosciences, medical imaging and image processing. The student will be expected to acquire additional training and skills related to this project and will be mentored by the PI and collaborators at IIT Bombay and other centres.

Project 2A: Deciphering 'B-others acute lymphoblastic leukemia': A prospective cytogenetic and molecular study for identification and evaluation of predictive prognostic markers.

PhD Guide: Dr.Dhanlaxmi Shetty PhD, Officer-In-Charge, Cancer Cytogenetics, ACTREC, TMC
Link: https://actrec.irins.org/profile/171452#education_information_panel
Co-Guide: Dr. Navin Khattry (Prof. Deputy Director, CRC)
Site of PhD work: ACTREC
Preferred education background of students: M.Sc / MTech in Lifesciences, Biotechnology or Bioinformatics

B cell precursor-ALL (BCP-ALL) comprises highly heterogeneous malignant hematological disorders accounting for 80% of ALL cases and survival rates of over 80% in children and ~45% in adults. The identification of recurrent cytogenetic aberrations has helped refine prognosis and guide management in B-ALL leading to improved outcomes seen in childhood ALL. However, ~ 30% of BCP-ALL patients remain unclassified at the genetic level and are assigned as intermediate risk, within the 'B-other-ALL' subgroup. Recently, several distinct, recurrent abnormalities have emerged from within this highly genetically heterogeneous subgroup replacing the default assignment of intermediate risk to these patients with increasingly more accurate prognostic information for improved treatments. It is important to continue to identify those patients who require less intensive therapy to achieve cure and to identify new targets for the development of novel, less toxic therapeutic agents. The gap in knowledge of the genomic landscape of B-Other 'ALL' subgroup in the Indian subcontinent will be addressed through our systematic, unified cytogenetic and genome wide approach with the aim to provide a complete genomic classification of BCP-ALL and define molecular targets for risk adapted therapies.

The study objectives:

- To identify ph-like patients using gene expression profiling in B-other-ALL.
- To detect copy number alterations, gene fusions and mutations in B-Other ALL.
- To identify genetic alterations in uncharacterized B-other-ALL using targeted RNA sequencing/Whole genome or transcriptome sequencing.
- To determine the clinical and prognostic impact of genetic aberrations in B-Other ALL cohort.

Novelty

Genomic profiling studies on 'B-other ALL' are limited and mostly from the West and mainly focused on childhood ALL; relatively little is known about its genomic characteristics in adolescents and young adults (AYA) or older adult ALL. Genomic profiling for identifying ph-like ALL patients will be standardized in India using microarray based approaches.

Outline of workflow and Methodology

- In patients classified as B-others-ALL, FISH would be carried out to determine ph like ALL and B-Other ALL genetic abnormalities. Genomic studies would be carried out in patients for complete characterization of B-Other ALL genome.
- Gene expression profiling will be performed and validated to identify Ph-like ALL gene expression profile.
- Conventional MLPA experiments will be performed to detect copy number alterations in prognostically relevant genes.
- Whole genome or Transcriptome sequencing will be carried out to detect novel aberrations.

Work done or leads

The prospective study will be carried out long term through in-depth follow-up to characterize 40.5% of patients as B-Other ALL patients and prognostic associations of genetic abnormalities and identify leads for translational studies.

Project 3A: Exploring Different Platforms of Product Development Processes for Cell Based Medicinal products (CBMPs) from bench-to-bedside with Comparative Analysis for Safety, Effectiveness, Biological Activity, and Translation to Scaled-up Production for Clinical Applications

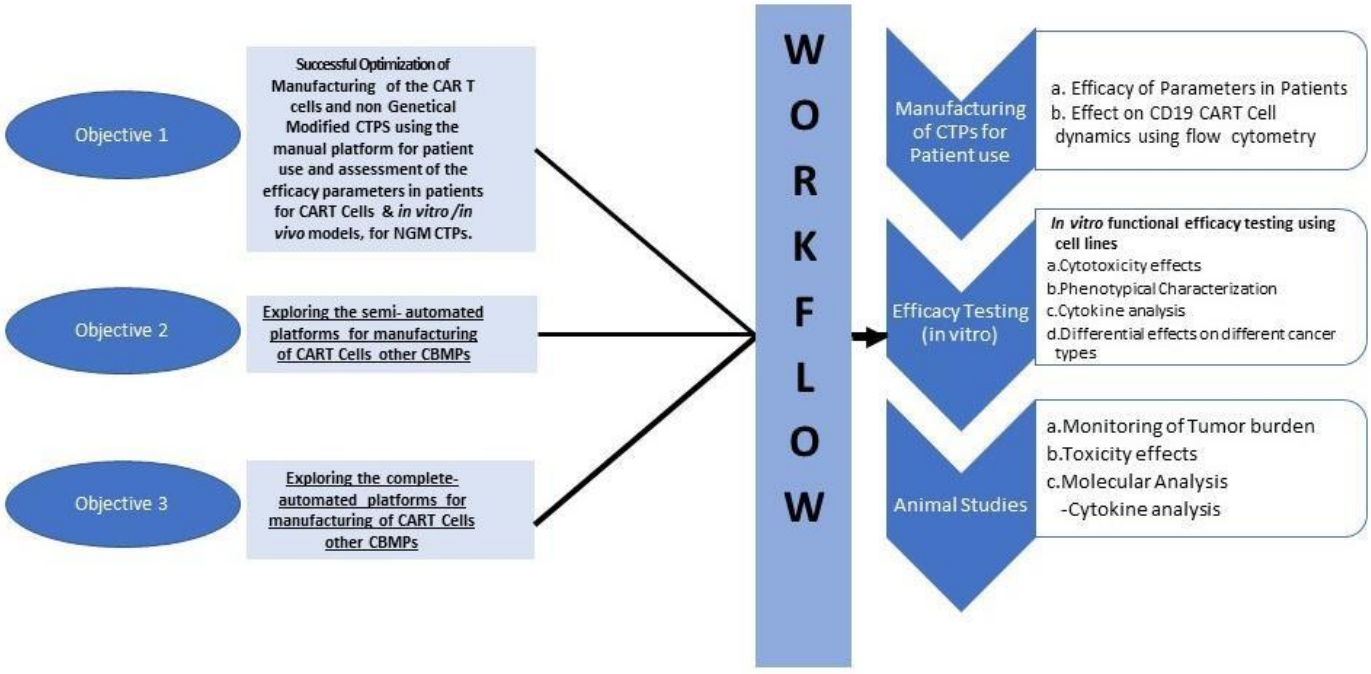
PhD Guide: Dr (Surg Cdr). Gaurav Narula, Professor Pediatric Oncology, TMC,
Email : narulag@tmc.gov.in, drgauravnarula@gmail.com, ctctc.tmc@gmail.com
Co-Guide: Dr. Navin Khatrry, Professor Medical Oncology, ACTREC
Site of PhD work: ACTREC & TMH
Preferred education background of students: Life Sciences / Medical

Cancer immunotherapy products has emerged a promising adjunct or even viable alternative to known therapies. CIPs includes modalities TILs, TCR-T cell therapy, CAR T-cells which precisely kill the cancer cells through tumor antigen recognition, NK cells, Virus Specific T cells & cancer vaccines made from patient-derived dendritic cells, tumor cell DNA/RNA, that can be tailored to individual cancers patients. We recently collaborated to develop a novel Chimeric Antigen Receptor (CAR) T-cell from design stage to human trials, and thus created infrastructure, expertise and pathways as a platform for other CAR and cell-therapy models like NK-cells, Virus Specific T-cells (VSTs), to treat Leukemias, Lymphomas, Myelomas, Solid Tumors, and severe infections of immuno-compromised patients. We are already developing some of these concepts at the CAR T & Cell Therapy Center (CTCTC) established at ACTREC Campus in Khargar, Navi Mumbai. These diverse strategies face common challenges needing similar development processes that are expensive, tedious and errorprone, and trod a path we have already established. One of the challenges is the development of efficient technologies and cost-effective clinical manufacturing platforms to support the later clinical trial phases and ultimately commercialization.

The first CART Cell Therapy Centre (CTCTC), a cGMP facility for CART and Cell Therapy has been designed and developed at ACTREC. At TMC, we are fully equipped for Clinical CAR T-Cell manufacturing and execution of the Clinical phase objectives for CAR T cell manufacturing, including Clinical Production, Quality management, Sterility Testing, CART administration and post CART Cell Management. Ongoing Phase 1 Clinical Trial, and already funded Phase II Clinical trial to study Biological endpoints as a basis for "back-to-bench" development.

This Ph.D project is to explore different platforms & novel strategies for cGMP grade manufacturing of the different cell based therapies including CART cells and non-genetically modified CTPs like NK Cells & VST and compare their effectiveness, applicability & up scale production at different time points for patient use.

OBJECTIVES & WORKPLAN



Previous Work Done : We are already in the process of manufacturing CD19 directed CART Cells for patient use in B-ALL & DLBCL at CAR T Cell Centre and the preclinical work on GD2 CAR T cells & NK cells is also ongoing, and we would explore the different manufacturing platforms, manual, semi -automated and fully automated closed systems for clinical-grade production of all these Cell therapy products. The current CD19-directed CAR Tcell project is funded by intramural grants, and a large grant by DBT-BIRAC National Biopharma Mission, in which transfer of technology will take place from innovator site- IIT-B, to the CTCTC at ACTREC. As part of this grant, DBT-BIRAC-NBM has already funded some automated and semi-automated platforms as exploratory objectives of our project. It is clinically relevant to study our production processes across platforms for wider applicability across the country and beyond. This will allow scaled-up production at our center for approved therapies requiring mass-production, and enable other centers to use CBMPs developed and validated at our Center on automated/semi-automated platforms, avoiding the need to replicate large GMP facilities like ours.

Novelty in the aspect of the project that will be done by the PhD student: The outcome of this PhD proposal is likely to result in force-multiplication of CBMPs by exploring and establishing novel safe and effective production processes.

Funding for the project : National Biopharma Mission, BIRAC, DBT, India & DAE (Intramural funds)

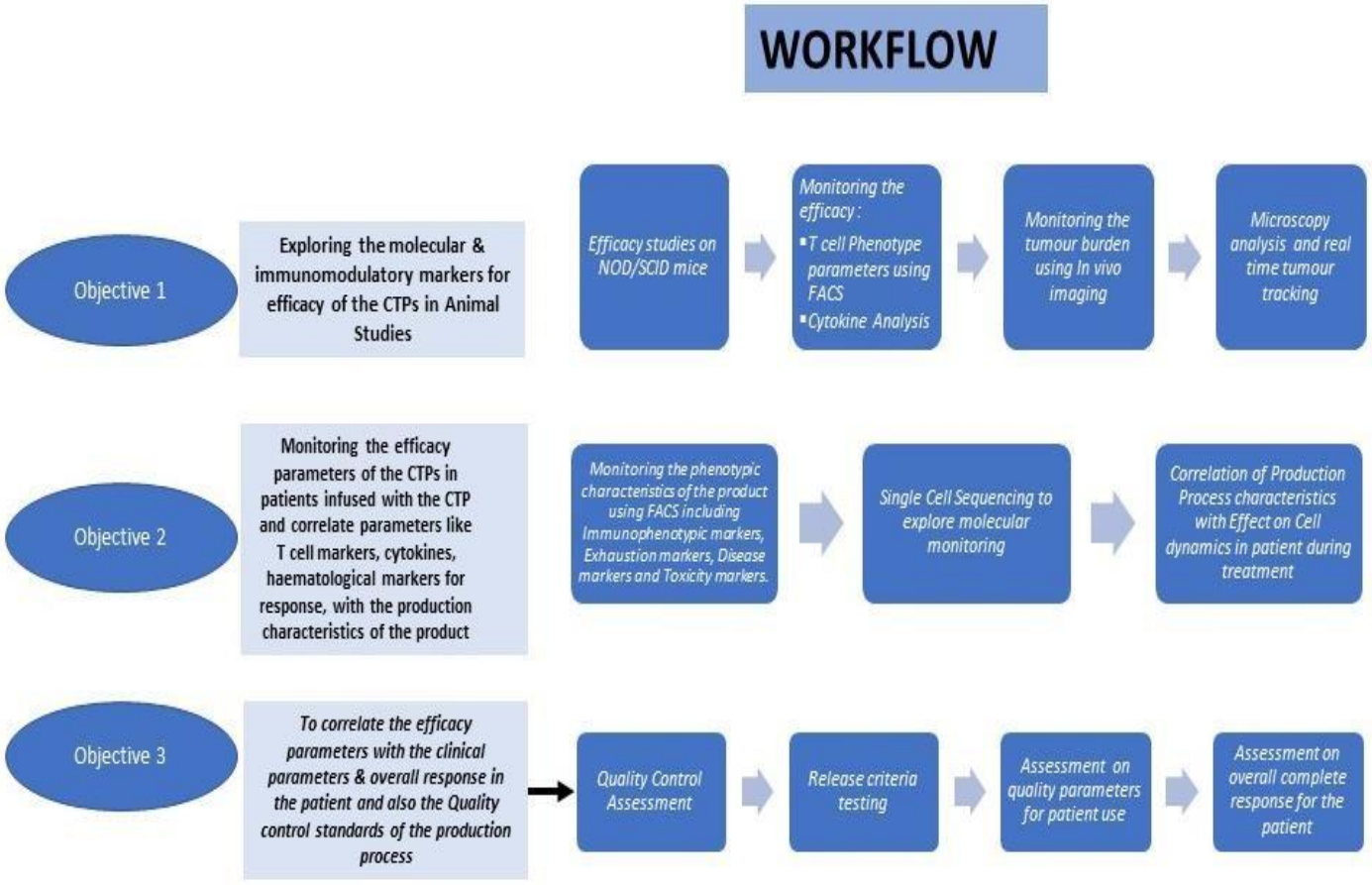
Project 3B: In vivo dynamics for Cellular Therapy Products with the Characteristics of the Manufacturing Process: Pre clinical & Clinical Studies

