



MAZUMDAR SHAW MEDICAL FOUNDATION

Annual Report 2022-23



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From the Managing Director's Desk



Mazumdar Shaw Medical Foundation (MSMF) is established to provide philanthropic support, medical outreach and translational research. Our goal is to make advanced healthcare accessible and cutting-edge Medical Science applications affordable. MSMF with Narayana Health has established a unique hospital-based ecosystem for bringing our shared vision to fruition.

Medical science has had the jurisdiction almost entirely for healthcare until now and has made remarkable progress possible. However, there is increasing recognition that physician-clinic-hospital centric healthcare must now make way for a broader Personal-Community- Physician- Hospital model of technology led healthcare. This shift mandates an approach that creates a culture of shared ideation between engineering and medical sciences. The expertise from disease biology encompassing genomic and molecular research, big data analysis, clinical research, must be now seamlessly integrated through advanced technology to socioeconomic factors to provide novel solutions of future healthcare.

With this in mind MSMF has created an ecosystem for guided innovation at multiple levels. Mazumdar Shaw Centre for Translational Research (MSCTR), Mazumdar Shaw Technology Business Incubator (MSMF-TBI) and the Mazumdar Shaw Cancer Outreach program (MSCOP) with Narayana Health- Mazumdar Shaw Medical Centre (MSMC) constitutes an eclectic, scientific network that provides an optimal setting for novel ideation, entrepreneurship and fast track bench-side discoveries to bedside applications and smart solutions.

Healthcare and the practice of medicine in recent times demands more than addressing the physiological and/anatomical anomalies. The knowledge base that includes the underlying molecular basis of disease conditions and its customization according to the genetic framework of each individual, can be leveraged to develop mandatory adjuncts that can enable precision medicine.

Application of advanced technologies for big data analysis, both molecular and clinic-pathological, is now a fast-advancing approach that can intelligently optimize existing information to obtain accurate diagnostics/prognostics. Finally, innovation is the primary strategy that can enable the translation of research findings to healthcare solutions. Fostering entrepreneurship in a healthcare set up will ensure a synergism with the clinicians fastening the process of deriving smart solutions. Given that medicine today demands a comprehensive understanding of all the various stakeholders, the Mazumdar Shaw Medical Foundation has a focus on integrative medicine that includes developing the knowledgebase, and encouraging innovation for its optimal application towards value addition in healthcare.

It is my view that we are embarking on one of the most exciting enterprises where clinicians, scientists and entrepreneurs can work under one roof for addressing healthcare issues by bringing in new tools and strategies in the fast-changing scenario of clinical practice.

Dr Paul C Salins

Managing Director, MSMF



Achievements

PhD Degree Awarded

PhD awarded to **Dr Sumsum Sunny** from Manipal Academy of Higher Education (MAHE) on the topic “*Molecular cytology of oral potentially malignant and malignant lesions*” under the guidance of Dr Amritha Suresh. Dr Sunny is the first Clinician-PhD to pass out of MSMF.

PhD awarded to **Nehanjali Dwivedi** from Manipal Academy of Higher Education (MAHE) on the topic “*Clinical Potential of LCN2 in Cancer Immunotherapy*” under the guidance of Dr Manjula Das.