PhD Guide: Dr (Surg Cdr). Gaurav Narula, Professor Pediatric Oncology, TMC,
Email : narulag@tmc.gov.in, drgauravnarula@gmail.com, ctctc.tmc@gmail.com
Co-Guide: Dr. Navin Khatrry, Professor Medical Oncology, ACTREC
Site of PhD work: ACTREC & TMH
Preferred education background of students: Life Sciences / Medical

The PhD is to study the different parameters of the In vivo efficacy of the Cell therapy products like CAR T Cells & VSTs and correlate the dynamics with the Manufacturing process of the therapeutic product. The proposal aims to cover the attributes of the CTPs in the patients and the animal studies also.

An immunotherapeutic "product" shown to be effective in the laboratory still needs to undergo a manufacturing "process" that meets clinical grade use norms in a standardized manner. The aim is to optimize a manufacturing process for maximum yield that meets safety standards and can consistently deliver successive batches of a final "cell- product" that meet safe clinical and regulatory requirements. The translation of this process to clinical-grade development is complex and often involves change of protocols and practices followed on the "bench", to those that are safer, and more likely to be consistently effective. This involves replicating processes and steps in "close-circuit" systems in GMP facilities that meet rigorous quality control standards of the manufacturing infrastructure, environment, equipment and consumables, in addition to the manufacturing steps and protocols, that meet compliance standards and scrutiny of regulatory agencies. By virtue of our "path-making" CAR T-cell project for a CD19-directed CAR that has successfully completed product and process development phases and already commenced First-in-Human clinical trial at the time of this submission, we are now uniquely poised to use this platform to adapt to a host of Cellular Immunotherapy concepts.

OBJECTIVES & WORKPLAN



Previous Work Done: The first CART Cell Therapy Centre (CTCTC), a cGMP facility for CART and Cell Therapy has been designed and developed at ACTREC. At TMC, we are fully equipped for Clinical CAR T-Cell manufacturing and execution of the Clinical phase objectives for CAR T cell manufacturing, including Clinical Production, Quality management, Sterility Testing, CART administration and post CART Cell Management. Ongoing Phase 1 Clinical Trial, and already funded Phase II Clinical trial to study Biological endpoints as a basis for "back-to-bench" development. We have performed similar studies in the ongoing CD19 CAR T cell Clinical trial, using FACS and cGMP process development processes at CTCTC. Similar studies have also been optimized for HCAR19 and the pre-clinical work in cell lines is ongoing.

Novelty in the aspect of the project that will be done by the PhD student: This study will be the first study in correlation of the efficacy characteristics of the CTP with the therapeutic efficacy and will give broads leads for improvising the efficacy of the CTP using the production parameters.

Funding for the project : National Biopharma Mission, BIRAC, DBT, India & DAE (Intramural funds)

Project 4A: Novel pharmacotherapeutic interventions to reduce neuro-inflammation & improve cognitive outcomes in Brain Tumors: A preclinical in vitro & In-vivo study

PhD Guide: Jayant S Goda MD, DNB, MRes, Professor & Scientific Officer 'G'
Co-Guide: Abhishek Chatterjee, MD Assistant Professor & Scientific Officer 'E'
PubMed: https://pubmed.ncbi.nlm.nih.gov/?term=goda+j, e-mail: godajayantsastri@gmail.com
Site of PhD work: ACTREC (if required IIT-Bombay& BARC)
Preferred education background of students: Life Sciences or Pharmacology

Radiotherapy forms an essential component of the multi-disciplinary management of low-grade brain tumors and is associated with high rates of durable local control. However, the control is achieved at the cost of significant radiation induced late toxicity, which is aggravated by the fact that most of the patients are children and young adults who have to live for decades with the debilitating consequences of irradiation of normal tissue in the Central Nervous System (CNS). Certain pharmacological interventions such as NMDA blockade with Memantine have been shown to confer benefit in terms of improving cognitive outcomes in a subset of patients receiving cranial irradiation. However, such drugs typically have been used in older patients with limited life expectancy (precluding study of long term drug induced toxicity) and have the additional disadvantages of multiple side effects and drug interactions which are not desirable .Importantly , such drug therapy does not address the core issue of neuroinflammation and consequent damage to glio-neuronal and microvascular structures that lie at the heart of radiation induced neurological toxicity in general and cognitive decline in particular.

Radiation Induced Cognitive Decline (RICD) is underpinned by multiple pathological processes (neuroinflammation, microvascular and glial cell damage and proliferation, impaired neuronal function with proliferation of inhibitory neurotransmission and reduced hippocampal neurogenesis). Two particular pathways, the Renin Angiotensin Aldosterone (RAS) system, and the Peroxisome Proliferator Activator Region (PPAR) are attractive targets on account of the key roles in neuroinflammation and readily available drugs for repurposing. Blockade of RAS in hypertensives has also been shown to improve cognition, suggesting a role in multiple neurodegenerative states. In addition, RAS blockade has also shown benefits in reducing the radiation induced downregulation of hippocampus specific genes involved in neuroplasticity. RAS blockade can be done by means of Angiotensin Converting Enzyme (ACE) inhibitors using Ramipril.PPAR agonists have also been shown to reduce neuroinflammation by inhibiting proinflammatory cytokine release from astrocytes and microglia. They also reduce radiation-induced decrease in new hippocampal neurogenesis. The PPAR agonist Pioglitazone has been demonstrated to be well tolerated & safe in brain tumor patients.

A compelling alternative exists in the form of extracts and components of phytochemicals which have formed a component of traditional systems of medicine. A particularly attractive candidate exists in the form of *Emblica Officinalis* (Amla). *E. Officinalis* and its constituents have been shown to have significant antioxidant, anti-inflammatory and neuroprotective properties. The potent antioxidative capacities of multiple constituent chemicals reduce NO, Oxidative stress and lipid peroxidation. In addition, constitutents such as 3,6-di-O-galloyl-d-glucose and Gallic acid present in E. Officinalis exert significant neuroprotective activity.

To conclude, investigation of these agents for ameliorating RICD in laboratory models of low-grade brain tumors would represent a novel and cost- effective method for mitigation of this debilitating toxicity in young survivors of this disease.

Gaps in literature: There is a paucity of data to suggest improvement in cognitive outcomes in laboratory models of cognitive decline, reduction in neuroinflammation and hippocampal neuronal loss or induction of hippocampal neurogenesis without concomitant tumor-protective effect in low grade brain tumors with the usage of RAAS modifiers like ARBs and PPAR-γ inhibitors .There is additional paucity of demonstration of such effects consequent to usage of *E.officinalis* extracts or active principles in low grade brain tumors. Therefore, our lab would like to study the neuroprotective effects of the above repurposed drugs when given concurrently with radiation therapy and/or chemotherapy in normal neuronal, astrocytic cells.

Study Objective & relevance

1. To establish the efficacy of test drugs for preserving neurocognition (losartan, pioglitazone, extract of P. embelica, gallic acid and 3,6-di-O-galloyl-d-glucose, GABA allosteric modifier-Diazepam) in reducing cellular markers of neuroinflammation, cell death, apoptosis on normal astrocytes and hippocampal stem cells.
   Aim A: to study the efficacy of these drugs on invitro cell lines
   Aim B: to study the efficacy of these drugs in tumor bearing mice models

2. To rule out tumor protective effect of the same compounds on GBM, Medulloblastoma and Ependymoma cell lines.
   A: To rule out tumor protective effect of the above compounds on GBM, Medulloblastoma and Ependymoma cell lines
   B: To rule out tumor protective effect of the above compounds on GBM orthotopic mice models.
3. To study the biodistribution of these drugs in the tumor vis-a-vis normal brain, plasma, other peripheral organs

Novelty

Neurocognitive pharmacotherapy has typically been demonstrated to have relatively modest benefits in patients of metastatic brain tumors who are typically older and have limited life expectancy. Therefore, the long- term sustained benefit of such interventions remain questionable. This study will aim to see the neuroprotective and pro-cognitive effect of the established (Memantine) drug and the experimental (Losartan, Pioglitazone, *E. officinalis* extracts or active principles) in gliomas for the first time and also have as its intended beneficiaries' young adults and children (long term survivors and thereby derive sustained benefits) for the first time.

Impact of the study

Robust lab data supporting a neuroprotective role for the investigational pharmaceuticals is likely to lead to Phase I-II studies investigating administration of the same to determine the MTD and lowest effective doses in conjunction with RT in order to produce meaningful arrest in the rates of cognitive decline as assessed by a suitable planned battery of age-appropriate cognitive tests. The consequent improvement in cognitive outcomes will lead to measurable improvements in Quality of Life (QOL) domains and likely successful social reintegration, education and gainful employment with reduction in Disability Adjusted Life Years (DALYs).

The lab has been working on drug development in glioblastomas for the last 7-8 years.

Outline of workflow and methodology in brief

Aim	Objectives	Expected work done	1st year	2nd year	3rd year	4th year	5th year
1	**In vitro experiments Tumour cell lines** (GBM, medulloblastoma, Ependymoma) **Normal cells:** Astrocytic cells, Neuronal cells,	Drug optimization studies					
		Clonogenic assays for cell survival					
		3.Invitro assays for neuroinflammatory biomarkers					
		4.Assay for Apoptosis					
		5.DNA Damage assays					
2	**In vivo Biodistribution and pharmacokinetics**	Pharmacokinetics and biodistribution studies in in vivo models					
3	**In vivo experiments** a. To study tumour protective effects of the drugs b.to study animal learning and memory behaviour c. Ultrastructural, IHC, Histopathology studies d. Exploratory PET imaging studies	Tumour imaging studies using bioluminescence imaging and micro-CT imaging to study tumour volumes following administration of the drugs					
		Learning and memory behaviour studies using water maze experiments					
		Histopathology and Immunohistochemical studies					
		Ultrastructural studies for axonal integrity using electron microscopy					
		Histopathological studies of normal tissues to study inflammatory changes, apoptosis/necrosis IHC of selected neuroinflammatory markers on the normal tissues pre therapy and post therapy with experimental drugs Exploratory study of using PET radiotracer (SV2A) Synaptic vesicle protein 2Afor measuring Neuronal density					

Project 5A: To identify the biomarkers for responsiveness to metronomic chemotherapy in Oral cavity cancer

PhD Guide: Kumar Prabhash, MD, DM, Professor of Medical Oncology, TMC
Site of PhD work: TMH & ACTREC
Preferred education background of students: Life Sciences, Bio-Informatics, Physics, Medical or Pharmacology

Metronomic chemotherapy is standard of care chemotherapy in palliative setting for oral cavity cancer in India. This treatment has been developed by us. We understand that 40-50% patients responds to this treatment. This suggests that almost 50% don't benefit with this treatment but they endure the side effects of the treatment. It is important to identify the biomarkers which may help avoid patients with toxic treatment and they may receive better treatment option. The objective of this project is to understand the biomarkers in genomic landscape of oral cavity cancer from base line to predict response to metronomic chemotherapy .