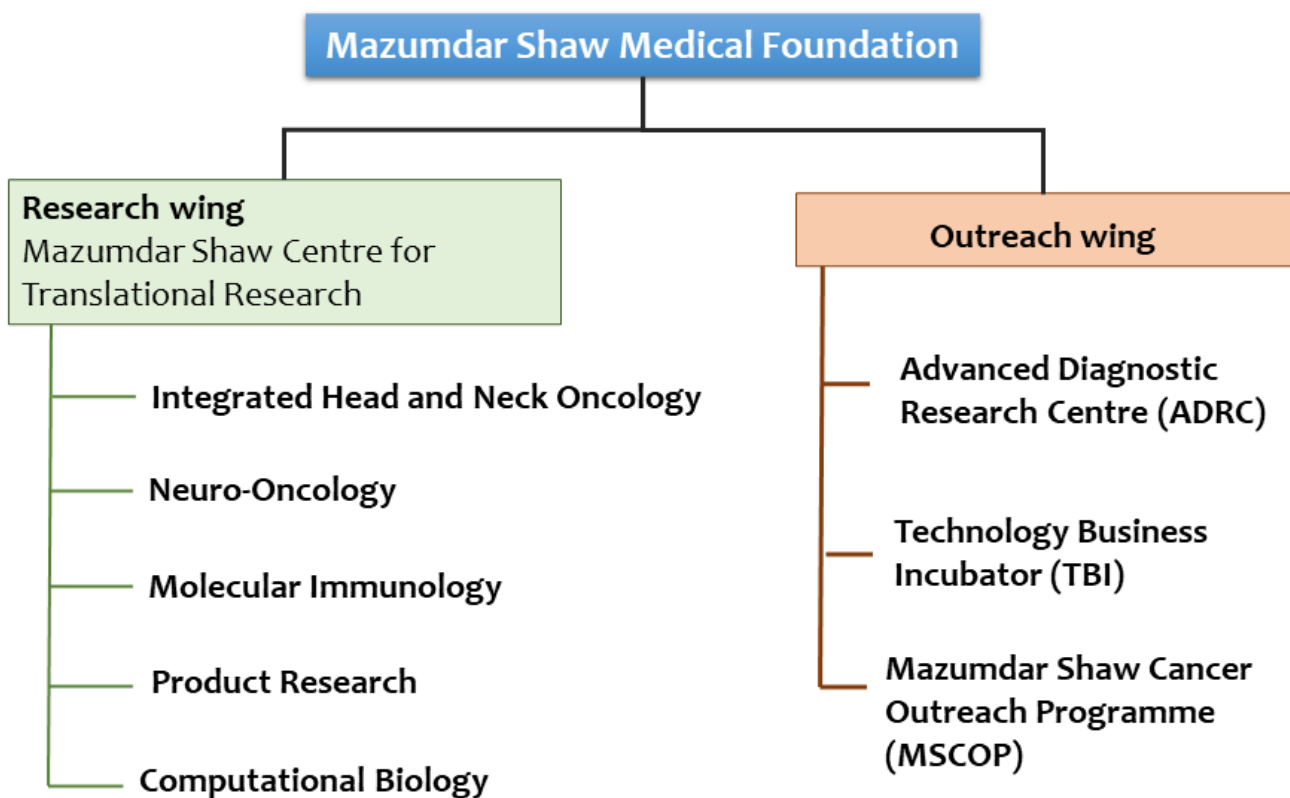
Conferences

Dr Sumsum Sunny

- Molecular markers in the detection of oral malignant and potentially malignant disorders: A systematic review, meta-analysis and validation (Narendra Desai Award for Best Basic Science Research).
- Detection of aberrant glycosylation for delineation of malignant thyroid nodules (First prize in poster presentation).

MSMF Structure

MSMF is organized into two broad wings. The research wing is called Mazumdar Shaw Centre for Translational Research comprising research programmes in multiple disease areas. The outreach wing of MSMF caters to broader society including the Advanced Diagnostic Research Center (ADRC), Technology Business Incubator (TBI) and Mazumdar Shaw Cancer Outreach Programme (MSCOP).



Integrated Head and Neck Oncology Program

The Integrated Head and Neck Oncology Program focuses on a multi-disciplinary approach towards addressing two grand challenges of down-staging oral cancer and the possibility of reversing treatment resistance in head and neck cancer. The team at MSCTR adopts a systems biology approach, exploring the cellular, molecular, biophysical and AI based parameters in tissues, cells and body fluids, such as saliva and blood. Our Cancer stem cell program explores the role of CSCs in the process of tumorigenesis, field cancerization, drug resistance and metastasis.