50 Oral cavity cancer patients tissue samples will be collected at the baseline. Blood will be collected at the baseline. Tissue sample will also be collected at the time of progression. Baseline samples and the sample at the time of progression will undergo whole exome and whole transcriptome sequencing, this will also be done on the samples collected at the time of progression.

The data obtained from this process will be validated with orthogonal tests on larger sample size.
This data will be correlated clinically. It will evaluate if genomic profiling of patients responding to the treatment is different. Once if find the biomarkers with the profiling then they will validated in bigger sample size with orthogonal tests.

We have project approved with this theme. We have already collected samples of 50 patients as part of this project. We have also got whole exome and whole transcriptome of these samples done.

The PhD Student will be doing the bioinformatic analysis of all the data, orthogonal testing and correlate the genomic data with the outcome of these patients.

Project 5B: To identify the biomarkers for responsiveness to neoadjuvant chemotherapy in Oral cancer

PhD Guide: Kumar Prabhash, MD, DM, Professor of Medical Oncology, TMC
Site of PhD work: TMH & ACTREC
Preferred education background of students: Life Sciences, Bio-Informatics, Physics, Medical or Pharmacology

Neo-adjuvant chemotherapy is standard of care chemotherapy in advanced stage setting for oral cavity cancer in India. This treatment has been standardised by us for these patients. We understand that around 40% patients responds to this treatment. This suggests that almost 60% don't benefit with this treatment but they endure the side effects of the treatment. It is important to identify the biomarkers which may help avoid patients with toxic treatment and they may receive better treatment option. The objective of this project is to understand the biomarkers in genomic landscape of oral cavity cancer from base line to predict response to metronomic chemotherapy .

50 Oral cavity cancer patients tissue samples will be collected at the baseline. Blood will be collected at the baseline. Tissue sample will also be collected at the time of progression. Baseline samples and the sample at the time of progression will undergo whole exome and whole transcriptome sequencing, this will also be done on the samples collected at the time of progression.

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The PhD Student will be doing the bioinformatic analysis of all the data, orthogonal testing and correlate the genomic data with the outcome of these patients.

Project 6A: Early T cell precursor acute lymphoblastic leukaemia: Unravelling mechanisms of resistance and development of novel therapeutic strategies

PhD Guide: Manju Sengar, MD, DM, Professor of Medical Oncology, TMC

Site of PhD work: TMH & ACTREC

Preferred education background of students: Life Sciences

Significance of the research question: Early T- cell precursor ALL (ETP-ALL), a novel subtype of T-cell ALL, exhibits significant resistance to chemotherapy and steroids resulting in high rates of induction failure and poor survival. Recent data suggest that treatment intensification with allogeneic stem-cell transplant and intensive chemotherapy can improve outcomes. However, the applicability of treatment intensification is limited by the costs involved and high early mortality due to uncontrolled disease. Thus, there is an urgent need for revamping the therapeutic strategies for ETP-ALL.

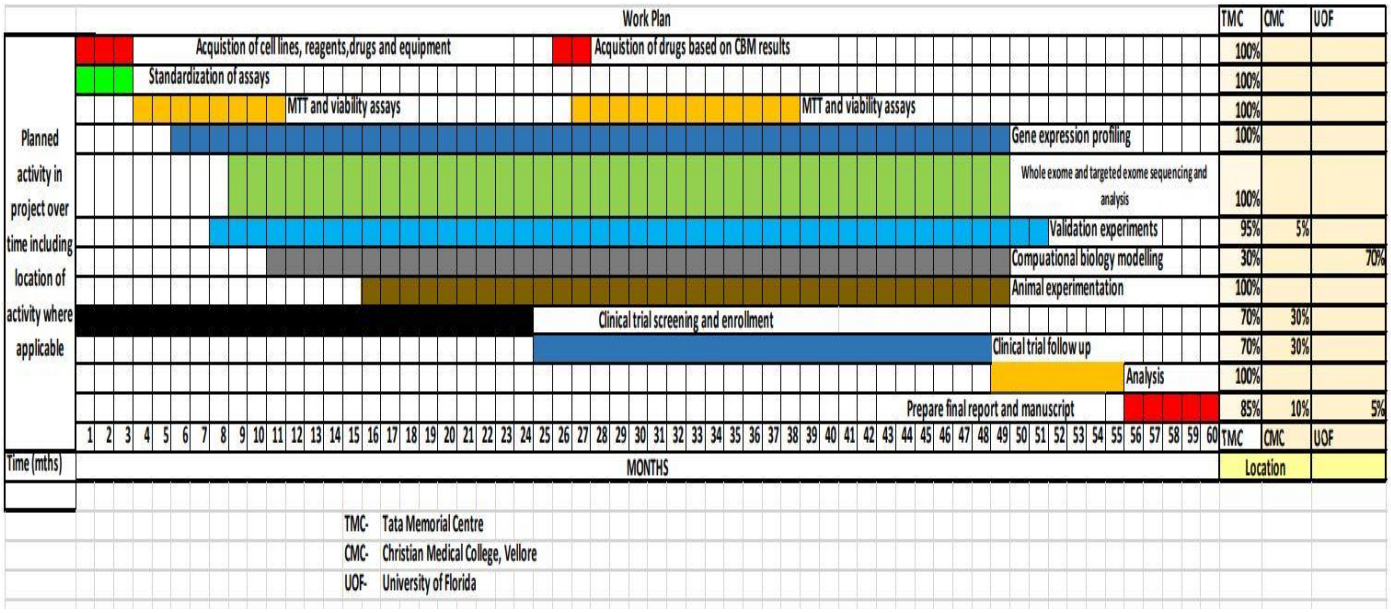
Objective 1: To demonstrate prednisolone resistance and its biological basis in ETP-ALL compared to non-ETP subtype of T ALL and to evaluate the impact of prednisolone exposure on the chemosensitivity to other drugs used in ALL induction therapy.

Objective 2: To demonstrate the exquisite sensitivity of ETP- ALL to 6-MP as compared to other ALL subtypes and its underlying biological basis**.**

Objective 3: In-vitro and in-vivo validation of sensitivity of ETP-ALL to FDA approved drugs as predicted by in-silico analysis.

Brief Methodology: We propose to evaluate the approach of drug-repositioning and repurposing in both preclinical and clinical trial to improve treatment outcomes. The drug-repositioning is based on our clinical observation of steroid resistance and exquisite sensitivity to 6-MP. Preclinical studies will address biological basis for these observations by evaluating these drugs in the preclinical studies including animal models.

PROJECT TIMELINES:



Project 7A: CORRELATION OF IMATINIB RESISTANT MUTATIONS (INCLUDING SPLICE SITE VARIANTS) WITH RESPONSE TO SUBSEQUENT THERAPIES IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

PhD Guide: Dr Navin Khattry, MD, DM, Professor Medical Oncology, ACTREC, TMC
Site of PhD work: ACTREC
Preferred education background of students: Health Sciences including Clinical Research or Lifesciences

Background and gaps in knowledge: Resistance to imatinib is a common clinical problem faced during treatment of CML. A large number of mutations associated with imatinib resistance are known. However, for many mutations, clinical data is lacking on the outcomes with different TKIs. Additionally, there are different methods for assessing for mutations in tyrosine kinase domain of BCR-ABL (which is one of the important mechanisms of imatinib resistance) and it needs to be explored which method is the most clinically relevant. With the advent of next-generation sequencing (NGS) for resistant mutation analysis, it has become essential to compare results obtained with NGS with those obtained with Sanger sequencing and determine the clinical relevance of the mutations identified by both these methods. Furthermore, recently additional somatic mutations have been described in CML which are of unknown prognostic value

Study objectives:

- Detect somatic mutations in patients of CML which are implicated in pathogenesis of myeloid malignancies.
- The objective of the proposed study is to evaluate the treatment efficacy of various tyrosine kinase inhibitors (including different doses) amongst patients with chronic myeloid leukemia (CML) harboring different tyrosine kinase domain mutations. Treatment efficacy will be assessed by attainment of cytogenetic / molecular responses at various time points, as well as assessment of progression free survival and overall survival.
- The goal of the study is to estimate if low level mutations which could have been missed by Sanger sequencing (<20% VAF) but detected with sensitive NGS are predictive of suboptimal responses.
- Additionally, we will also study the baseline samples of these patients to see if these mutations were present at baseline and explain the clonal evolution of these mutations under selective pressures by TKIs.
- Scientific relevance, expected impact and novelty: Resistance to imatinib is a common clinical problem faced during treatment of CML. A large number of mutations associated with imatinib resistance are known. However, for many mutations, clinical data is lacking on the outcomes with different TKIs. Approximately, we have >2000 IRMA analysis done in the above period and with available follow up of the majority of these patients, this study will help the get the much-needed clinical data on the responses with different TKIs in such patients. This will help in guiding treatment for such patients. This study will also help to compare the results of Sanger's sequencing versus NGS as a method to detect mutation analysis and to identify which of these results are clinically more relevant. Also, because we will process baseline samples for NGS, we will be able to find out whether these clones existed at baseline. This will help us to find out whether doing a baseline NGS has any predictive and / or prognostic significance. We will also determine if somatic mutations are detected at diagnosis are predictive of outcomes.

Outline of workflow and methodology in brief:

- Study design: This will be an ambispective cohort study of patients who have undergone imatinib resistance mutation analysis (IRMA) at our institute between June 2013 to June 2022 (tentative).
- Patient population
  - For objective 1 - all patients with CML who have undergone IRMA testing in the above period.
  - For objectives 2 and 3, we will take only those patients who have a back-up sample available for running the IRMA test by NGS.
  - For objective 4, we will include only those patients who have a back-up of the baseline sample for processing by NGS.
- Inclusion and exclusion criteria: Any patient with CML with age >18 years at study entry who has a mutation analysis done for imatinib resistance will be eligible for inclusion in the study irrespective of the phase of the CML. Patients who have received concomitant chemotherapy at time of change of TKI or within 30 days would be excluded as that would blur the response assessment.

Project 7B: Developing Natural Killer cell-based Immunotherapies for Cancer

PhD Guide: Dr Navin Khattry, MD, DM, Professor Medical Oncology, ACTREC, TMC
Site of PhD work: ACTREC & TMH
Preferred education background of students: Lifesciences or Biotechnology

Acute myeloid leukemia is the most common acute leukemia in adults and has a poor prognosis. With advanced therapies about 60 to 70% will achieve a remission but only 25% of adults with AML are expected to survive at 3 years from diagnosis. Further intensification of chemotherapy is not an option. Newer therapies like Venetoclax might help certain patients achieve a prolonged remission but eventually many of them relapse. Harnessing the body's immune system might be a way to further improve outcomes.

NK cells are the most potent effector cells against AML blasts as seen from studies in patients who have undergone T cell depleted hematopoietic stem cell transplant. To enhance the efficacy of NK cell therapy, the NK cells need to be expanded in vitro or in vivo to achieve a better effector to target ratio.

In our lab we are working to enhance the efficacy of NK cells by generating a memory like phenotype so that the NK cells will expand in vivo after infusion into the patient. This therapy has shown promise in some phase I trials by investigators from Dana Farber and St. Louis.

We have preliminary data on generating these memory-like NK cells in the lab with enhanced cytotoxicity. We plan to translate these findings to the clinic by generating clinical grade memory like NK cells for infusion into patients with AML in a phase I clinical trial.

Study objective:

1. Generation of clinical grade cytokine induced memory like NK cells.
2. To study the safety and efficacy of cytokine induced memory like NK cells in patients with relapsed refractory AML.
3. Study the engraftment kinetics and persistence of cytokine induce memory like NK cells after infusion into the patient.
4. Study the immune escape mechanisms of AML blasts after NK cell infusion between responders vs non responders.

Scientific relevance, expected outcome and novelty:
AML blasts escape the body's immune surveillance by inhibiting NK cell function. Understanding the mechanism of immune escape will help improve the efficacy of allogeneic cytokine induced memory like NK cells and identify mechanisms to harness the cytotoxic potential of autologous NK cells for AML and NK sensitive solid tumors.

We plan to develop cGMP grade cytokine induced memory like NK cells and explore their efficacy in a phase I clinical trial. Since escalation of chemotherapy has no added advantage exploring immune based approaches to overcome relapsed refractory disease is a novel approach in the management of Acute myeloid leukemia.

Outline of workflow and methodology in brief:
Allogeneic NK cells are harvested from a haploidentical donor by apheresis and purified using magnetic bead separation. These NK cells are incubated with a cytokine cocktail to generate a memory like phenotype.
In a phase I clinical trial using dose escalation 3 + 3 design the safety and efficacy of the cytokine induced memory like NK cells will be tested for safety and efficacy. AML patients not in morphological remission at the end of 2 cycle of hemotherapy and relapsed patients with AML will be eligible for the trial. The engraftment kinetics and persistence of the NK cells will be studied using flowcytometry techniques. Further studies to identify immune escape of AML blasts from these allogeneic NK cells will be studied to further enhance the efficacy of NK cells in future trials.

The PhD project involves manufacturing clinical grade NK cells for the clinical trial, studying the persistence of NK cells after infusion into the patient and understanding the immune escape mechanisms in AML in responders vs non-responders. Experience cGMP facility is desirable

Project 8A: Understanding Clonal Evolution of Acute Myeloid Leukemia at a Single Cell Resolution

PhD Guide (& Co-Guide): Dr Nikhil Patkar, MD.DNB, Professor & Clinician Scientist, Wellcome-DBT Senior Fellow, ACTREC, Tata Memorial Centre
Website: https://nvpatkar.wixsite.com/research-group
Site of PhD work: ACTREC
Preferred education background: LifeSciences, Biotechnology, Bioinformatics, Computational Biology, Computer Science, Engineering

This project is part of a larger five-year prospective study on acute myeloid leukaemia. The project is funded by Wellcome Trust – DBT (India Alliance). The project leverages the strength of a fully characterized adult AML cohort based on past work carried out by Dr Patkar and his research group in this field.

Background and important gaps in knowledge:
AML is a heterogeneous disease with subsets of varying prognoses with current therapy. Cellular heterogeneity, a hallmark of AML, is masked by analysis of bulk populations. Although relapse occurs from genetically distinct sub-clones, current therapies do not account for this heterogeneity. Single-cell DNA sequencing (scDNAseq) has allowed us to genotype individual leukemic cells revealing complex clonal relationships between AML blasts and clonal hematopoiesis. It has helped decipher mutational epistasis, mutational co-occurrence, and mutational exclusivity in AML. It is possible that clonal heterogeneity in AML is possibly dynamic and can evolve during therapy. It thus becomes vital to elucidate the clonal nature of MRD populations and their evolutionary trajectories. ScDNAseq based MRD has yielded encouraging results on small patient cohorts (n=14) where the persistence of leukemic clones at morphological remission was associated with a higher risk of relapse. Current commercial methodologies (10X Genomics, BD Rhapsody, Mission Bio) enable an analysis of either RNA or DNA (±protein). A true single-cell "multi-omics" approach is desirable, which links the mutational state with its transcriptional phenotype within the same analysis. This would allow us to detect mutant clones, identify cellular phenotype and detect transcriptional abnormalities from diagnosis to MRD time points and relapse. This concept is in a nascent stage. Some DNA/RNA co-assays require physical separation of DNA and RNA, resulting in loss of analytical sensitivity, whereas others have limitations in the number of analyzable genes (https://teichlab.github.io/scg_lib_structs/). Recently described techniques such as TARGET-seq rely upon cell sorting for single-cell isolation, making them cumbersome and low throughput (hundreds of cells). In that context, commercial single-cell platforms also have a limited throughput of ~6,000-8000 cells per sample. This low throughput is a hurdle for MRD testing as nearly 90% of an AML MRD sample comprises erythroid cells, T/NK/B cells, and granulocytes.

Study objectives, scientific relevance, novelty and likely impact:
Establish a single cell targeted DNA-RNA co-assay for simultaneous detection of expression and sequence level abnormalities in AML and evaluate its clinical relevance towards the detection of MRD

This co-assay will *simultaneously* target somatic mutations, expression abnormalities, and yield cellular phenotype in single cells while overcoming the need for separate instrumentation and analyses for these assays. We will leverage sci-L3-target-seq to track the clonal trajectories of somatic mutations and transcriptional abnormalities in AML blasts from diagnosis to MRD time points in a prospective study. In parallel, we will also assess MRD measured by NGS and multicolor flow cytometry on a cohort of patients treated with standard therapy. We hope to understand the clonal composition of MRD populations and evolutionary trajectories of treatment-resistant clones (including subclones) from diagnosis to MRD and finally relapse. Lastly, we will also understand if MRD detected by sci-L3-target-seq has a bearing on disease outcome.

Outline of workflow and methodology in brief
We will develop a relatively low-cost, high throughput, single-cell targeted AML MRD assay called sci-L3-target-seq. Sci-L3-target-seq is a targeted DNA-RNA co-assay built on the concept of split-pool barcoding, combinatorial indexing, and linear amplification via engineered transposons.

Work Done so far: I have a track record of genomics and AML research which have laid the foundation of this current proposal. Complete and updated list of 80+ publications can be seen here: https://pubmed.ncbi.nlm.nih.gov/?term=Patkar+Nikhil&sort=date

Project 8B: GENOME SEQUENCING-BASED PROGNOSTICATION AND MONITORING OF ACUTE MYELOID LEUKEMIA

PhD Guide (& Co-Guide): Dr Nikhil Patkar, MD.DNB, Professor & Clinician Scientist, Wellcome-DBT Senior Fellow, ACTREC, Tata Memorial Centre
Website: https://nvpatkar.wixsite.com/research-group
Co-Guide: Dr Rahul Kumar, Assistant Professor, IIT Hyderabad
Site of PhD work: ACTREC , IIT Hyderabad
Preferred education background of students: Life Sciences/Biotechnology/Bioinformatics/Computational Biology /Computer Science/Engineering

This PhD project is part of a larger five-year prospective study on acute myeloid leukaemia. The project is funded by Wellcome Trust – DBT (India Alliance). The project leverages the strength of a fully characterized adult AML cohort based on past work carried out by Dr Patkar and his research group in this field.

Background and important gaps in knowledge:
AML is a heterogeneous disease with subsets of varying prognoses with current therapy. Traditional chromosomal karyotype (cytogenetics) based risk stratification, though highly predictive of outcome, is not always accurate as subsets of patients in favourable karyotypic risk behave poorly and those in intermediate risk have comparable or better prognoses than favourable karyotype. In the last few years, our understanding of AML biology has greatly changed due to improvements in genome sequencing technologies. Exome and other targeted sequencing studies demonstrated that genetic alterations in key coding genes are recurrent and subsequent studies demonstrated that they predict outcomes.

A very important predictor of outcome in AML is the response to chemotherapy, as evident by measurable residual disease (MRD). Our group has led pioneering work (Patkar, *Leukemia* 2021) to demonstrate that corrected sequencing-based molecular MRD is highly predictive of AML outcomes.

Study objectives, scientific relevance, novelty and likely impact:

- Analyze whole genome sequencing (WGS) data on a characterized cohort of AML and understand the genomic landscape of AML
- Develop and refine genomics-driven risk stratification schemes for AML.
- Explore and develop genomics methodologies at the frontier of sequencing-based MRD detection and rare variant detection in AML such as phased and duplexed sequencing. Evaluate the clinical relevance of these techniques to predict outcomes in AML.

Outline of workflow and methodology in brief

- Develop and clinically validate an affordable, high-efficiency, accurate duplex consensus sequencing (DCS) assay for the detection of AML MRD. We will develop an efficient DCS assay that corrects for sequencing errors in singleton reads and retains them instead of discarding these reads. This rescue of singletons will reduce redundancy typically seen with conventional DCS while allowing for larger MRD panels without compromising on the performance advantages of DCS. This MRD assay will be clinically validated on a cohort of adult AML that has been comprehensively characterized for baseline cytogenetic and genomic risk, FCM-MRD, leukemic stem cell MRD, (smMIPs based) NGS-MRD, and clinical outcomes. We estimate that this research will help identify an optimal approach or a combination of approaches for monitoring responses to chemotherapy in AML.
- Develop analytical tools as well as computational pipelines to analyze WGS data on the AML cohort. Determthe ine clinical relevance of regulatory as well as non-coding regions in AML.
- Techniques involved here would be computational biology as well as wet lab genomics methods development.

Work Done so far: I have a track record of genomics and AML research which have laid the foundation of this current proposal. Complete and updated list of 80+ publications can be seen here: https://pubmed.ncbi.nlm.nih.gov/?term=Patkar+Nikhil&sort=date

Project 8C: Deciphering the molecular heterogeneity in Acute Leukemia of Ambiguous Lineage at a cellular level

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PhD Co-Guide: Dr Gaurav Chatterjee, MD, MRes, Associate Professor, Hematopathology Laboratory,
Site of PhD work: ACTREC
Preferred education background of students: Life Sciences/Biotechnology/Bioinformatics/Computational Biology /Computer Science/Engineering:

- **Background and important gaps in knowledge**: Acute Leukemia of ambiguous lineage (ALAL) refers to a group of relatively rare acute leukemia with poor prognosis that are defined by genomic and phenotypic heterogeneity. Together, ALAL amounts to ~5% of all AL and are traditionally classified based on the combination of phenotypic commitment as determined by multicolor-flowcytometry (MFC) to T/B/myeloid lineages. Such an immunophenotypic classification schema is inadequate to capture the remarkable clonal and cellular heterogeneity of ALAL. Published works on genomics of ALAL are limited and have used broad-spectrum bulk sequencing approaches such as WES/WTS. These approaches do not provide cellular/clonal level genomic changes and are unable to decipher correlation between particular mutational profile and expression levels. The limitations of currently established techniques to capture the complete spectrum of heterogeneity in ALAL are reflected in that the patients with ALAL are uniformly treated with ALL-like therapy. An immunophenotype-based treatment algorithm has been recently proposed in children but is still not widely adopted in clinical practice and is inadequate to completely address the potential need of AML-like/novel therapy in a subset of these patients.
- **Preliminary work of our laboratory**: We have already performed targeted DNA and RNA sequencing using in-house designed panels in a cohort of 106 patients with ALAL (52 pediatric, 54 adults). A total of 190 different SNV/indels and 24 fusions were identified in this cohort. Commonly mutated genes included *NRAS, FLT3, RUNX1, WT1, TP53, EZH2, KRAS, CSF3R, NF1* and *PHF6*. The variant allelic frequency (VAF) ranged from 1.3-98% (median 33.9%). Interestingly, 58/190 (30.5%) of mutations had a VAF of ≤20%, indicating a possible subclonal distribution further reiterating the need of high-throughput molecular techniques able to characterize cellular-level heterogeneity. This is especially relevant in patients of ALAL as genetically distinct sub-clones may be associated with distinct phenotypic patterns and may influence treatment-response and relapse differentially. Therefore, single-cell sequencing technologies combining data from mutations, fusions, and expression patterns in terms of lineage commitment is desirable for comprehensive understanding of biology of these neoplasms. Current commercial methodologies (10X Genomics, BD Rhapsody, Mission Bio) enable an analysis of either RNA or DNA (±protein), and are limited in terms of throughput as these assays need physical separation of DNA/RNA.
- **Study Objectives and relevance**: We aim to establish a single cell RNA/DNA co-assay using combinatorial indexing and linear amplification for simultaneous detection of expression and sequence level abnormalities in ALAL. This will be a relatively low-cost, high throughput, single-cell assay built on the concept of split-pool barcoding, combinatorial indexing, and linear amplification via engineered transposons. This assay will simultaneously target somatic mutations/indels, expression abnormalities, and yield cellular phenotype in single cells while overcoming the need for separate instrumentation.
- **Study methodology**: In this pilot, exploratory, observational, prospective study, we will leverage sci-L3-RNA/DNA co-assay to decipher the clonal and cellular heterogeneity of ALAL. After informed consent, all consecutive patients of ALAL (50 patients) who opt for the standard treatment at TMC will be recruited. Each sample will be characterized for morphology and immunophenotyping using a 5-tube 10-13 color flowcytometry panel, standard Cytogenetics and FISH, routine mutations and chimeric fusions. In addition, the sci-L3-RNA/DNA co-assay will be performed at diagnostic, after initiation of therapy and relapse timepoints. The cohort will be characterized for clinical and demographic variables including death, relapse of leukemia and secondary malignancies. We hope to understand the genomic-phenotypic correlation of various subclones seen in ALAL, especially in the context of lineage commitment. Lastly, we will also determine the clinical significance of cellular-level clonal architectural patterns by comparing them with flowcytometry and evaluating its clinical impact on overall survival, event-free survival and cumulative incidences of relapse.
- **Potential impact**: This proposed work is expected to provide cellular/clonal level information on the biologic heterogeneity and clonal evolution with therapy in ALAL at unprecedented detail. These findings may form rationale for refining biologic classification of ALAL and may provide basis for biology-driven therapy selection in a subset of these patients.

Project 9A: OVOL-α-Actinin 4 crosstalk in EMT & Breast cancer stemness

PhD Guide (& Co-Guide): Dr Omshree Shetty, PhD., Molecular Pathology Lab, Tata Memorial Centre
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Site of PhD work: TMH
Preferred education background of students: Life Sciences/Biotechnology

Epithelial-mesenchymal transition (EMT) is the process by which epithelial cells lose their cell-cell junctions and polarity and produce migratory mesenchymal cell types, and is thought to be necessary for initiation of cancer metastasis. EMT is also thought to give rise to cancer stem cells (CSCs) which possess tumorigenic potential, are associated with distant metastasis and therapeutic resistance. While breast CSCs were initially shown to possess high levels of CD44 and low or negligible expression of CD24 and are typically mesenchymal in nature, recent studies suggest that cells with a hybrid epithelial/mesenchymal (E/M) phenotype associated with $CD44^{+}CD24^{+}$ expression profile can display up to 50 times higher tumor-formation potential as compared to individually migratory mesenchymal cells. Therefore, characterizing the hybrid E/M phenotype can contribute to a full understanding of the role of EMT in metastasis and chemo resistance. OVOL and α-actinin 4 (ACTN4) are two proteins that have been shown to have opposite effects in inducing EMT with high OVOL expression driving cells to an epithelial phenotype and high ACTN4 expression driving cells to a mesenchymal phenotype. We hypothesize that the hybrid epithelial/mesenchymal (E/M) phenotype is sustained by a sub-population of CSCs expressing moderate levels of OVOL and ACTN4, which collectively impart increased tumorigenic potential and increased invasiveness.

Objectives

Objective 1: To correlate OVOL/ACTN4 expression with invasiveness and mode of invasion in breast cancer cell lines and patient derived tumor cells

Objective 2: To probe OVOL/ACTN4 levels in CSCs/hybrid CSCs and their invasiveness/mode of invasion

Objective 3: To probe the role(s) of OVOL/ACTN4 in maintenance of cancer stemness, EMT phenotype & cancer invasiveness.

Broad Work Plan

1.1. Maintenance of TNBC cell lines/Isolation of primary cells from TNBC patient derived tumors.
1.2. Transcriptomic and expression profiling of Tumor Tissue
1.3. Assessment of invasiveness of cancer cells using 3D collagen gels
2.1. Isolation of CSCs and hybrid-CSCs using flow cytometry
2.2. Assessment of OVOL/ACTN4 levels and localization in CSCs and hybrid-CSCs
2.3. Correlation of invasiveness of CSCs and hybrid CSCs with OVOL/ACTN4 levels
3.1. Establishment of ACTN4 and OVOL knockdown cells
3.2. Effect of knockdown on fraction of CSCs/hybrid CSCs and their EMT profile
3.3. Mammosphere formation ability of knockdown CSCs/hybrid CSCs:
3.4. Characterization of invasiveness of knockdown CSCs:
3.5. Establishment of ACTN4/OVOL knock-in cells
3.6. Effect of knock-in on fraction of CSCs/hybrid CSCs and their EMT profile

Project 10A: Evaluation of intraoperative Short latency responses (SLR) and Long latency responses (LLR) to map the motor speech brain areas and compare with traditional direct electrical stimulation (DES) technique to predict postoperative speech functions in patients undergoing for the brain tumors removal surgery.

PhD Guide: Dr. Parthiban K Velayutham, Assistant Professor & Clinical Neurophysiologist, TMH & ACTREC.
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Co- Guides: Dr. Aliasgar Moiyadi, Professor of Neurosurgery, TMH& ACTREC
Dr. Prakash Shetty, Professor of Neurosurgery, TMH & ACTREC
Site of PhD work: TMH & ACTREC
Preferred education background of students: M.Sc – Neuroelectrophysiology / Neurosciences / Life Sciences

Background and important gaps in knowledge: The structural and functional organization of brain networks sub serving daily basic activities like speech, visuo-spatial cognition, movement and semantics etc. This task based brain networks are highly interconnected especially the crucial cortical areas for the expression of speech. Preservation of this anatomical substrate is more important during the removal of opercular tumors such as gliomas in neurosurgery. In this context, the application of direct electrical stimulation (DES) at cortical and subcortical level is used as the gold standard method for identification of motor speech areas along with other functional areas in awake surgery with limited clinical and technical reasons. Thus, a method for the intraoperative mapping of speech functions using neurophysiological markers under general anaesthesia (GA) would be of great potential use. Recently, high frequency electrical stimulation induced short latency response (SLR) from M1 motor area of the laryngeal muscles has accompanied with dysarthria (slurring of speech) and long latency response (LLR) from the caudal opercular part of the inferior frontal gyrus accompanied with semantic paraphasia (word errors) has been documented. However, information on the usefulness of SLR and LLR markers based mapping and preservation of speech functions in brain tumors removal surgery under general anaesthesia (GA) is sparse. Thus, in this study, we would like to establish the SLR and LLR technique to preserve the speech functions under GA and compare its efficacy with traditional DES stimulation in awake surgery to predict postoperative speech and its associative functions.

- **Study objectives, scientific relevance, novelty and likely impact:**
  1. To standardize the method for intraoperative motor speech mapping under general anaesthesia using SLR and LLR markers in patients with brain tumors removal in and around the speech related cortical areas.
  2. To evaluate the preoperative MRI and intraoperative navigation tool markers and correlate intraoperative SLR and LLR markers with respect to postoperative speech outcome.
  3. To study the association of intraoperative SLR and LLR markers with perioperative neuropsychological assessment and predict the postoperative speech presentation.
  4. To compare the SLR and LLR techniques under general anaesthesiaVs gold standard (60Hz stimulation) technique in awake surgery by assessing the intraoperative and postoperative speech functional outcome.
- **Hypothesis:** To establish and set the SLR and LLR stimulation and recording parameters that would help in preservation of Motor, sensory and Speech functions in patients whom undergoing surgery for the removal tumors in and around motor and speech related cortical areas.
- **Outline of workflow and methodology in brief:** 1. Preoperative clinical and Neuropsychological evaluation 2. Intraoperative DES, SLR and LLR electophysiological study 3. Correlation of intraoperative DES, SLR and LLR sites with preoperative MRI data's 4. Postoperaitve clinical and Neuropsychological evaluations.

- **Preliminary work:** We have an extensive experience on DES in awake craniotomy conditions and preserved speech and language associated functions intraoperatively during the tumor resection surgery. However, this DES in Awake craniotomy is not possible in many circumstances due to many perioperative clinical and technical reasons. Thus, this current project is aimed to use the newly emerging SLR and LLR techniques to assess the speech and language functions under general anaesthesia intraoperatively.

Project 11A: Developing precision immunotherapy for value-based treatment of lung cancer in India

PhD Guide: Dr. CS Pramesh, MS. MCh. Professor, Surgical Oncology, TMC
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Site of Ph.D. work: ACTREC and TMH

Preferred education background of students: (e.g. Life Sciences / Biotechnology / Medical etc)

Project Summary:

Background: Checkpoint immunotherapy that blocks the inhibitory receptor, programed cell death-1 (PD-1), pathway has revolutionized treatment of many cancers, most importantly lung cancer. While these therapies have shown promising clinical results in a significant number of cancer patients, some patients progress despite showing favorable responses initially and others show no response to the therapy. It is therefore important to monitor the patients' immune responses during treatment to understand why in some cases anti-tumor responses are shortlived, and subsequently develop strategies to promote more effective and durable responses. In this proposal, we will identify predictive biomarkers and characterize novel stem-like immune cell populations that determine clinical responses to PD-1-targeted immunotherapy in lung cancer patients in India.

Objective:

1. To determine if blood immune biomarkers can predict the response to immunotherapy in Indian patients with NSCLC. To study kinetics of proliferating PD-1 CD8 T cells, their phenotypes, functions and tissue homing markers.
2. To characterize the basic mechanisms involved in an immune response to lung cancer, and determine if these parameters maybe used to predict response to therapy.
3. To perform immunotypic, genetic and epigenetic characterization of tumor infiltrating (TIL) and circulating stem like cytotoxic T-cells using immunophenotyping, RNA-sequencing and ATACseq analysis.
4. To determine the utility of markers mentioned above in the evaluation of response to immunotherapy.

Methods:

- Flow Cytometric immunophenotyping for immune cell subset analysis including immune checkpoint-protein expression/T-cell functions) and to investigate expression pattern of CD3, CD8, PD-1, TIM-3, CTLA-4, TCF-1, Bcl-6, Blimp-1, granzyme B, perforin, CD73, CD39, CD62L, CD127, KLRG-1, and CD28, gamma-deltaTCRs, NKG2D and CD226 using high-dimensional multicolor flow cytometry.
- Gene expression profile of immune surveillance genes in tumor-infiltrating lymphocytes (TIL) and circulating stem like cytotoxic T-cells will be studied using RNA sequencing, scRNA sequencing.
- Epigenetic characterization of immune cells will be performed using ATACseq analysis in FACS-sorted cells.

Expected outcome and translatability: The results of this study will provide detailed immune-cell signatures and immunogenetics in Lung cancer patient taking anti-PD1 therapy and kinetics of various immune-cell subsets at subsequent intervals. The study will provide an essential data to optimize treatment efficacy, and thereby, predictive biomarkers to choose better treatment options given that immunotherapies are prohibitively expensive in India.

Project 11B: Understanding the effect Aspirin on disease recurrence and outcome in patients with Gastro-Oesophageal cancer in India

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Co-Guide: Dr. Kinjalka Ghosh, Assoc Professor-Department of Biochemistry, TMH
Site of PhD work: TMH
Preferred education background of students: Life Sciences, Biochemistry, Biotechnology, Pharmacology, Medical

Add-Aspirin is a phase III, double-blind, placebo-controlled, randomised multicentric trial (co-developed between TMC and the Medical Research Council Clinical Trials Unit (MRC CTU) assessing the effects of aspirin on disease recurrence and survival after primary therapy in common non-metastatic solid tumours (breast, colorectal, prostate and gastro-oesophageal (GO) cancer).Participants take open label 100mg aspirin daily for an 8-week run-in period followed by random assignment to 100mg or 300mg aspirin or matched placebo daily for 5 years.

The trial hypothesis is based mainly on epidemiological data and the precise mechanism underlying aspirin's anti-cancer effects is unclear. Recent data from UK participants within the Add-Aspirin study show that platelet activation is increased at baseline compared to healthy individuals, is inhibited by Aspirin, and is most marked in the GO cohorts, independent of other baseline variables, where the epidemiological effects for the anti-cancer effects of aspirin are strongest. Platelet activation can be measured through the urinary thromboxane metabolite 11-dehydro-thromboxane B2 (U-TXM).

Data has shown high U-TXM are associated with an increased risk of cancer mortality (XX), as well as cardiovascular and all-cause mortality. Hence anti-cancer effects of aspirin may be mediated through the reduction of Thromboxane which enhances T-cell cancer clearance.

To establish a sub-study within the Add-Aspirin trial in India to explore:

1. Whether serial urine and plasma biomarkers predict the recurrence of GO cancer.
2. Are there tissue markers that can predict response to Aspirin and indicate poor outcomes

Currently, ~10,000 participants have been registered in Add Aspirin study with 987 from India, including 332 in the GO cohort. This provides an opportunity to explore urine and plasma biomarkers, genomics and other -omics technologies and tissue biomarkers to elucidate the anti-cancer mechanism of action of aspirin particularly in the GO cohort. As recruitment is ongoing in India, U-TXM levels will be measured over a longer period time which will be a unique data set. The study will also add significantly to the gastro-oesophageal data where the UK numbers were limited.

Novel insights into GI cancer which would inform mechanism of action in Indian patients (India is potential recruiter in the GE cohort of Add Aspirin study. **On a broader note, the results of this PhD project (correlating the genetic expressions with the recurrence in GO cancer) may be extrapolated to substantiate the use of Aspirin (which is a low-cost generic) as an adjuvant treatment option.**

Outline of work
The project includes 4 phases:
Phase I: Retrospective evaluation of panel of biomarkers (including MMRd, PIK3CA, HLA Class I and other immune markers) in GO patients who have undergone surgery at TMH between 2010-2022 *(excluding Add-Aspirin participants)*
Phase II: Prospective collection of serial urine, plasma and whole blood samples from the Add-Aspirin participants in the GO cohort (2022-2025) and biomarker profiling (including urinary Thromboxane). EIA measurements of Urinary 11-dh-TXB_2 would be undertaken.
*Phase III:*Tissue profiling of Add-Aspirin participants(markers outlined in *Phase I*) from the GO cohort to identify predictive/prognostic markers of recurrence (2015-2025)
*Phase IV:*Exploring the possibility of building algorithm combining the biomarkers as predictive/prognostic markers for GO cancers.

Project 12A: Role of immune surveillance in the persistence of minimal/measurable residual disease (MRD) and pathogenesis of relapse in adult B-cell acute lymphoblastic leukemia (B-ALL)

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Site of Ph.D. work: ACTREC (Hematopathology Laboratory and Dutt Laboratory)

Preferred education background of students: Life Sciences, Biotechnology, Medical etc)

Background: B-cell acute lymphoblastic leukemia (B-ALL) is the second commonest acute leukemia in adults with a 5-years disease-free survival of only ~40-45%. Of the many high-risk factors, the persistence of measurable/minimal residual disease (MRD) has been shown as the most important risk factor. Hence, the latest therapeutic focus is shifted to MRD-guided approaches including the addition of novel immunotherapies. While these approaches may improve clinical outcomes, they also add to the therapy-related toxicities and expenses, making them less affordable in resource-limited regions. Currently, there is no definitive way to identify MRD-positive patients who will benefit from the addition of immunotherapy. Interestingly, relapse of leukemia has been reported in only 50-60% of MRD-positive patients (not all MRD+ patients) indicating the role of other factors in the progression of MRD to relapse. Several studies have shown that weakened immune surveillance is a major factor responsible for recurrence/progression in solid malignancies, myeloid neoplasms, and B-cell NHLs.

Novelty: Currently, prospective studies highlighting the role of dysregulated immune surveillance in the persistence of MRD and pathogenesis of relapse in adult B-ALL are lacking. This project will study in-depth immune signatures including immune checkpoint protein (ICP) expression and T-cell functions, immune activating/inhibitory molecules in B-ALL blasts, and expression/mutation profile of the immune surveillance genes in B-ALL. We will study a relation between baseline immune perturbations and underlying high-risk genetic alterations. We also propose to study the kinetics of the dysregulated or exhausted immune subsets at multiple points and their relation to the persistence of MRD and the eventual relapse of leukemia.

Objective: To study the immune signature (immune checkpoint-protein expression and expression of immune-activatinginhibitory molecules) and immunogenetics (gene expression/mutation profile) in tumor cells at baseline, MRD timepoints, and relapse in adult B-ALL.

Methods: *Immune cell profile* (including immune checkpoint-protein expression/T-cell functions; at baseline, MRD & relapse) and expression of immune activating/inhibitory molecules in blasts (at baseline & relapse) will be studied using high-dimensional 16-color flow cytometry.
Immunogenetics: Gene expression/mutation profile of immune surveillance genes in tumor cells will be studied by transcriptome analysis at baseline, in FACS-sorted MRD-positive samples, and relapsed disease using RNA sequencing. Sequencing data will be analyzed using pipelines developed by Dr. Amit Dutt from Dutt laboratory in ACTREC.

Expected outcome and translatability: The results of this study will provide detailed immune-cell signatures and immunogenetics in adult B-ALL at baseline and kinetics of various immune-cell subsets at MRD intervals. The study will investigate the relation between immune-cell signatures and immunogenetics and MRD persistence in B-ALL. Additionally, transcriptome sequencing will help us study the trajectory of genetic aberrancies in baseline to MRD to relapse samples and detect novel genetic aberrancies in Indian patients.

Project 13A: Functional characterization of ethnic specific alterations in human cancer

PhD Guide (& Co-Guide): Pratik Chandrani, PhD (Assistant Professor and PI, Medical Oncology Molecular Lab and Centre for Computational Biology Bioinformatics, Crosstalk Lab) https://pratikchandrani.github.io/
Site of PhD work: ACTREC & TMH
Preferred education background of students: Life Sciences, Biotechnology

Background:

Given the perplexing diversity of cancer, investigation of a universal and effective therapeutic solution is a daunting task. Cancer is generally understood as a group of genetic diseases that result from changes in the genome of cells, leading them to grow uncontrollably. Most of these genetic changes are somatic and stochastic. Evolutionary selection pressure leads to a definitive selection of a few genetic alterations, aka "driver oncogenic alterations", against the background of vast majority of "passenger alterations" [1]. The driver alterations provide addiction to the cancer cells which makes them a therapeutic target as well. The types of oncogenes, incidence and response to targeted therapeutics are further affected by race, ethnicity, and lifestyle of world populations [2]. The targeted therapy has shown remarkable response in clinics for several classical oncogenes [3,4]. While a repertoire of targeted therapy option is available today, the real-world benefits to global populations are constrained due to several technological and practical limitations [5,6], with the apparent limitations in underdeveloped nations.

Broad work plan:

This 5-year PhD project will attempt to characterize the ethnic specific alterations obtained from about 500 tumour samples of the Indian origin cancer patients. PhD student will essentially validate key genomic alterations using orthologous techniques. Furthermore, we will take isogenic cell lines as an *in-vitro* model system. The isogenic cells will be used for cell growth assay, signalling pathway assays, and drug sensitivity assay [7] to determine the functional role of candidate genes. Altogether, this study will help us understand the genomic markers of cancer in Indian ethnicity and their functional biological role in the cancer.

Objectives:

1) Establishing molecular correlates of cancer related signalling pathways and phenotype
2) Establishing molecular correlates of ethnicity in cancer patients of Indian origin
3) *In-vitro/in-vivo* characterization of potential molecular alterations

Key references:

1 Meyerson, M., Gabriel, S. & Getz, G. Nat Rev Genet 11, 685-696, doi:10.1038/nrg2841 (2010).
2 Tan, D. S., Mok, T. S. & Rebbeck, T. R. J Clin Oncol 34, 91-101, doi:10.1200/JCO.2015.62.0096 (2016).
3 Sudhakar, A. J Cancer Sci Ther 1, 1-4, doi:10.4172/1948-5956.100000e2 (2009).
4 Chin, L., Andersen, J. N. & Futreal, P. A. Nature medicine 17, 297-303, doi:10.1038/nm.2323 (2011).
5 Hyman, D. M., Taylor, B. S. & Baselga, J. Cell 168, 584-599, doi:10.1016/j.cell.2016.12.015 (2017).
6 Marquart, J., Chen, E. Y. & Prasad, V. JAMA Oncol 4, 1093-1098, doi:10.1001/jamaoncol.2018.1660 (2018).
7 Chandrani, P. et al. Ann Oncol 28, 597-603, doi:10.1093/annonc/mdw636 (2017).

Project 13B: Integrative characterization of human cancer – a computational approach

PhD Guide (& Co-Guide): Pratik Chandrani, PhD (Assistant Professor and PI, Medical Oncology Molecular Lab and Centre for Computational Biology Bioinformatics, Crosstalk Lab) https://pratikchandrani.github.io/
Site of PhD work: ACTREC & TMH
Preferred education background: Life Sciences with knowledge in basic computational biology, programming

Background:

Cancer is a group of complex diseases that result from changes in the genome of the cells, leading them to grow uncontrollably. Most of these genetic changes are somatic (~ 85%) and stochastic in nature. However, evolutionary selection pressure leads to a definitive selection of a few genetic alterations, aka "driver alterations", against the background of vast majority of "passenger alterations" [1]. Driver genes providing selective growth advantage through activating mutations are identified as oncogenes while those providing selective growth advantage through inactivating mutations are identified as tumour suppressor genes. The types of oncogenes, incidence and response to therapeutics are further affected by a complex combination of multiple alterations [2]. To understand the multitude of these factors, proposed PhD candidate will take computational biology approach to study the complex interplay of molecular markers.

Broad work plan:

The PhD candidate will focus on development of computational program for analysis and normalization of heterogenous genomics data and *in-silico* cancer model development. Briefly, a repertoire of molecular markers will be derived from in-house and public genomics dataset. The molecular data will be systematically normalised and deposited in the form of a computational database. Furthermore, we will develop a computational model establishing the integrative network of cancer cells using computational/systems biology approach [3]. The network will be propagated to computationally simulate the impact of molecular changes on signalling pathways. Key molecular changes identified to be driver of network components related to tumorigenesis will be further validated [4]. Altogether, this study will help us develop a complex molecular signalling network which will be further used to simulate and identify key driver genes amongst the complex cancer related signalling pathways.

Objectives:

1) Establishing computational program to model cancer using multitude of molecular data
2) Determination of key molecular markers in the multi-dimensional model
3) *In-silico* characterization and validation of potential molecular alterations

Key references:

1 Meyerson, M., Gabriel, S. & Getz, G. Nat Rev Genet 11, 685-696, doi:10.1038/nrg2841 (2010).
2 Kuenzi, B. M. & Ideker, T. Nat Rev Cancer 20, 233-246, doi:10.1038/s41568-020-0240-7 (2020).
3 Chandrani, P. et al. BMC Genomics 16, 936, doi:10.1186/s12864-015-2138-4 (2015).
4 Chandrani, P. et al. Ann Oncol 28, 597-603, doi:10.1093/annonc/mdw636 (2017).

Project 14A: Longitudinal Follow up to determine the malignant transformation of oral leucoplakia and concordance of clinical diagnosis and the definitive histopathologic diagnosis for Risk Stratification for oral cancer risk assessment.

PhD Guide (& Co-Guide): Dr Sharmila Pimple MD, Professor , Department of Preventive Oncology, Centre for Cancer Epidemiology (CCE), Tata Memorial Centre. pimplesa@tmc.gov.in
Co-Guide : Dr Manoj Mahimkar PhD, Mahimkar Lab [Tobacco Carcinogenesis lab], CRI, ACTREC
Site of PhD work: TMH
Preferred education background of students: Life Science, Medical, Dental, Oral pathology

Background and important gaps in knowledge
Oral cancer has a well-documented long preclinical phase, starting with an oral pre-invasive lesion (OPL) those progresses from hyperplasia through the increasing degrees of dysplasia, and finally develops into invasive oral squamous cell carcinoma (OSCC). Leukoplakia is the most predominant pre-invasive lesion. Up to 67% of OSCC are preceded by oral leukoplakia (OLP) which often occur years before diagnosis of the invasive carcinoma. The early identification of OLP with a high risk of malignant transformation is therefore an important clinical issue. OSCC occur on the basis of OLP. Oral Leucoplakia have been reported with variable transformation rates and the ability to predict the malignant potential of these lesions based on histopathological data is limited. There is, to date, insufficient evidence concerning the malignant potential of oral leucoplakia. Exogenous factors such as Tobacco smoking and alcohol intake have been attributed as major risk factors contributing to the malignant transformation

Study objectives
1. To assess the persistence and/or regression of OLP at yearly intervals for 5 years
2. To determine the rate of malignant transformation and assess the risk factors associated with this transformation in the longitudinal follow up for 5 years.
3. Determine the concordance between the clinical diagnosis and the definitive histopathologic diagnosis for Risk Stratification

Wide range of molecular changes are also associated with progression of dysplasia to squamous cell carcinoma was found. As the oral carcinogenesis is a multifactorial process in which both environmental and genetic factors contribute in the disease development. Among the environmental risk factors use of tobacco is the majorly of the disease and limited information exists about comprehensive role of tobacco habits and molecular genetic alterations in the development of oral precancerous lesions which is another the aim of the present study.

Outline of workflow and methodology in brief
Methodology: Present study is cross sectional study of populations**.** Subjects recruited with clinically suspected OPLs from a clinic based oral cancer screening program among men and women in the age group from 18- 60 years in Mumbai, will be invited to participate in the study.
Biological sample collection: Oral Punch Biopsy: Biopsy specimen will be obtained from the subjects with OPLs

Follow-up: Patient will be prospectively followed up annually for average period of 5 years with. As a part of patient care at the time of follow up, additional biopsy will be taken from high risk suspicious lesions which have been persistent / newly formed after the treatment to rule out the disease progression.
Clinical, histopathological and risk factor data will be recorded at baseline. One of three clinical endpoints will be determined: malignant transformation, progression of dysplasia grade, remission/stable dysplasia grade.

Preliminary work done: Accrual of leukoplakia cases was initiated on 16th July 2018 after completing all the IEC related formalities; active accrual is ongoing in Preventive Oncology, TMH). Total of 405 tobacco users were screened and 304 OPL cases recruited. Initiated the blinded histopathological analysis of the OPLs as per the study plan.

The proposed study project objectives are part of the larger IRB approved project IEC Project No.268, ACTREC with Broad objectives to perform array based DNA methylation analysis and evaluate association of DNA methylation pattern with disease progression, clinicopathological parameters and clinical outcome in oral cancers.

The project is fully funded under Teri Fox Grants, Terry Fox Research International program

Project 15A: Establishing patient derived pre-clinical in-vitro and in-vivo models of endocrine hormone therapy sensitivity and resistance in breast cancer Preferred education background of students:

PhD Guide: Dr Sudeep Gupta, Professor, Medical Oncology, Tata Memorial Hospital, Mumbai, and Principle Investigator, Clinician Scientist Lab (Hypoxia & Clinical Genomics Lab), ACTREC

Site of PhD work: TMH & ACTREC. *This is a fully funded project supported by a DBT& Intramural grant. Prospective applicants can mail csl.actrec@gmail.com for any technical queries related to this project*

Preferred education background: Lifesciences, bioinformatics, bioanalytical, biomedical instrumentation

Background: ER/PR positive Breast Cancer remains the commonest subtype in India (~50-60%). Approx. 20-40% of all women diagnosed with this subtype eventually develop recurrent/metastatic disease. ~30% of metastatic patients have intrinsic resistance to selective estrogen receptor modulators (SERM) or aromatase inhibitors (AI) & up to 40% of patients receiving adjuvant endocrine therapy eventually develop recurrent/metastatic tumors. Significantly, most patients with ER+ metastatic breast cancer eventually develop resistance to endocrine hormone therapy (EHT). EHT resistant, HER2 -ve & ER &/or PR +ve breast cancer are thus responsible for a considerable fraction of deaths due to this disease. Thus, understanding the mechanisms of resistance & developing therapeutics for EHT resistant breast cancer is critical to improving patient survival. However, very few pre-clinical models accurately recapitulate this clinical phenotype. We therefore propose establishing Patient derived cell lines (PDC) and Patient derived xenografts (PDX) from HER2 -ve & ER &/or PR +ve Breast cancer patients who have relapsed on EHT within 2 year of therapy start. We propose using various biochemical & functional assays to characterize their biology. We will then subject these models and cells derived therefrom to Kinome-wide Crispr-Cas9 profiling to identify kinases involved in cellular dependency. Concurrently, these cells will also be subjected to a AYUSH formulation based drug screen to identify novel compounds that may ameliorate resistant to therapy. These pre-clinical (pre-treated and treated,) will then be subjected to whole genome, transcriptome, methylome & acetylation, copy number SNP profiling, & proteomic analysis to identify molecular pathways involved in response to therapy. We have published preliminary work in collaboration with a group at KIIT, Bhubaneswar, reporting the establishment of the first metastatic liver PDX model from an EHT patient in India (https://www.spandidos-publications.com/10.3892/or.2023.8536)

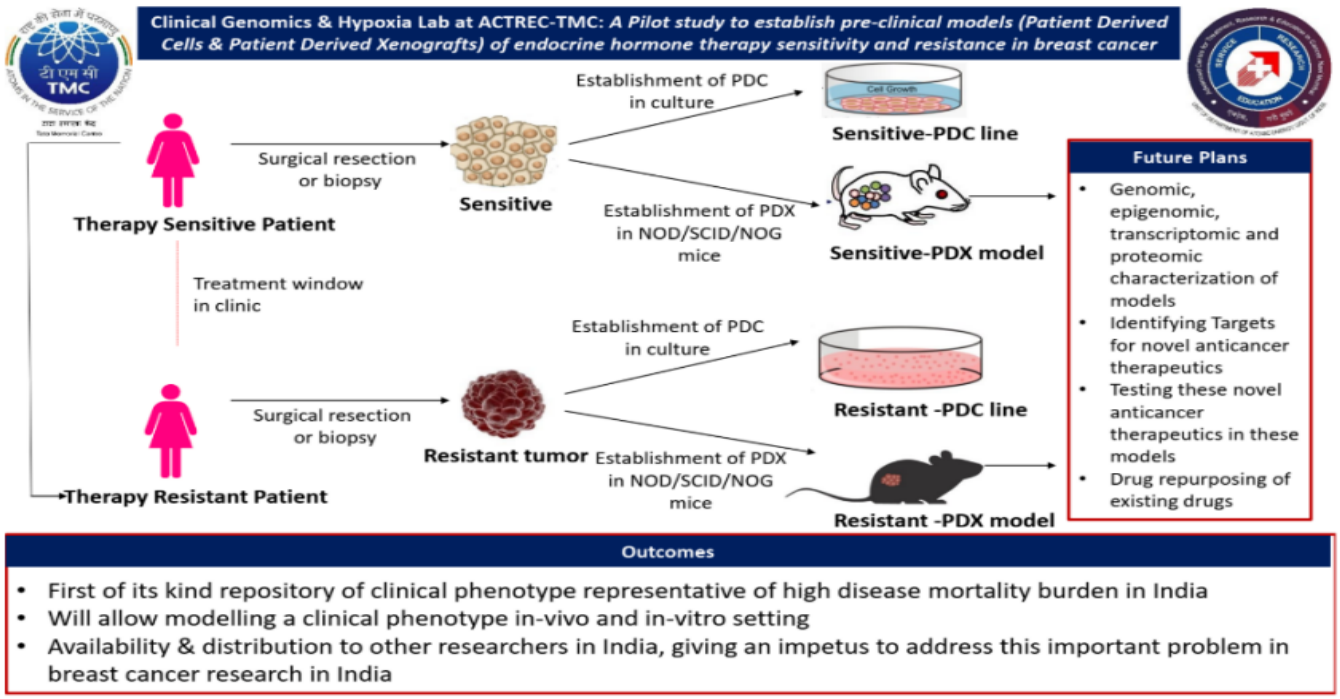
Objectives:

a. Establish models of Patient Derived Cells and Mice Xenografts from patientssensitive and resistant to endocrine hormone therapy

b. Subject these cell models to Kinome-wide Crispr-Cas9 profiling to establish kinases in cellular dependency

c. Drug screening using AYUSH based formulations

d. Subject treatment naïve and treated cells to Whole Genome Sequencing, Whole Transcriptome Sequencing, Whole Methylome Bisulfite Sequencing and Phospho-Proteome Profiling to identify molecular pathways responsible for resistance and drug response



Project 15B: Characterizing clonal evolution of endocrine hormone therapy resistant breast cancer

PhD Guide: Dr Sudeep Gupta, Professor, Medical Oncology, Tata Memorial Hospital, Mumbai, and Principle Investigator, Clinician Scientist Lab (Hypoxia & Clinical Genomics Lab), ACTREC

Site of PhD work: TMH & ACTREC. *This is a fully funded project supported by a DBT& Intramural grant. Prospective applicants can mail csl.actrec@gmail.com for any technical queries related to this project*

Preferred education background: Lifesciences, bioinformatics, bioanalytical, biomedical instrumentation

Background: ER/PR positive Breast Cancer remains the commonest subtype in India (~50-60%). Approximately 20-40% of all women diagnosed with this subtype eventually develop recurrent/metastatic disease. ~30% of metastatic patients have intrinsic resistance to selective estrogen receptor modulators (SERM) or aromatase inhibitors (AI) & up to 40% of patients receiving adjuvant endocrine therapy eventually develop recurrent/metastatic tumors. Significantly, most patients with ER+ metastatic breast cancer eventually develop resistance to endocrine hormone therapy (EHT). EHT resistant, HER2 -ve & ER &/or PR +ve breast cancer are thus responsible for a considerable fraction of deaths due to this disease. Thus, understanding the mechanisms of resistance & developing therapeutics for EHT resistant breast cancer is critical to improving patient survival. We propose to recruit patients in an ongoing multi-institutional study where we are the lead co-ordinators. We would then subject tumour samples before therapy and samples at resistance from the same patient to whole genome, transcriptome, methylome & acetylation, copy number SNP profiling, & proteomic analysis which can be co-related to the PDX and PDC cells established from these primary tumours. Such analysis of PDX models evolving over subsequent generations will allow us to study clonal evolution of tumours bereft of pressures of therapy, & modulate the disease trajectory with novel therapeutics in the in-vivo & in-vitro pre-clinical models.

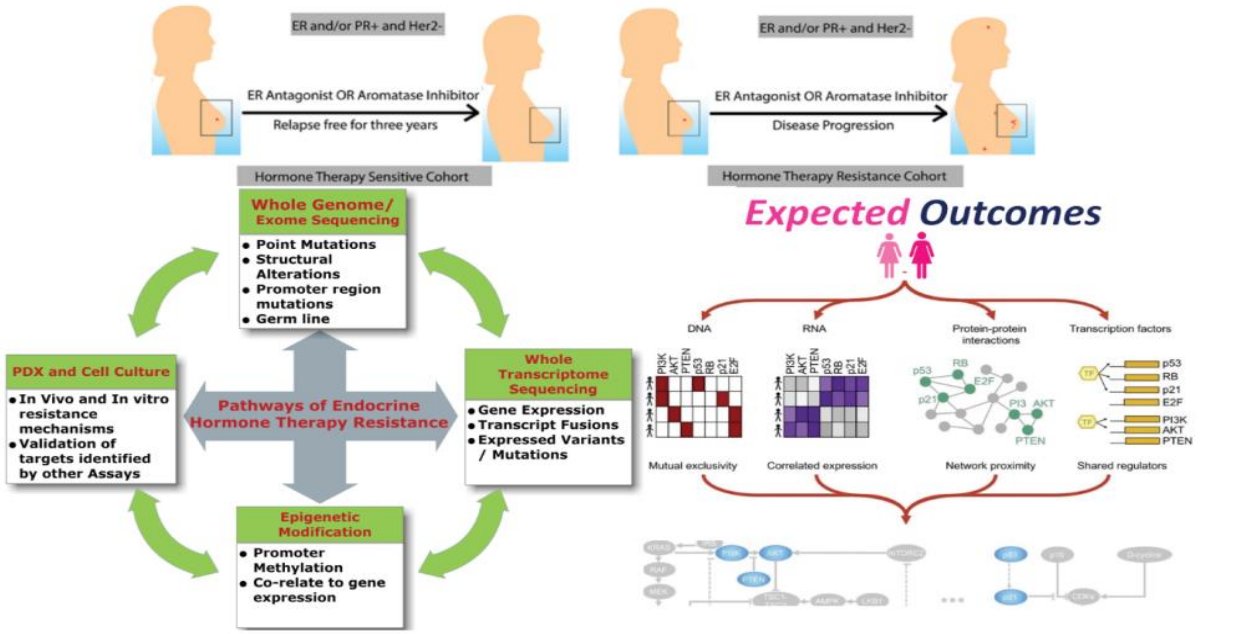
Objectives:

a. Subject tumours and tumour adjacent normal from endocrine hormone therapy sensitive patients at diagnosis to Whole Genome Sequencing, Whole Transcriptome Sequencing, Whole Methylome Bisulfite Sequencing and Phospho-Proteome Profiling (n= 30 patients)

b. Subject tumours and tumour adjacent normal from endocrine hormone therapy resistant patients at diagnosis and at relapse to Whole Genome Sequencing, Whole Transcriptome Sequencing, Whole Methylome Bisulfite Sequencing and Phospho-Proteome Profiling (n= 20 patients)

c. Subject corresponding PDC / multi generation PDX developed from resistant patients to Whole Genome Sequencing, Whole Transcriptome Sequencing, Whole Methylome Bisulfite Sequencing and Phospho□Proteome Profiling (n= 20)

d. Integrate data from primary tumours, PDC and PDX multigeneration models to Identify mechanisms of resistance that clonally evolve over time using statistical method



Project 15C: Identifying & characterizing dormant cellular subpopulations of human breast cancer cells

PhD Guide: Dr Sudeep Gupta, Professor, Medical Oncology, Tata Memorial Hospital, Mumbai, and Principle Investigator, Clinician Scientist Lab (Hypoxia & Clinical Genomics Lab), ACTREC

Site of PhD work: TMH & ACTREC. *This is a fully funded project supported by a DBT& Intramural grant. Prospective applicants can mail csl.actrec@gmail.com for any technical queries related to this project*

Preferred education background: Lifesciences, bioinformatics, bioanalytical, biomedical instrumentation

Cancer metastases is the main cause of death among breast cancer patients worldwide. Metastatic recurrence of these breast cancer cells at distant sites can even occur several years after eliminating the primary tumour. Late recurrence is a consequence of induction of prolonged quiescence in the disseminated tumour cells (DTCs). It is hypothesized that DTCs may lodge in a non-proliferative state at the site of metastasis, called cellular dormancy. Cellular dormancy is where predominantly single DTCs exist in a state of reversible growth arrest (G0/G1) controlled by the crosstalk between DTCs and the microenvironment. Not all cells that disseminate to the secondary organ are competent to colonize and proliferate into overt lesions. Literature suggests that extracellular matrix (ECM) plays a pivotal role in deciding the fate of DTCs during metastasis. The role of hypoxia signaling in the induction and maintenance of quiescence in the DTCs is relatively less studied. We aim to develop an understand the biology of an in-vitro model of breast cancer cells disseminated in the bone marrow which may help in facilitating a better understanding of the microenvironmental cues necessary for regulating dormancy.

Objectives a) Investigating the bone marrow disseminated tumour cell dormancy system to identify microenvironmental cues necessary for regulating dormancy. b) Investigation of dormancy associated regulatory genes. c) Characterization of culture conditions to generate a 3D in vitro model system for breast cancer cellular dormancy. d) Identification of dormancy regulatory gene specific agonist for suppressing metastasis.

Brief Methodology

a) Generation of 2D in vitro model system: Optimizing 2D the culture conditions for dormancy induction and escape in breast cancer cells under normoxic and hypoxia using various ECM proteins, growth factors, cytokines, etc. present in the tumor microenvironment.

b) Phenotypic characterization of dormant cells: Using various cell based and biochemical assays to establish phenotypic character of these cells

c) Genotypic characterization of dormant cells: Identifying Molecular pathways of dormancy using genome wide RNA-Seq based high throughput analysis, Immunofluorescence and molecular biology-based analysis to establish a robust profile of dormancy associated signature genes and their protein expressions.

d) Knockdown and reconstitution assays: Using crispr-cas9 based knockdown screens to identify genes that are responsible for cellular dependency of dormancy. Using cloning techniques to introduce these changes in a proliferating cell to evaluate if it induces dormancy.

A poster for this study from preliminary work conducted was presented at the Annual Meeting of the American Association for Cancer Research 2022 & the work was very well received https://aacrjournals.org/cancerres/article/83/7_Supplement/78/719560/Abstract-78-Microenvironmental-cues-that-regulate

Outcomes: It is expected that the successful candidate will have developed skillsets in a wide variety of biochemical and cell-based assays, microscopy and imaging, and Omics data generation and analysis

Project 16A: Evaluation of the role of the immune cell profile in the persistence of minimal residual disease and dynamics of the relapse in childhood T-cell lymphoblastic leukemia: a prospective observational study.

PhD Guide: Dr. Sumeet Gujral, MD, Professor of Pathology, TMH
Editor, WHO Hematolymphoid neoplasms classification 2022 Email ID: flowtmh@gmail.com Mob: 9820523962

Co-Guide: Dr. Prashant Tembhare, MD (Path), Clinician Scientist & Professor, Flow Cytometry & Hematopath, TMC, Email: docprt@gmail.com. ResearchGate: https://www.researchgate.net/profile/Prashant-Tembhare

Site of PhD work: ACTREC

Preferred education background of students: Life Sciences, Biotechnology, Medical etc

Background: T-cell acute lymphoblastic leukemia (ALL) is high-risk leukemia with a higher rate of measurable/minimal residual disease (MRD) positivity. MRD is a well-established source of disease relapse and poor survival. On other side, weakened immune-cell surveillance has been proven a major factor responsible for the recurrence/progression of cancer. However, the biological relationship between the weakened immune system and MRD positivity in T-ALL has not been studied. There is no data on the role of immune escape in the pathogenesis and progression of disease in T-ALL.

Novelty: Currently, there is no data that indicate the role of immune-cell profile in the pathogenesis of relapse of T-cell ALL. This project will first-time provide novel evidence on the association between immune exhaustion, disease immune escape and the progression of MRD to relapse in T-ALL.

Objective: To study an in-depth immune-cell profile in T-cell ALL patients at multiple time points during the disease course and to find the complex immune signature as well as the immunogenetic profile associated with relapse.

Methodology: *Immune profile*: A detailed quantitative and functional assessment of immune signature in peripheral blood and bone marrow samples will be performed with 80 immune markers including 8-immune checkpoint proteins using high-dimensional flow cytometry. It will study T-cell subsets (naïve/memory, effector/central, activated/resting CD4+T and CD8+T, T-reg, Th1/Th2/Th9/Th17/Th22 cells, γδ-T-cells), NK cells, B-cell subsets, dendritic cells, monocytes and MDSC, etc. This includes the study of immune checkpoint proteins like PD-1, PDL-1, CTLA-4, Tim-3, lag3, OX40, OX40L etc. The study involves high-dimensional 16-color flow cytometry and cell sorting techniques.

Immunogenetics will be studied using transcriptome analysis (gene expression/mutation profile) using RNA sequencing in a representative cohort.

Expected outcome and translatability:

- This study will provide in-depth data on the immune signature in childhood T-ALL. It will provide biological insight into the association between levels of immune signature and relapse of leukemia.
- This study will provide a novel biomarker for the prediction of relapse in MRD-positive T-ALL children.
- It will provide unique data which will provide a rationale for using immune therapy in T-ALL.

Project 17A: Systemic and Tumor Immune Response during pelvic radiation and /or brachytherapy for cervical cancer

PhD Guide (& Co-Guide): Dr Supriya Chopra, MD, DNB. Professor, Radiation Oncology, TMC
Google Scholar: H-Index: 29; i-10 index: 69 https://scholar.google.com/citations?user=zJS0QnUAAAAJ&hl=en
Pubmed: https://pubmed.ncbi.nlm.nih.gov/?term=supriya+chopra&sort=date
Expertscape: Rated as top 0.02% of world experts in cervical cancer. PI has been the international coordinator for largest collaborative project on understanding of impact of biomarkers for clinical outcomes of cervical cancer. (BIOEMBRACE) and has studied impact of stem cells and tumour heterogeneity on clinical outcomes.
Site of PhD work: TMH & ACTREC
Preferred education background of students: Life Sciences / Medical

Background: Multiple clinical trials of combination immunotherapy and radiation are ongoing for cervix cancer. However, the mechanism of immunotherapy and radiation interaction are not completely understood. Unlike other tumor sites, there are key components of gynecological radiation therapy that may lead to a very different immune response from other body sites. Pelvic bones, lower lumbar and sacral vertebra that house 60% of bone marrow receive high doses of radiation during cervix cancer definitive radiation. In addition ileocecal junction, liver, spleen that are immune reservoirs receive very low dose spillage (especially when treating abdominopelvic targets). Pelvic radiotherapy may lead to lymphopenia and depletion of other key hematopoietic elements leading to immunosuppressive microenvironment impacting patients immune system for years after treatment and also possibly development of metastasis and relapse. Also different from other body sites is the additional use of brachytherapy (internal radiation) in cervical cancer where a high dose per fraction is prescribed to the periphery of the tumour (7-9 Gy) and even higher doses are received by a part of the target (14-18 Gy in 1 sitting over few minutes).

The impact of such different doses on tumour and systemic microenvironment needs further investigation. Through this research proposal we intend to undertake a systematic investigation of immune response during the course of radiotherapy. With available multi time point tissue, blood samples, PET imaging and radiation dosimetry and longitudinal clinical follow up a serial investigation of impact of external radiation and brachytherapy on immune milieu is envisaged.

Study objectives, scientific relevance, novelty and likely impact: The study is novel and the topic is not previously studied within domain of gynecological malignancies and will provide new information about impact of radiation on immune microenvironment and tumour response. We envisage development of immune reservoir sparing techniques from information gained through this protocol and better understanding of sequencing of immunotherapy for gynecological cancers.

Objectives
1. To evaluate the impact and kinetics of lymphocyte depletion in reference to field size, radiation fraction, prescription dose and irradiated marrow dose.
2. To evaluate changes in pelvic and systemic immune reservoirs through paired FDG and FLT PET scans in patients undergoing pelvic radiotherapy.
3.To investigate the impact of brachytherapy dose, heterogenous volume, and technique on tumoral immune response.

Outline of workflow and methodology in brief: The study will involve using the available patient tissue material, blood samples, images in repository, clinical outcome reports to execute objective 1-3. The work will involve investigation of blood lymphocytes, circulating blood volume, immunohistochemistry for CD4, CD8, PDL-1 expression, radiation doses to immune reservoirs and their correlation with clinical outcomes.

Project 17B: Developing normal tissue complication probability (NTCP) models for prediction of individual or clustered late adverse events in patients undergoing radiotherapy for cervical cancers for selection of patients for proton therapy.

PhD Guide (& Co-Guide): Dr Supriya Chopra, MD, DNB. Professor, Radiation Oncology, TMC
Google Scholar: H-Index: 29; i-10 index: 69 https://scholar.google.com/citations?user=zJS0QnUAAAAJ&hl=en
Pubmed: https://pubmed.ncbi.nlm.nih.gov/?term=supriya+chopra&sort=date
Expertscape: Rated as top 0.02% of world experts in cervical cancer. PI is a recognized international expert who has worked towards reducing late effects in cancer
Site of PhD work: TMH & ACTREC
Preferred education background of students: Medical Physics/ Computing/ Statistics/ Life Sciences. Previous experience in data analytics is desirable.

Background: Radiation treatment delivery for solid tumours has relied on using broad guidelines about tolerance of normal organs. For any normal organ system the radiation tolerance doses for various adverse events need large scale validation. This becomes more relevant as the use of advanced techniques is increasing and there is evolving rationale to utilise high precision techniques for treatment execution (e.g protons). However, before moving ahead with proton treatments it will be important to define these normal tissue complication probability using photon IMRT techniques that will serve as reference for future work.

PI has access to structured RT DICOM, dosimetric and clinical databases of patients treated in the last 10 years within clinical trials for cervix cancer. Furthermore, all additional information that may be needed to compute risk of toxicity (age, body mass index, presence of comorbidities, tobacco use, previous surgical history, dose of chemotherapy and radiation dose maps (dose volume histograms and actual dose data) is available. Also in a very structured method, there is access to clinically significant adverse effects not only in classical method (CTCAE) but also as numerical multiorgan summated mathematical score (MOSES). (Chopra JCO 2021, Ranjan Lancet E Clin Medicine 2022). This available data can be used to construct patient outcome prediction models. NTCP modelling hinges on combining external radiation and brachytherapy doses (dosimetric data) and in first step correlating with available adverse event data or symptom score. In parallel we intend to integrate other parameters (patient and treatment related) and proceed also to symptom cluster analysis. Such NTCP plots when developed will help not only in risk prediction but also prospectively identifying patients who may benefit from advanced beams (protons).

Study objectives

Aim 1 : *To use existing databases and radiation dose maps of patient treatment parameters and adverse events for developing normal tissue complication plots.*

Aim 2: *To develop predictive models to assist for patient selection for Proton Treatment for gynecological cancers.*

Scientific Relevance

Gynecological cancers are highly curable. However, due to high doses of radiation used patients can have significant burden of side effects. These side effects can be reduced by selecting appropriate treatments. However, such prediction and selection models are lacking in gynecological literature. As PI has access to structured longitudinal data Tata Memorial Centre is in a unique position to create such prediction models that will be of use to international community as well.

Preliminary work: PI has worked on standard predictive models and NTCP models is the next step. This is a part of broader work to develop prediction models. The work of the selected student will be related to listed aims.