Given that over two-thirds of the patients with head and neck cancer present at advanced stages III/IV, with an overall survival rate of less than 20%, early detection is the key. Secondly, about 50% of all head and neck cancers recur after 'curative intent treatment'. As in the majority of solid tumors, once the disease recurs or develops distant metastasis, there are no curative treatment options. ***Screening and early detection, accurate prognostication, and reversing resistance is hence an immediate need.***

Research Highlights

- Novel dual-modality based imaging system for oral cancer screening/detection
- Biomarker database for detection of thyroid nodules
- Novel candidate markers for detection, targeting in head and neck cancer
- Biomarker database for nodal metastasis

Oral Cancer Control Program

The Oral Cancer Control Program in the group focused on two primary aspects i) advancing the technological innovations that can help in improving the surveillance and screening of oral cancer and ii) integrated omic profiling of oral potentially malignant lesions, and deconvolution of the immune landscape

Technological Innovations in screening and surveillance of Oral Cancer: Seventy percent of the oral cancers India are diagnosed in the advanced stages, resulting in poor prognosis. Eighty percentages of oral cancer arises from precursor lesions called Oral Potentially Malignant Disorders (OPMD) and *there is a need of minimally invasive and automated technique to detect the early cancer/high-grade OPMDs.*

We have performed a systematic review and meta-analysis (2000 to 2020) to evaluate the effectiveness of imaging techniques in detecting dysplastic-OPMDs and OSCC. Out of 529 articles evaluated for eligibility, 56 satisfied the pre-determined inclusion criteria, including 13 varying imaging techniques. Meta-analysis consisted 44 articles, wherein majority of the studies reported Autofluorescence (AFI-38.6%) followed by Chemiluminescence (CHEM), Narrow Band Imaging (NBI), Fluorescence Spectroscopy (FS), Diffuse Reflectance Spectroscopy (DRS), and 5aminolevulinic acid (5ALA). Higher sensitivities and specificities were obtained using FS (Sen:74%, Spe:96%, SAUC=0.98), DRS (Sen:79%, Spe:86%, SAUC = 0.91) and 5 ALA induced PPIX (Sen:91%, Spe:78%, SAUC = 0.98) in the detection of dysplastic OPMDs from non-dysplastic lesions(NDLs). AFI, FS, DRS, NBI showed higher sensitivities and SAUC (>90%) in differentiating OSCC1 (Figure 1).

AI integrated image-based method empowered the FHWs in early detection of oral cancer. We have developed an AFI-based dual Point-of-Care, AI-integrated, imaging device to assist FHWs in identifying high-risk oral lesions (Figure 2). This study was conducted to validate the accuracy of Convolutional Neural Network (CNN) enabled m(mobile) Health device deployed with FHWs for delineation of suspicious oral lesions (malignant/OPMDs). The effectiveness of the device was tested in tertiary care hospitals and low resource settings in India. The subjects were screened independently, either by FHWs alone or along with specialists. This device was validated on 5,025 participants and accurately delineated suspicious oral lesions using cloud-based AI (Figure 3:VGG19: sensitivity= 87%). This research was funded by NIH (2016-21) and received additional funding for commercialization (2021-2023).

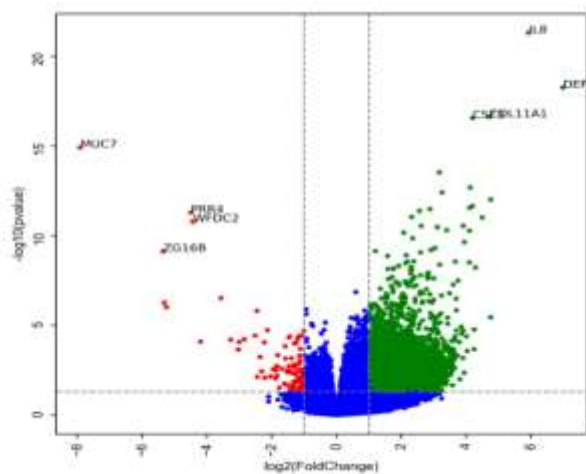
Molecular markers integrated with AI for delineation of high grade dysplastic and neoplastic oral lesions: We have improved oral cytology in the early detection by integrating molecular markers and AI. The marker based cytopathology images were segmented using U-Net (Figure 4) and features were extracted for developing classification model³. The study, supported by Wellcome-Trust DBT India Alliance (2016-19) and BIRAC (2019-21), used two-biomarker panel (CD44/SNA-1) integrated with clinical parameters or SNA-1 with automated image analysis (Sensitivity >85%) or multiplexed two-marker panel analysis (Sensitivity: >90%) provided efficient risk stratification of oral lesions (Figure 5). Currently we are developing a multimodal approach combining imaging and molecular cytopathology for accurate risk stratification of oral lesions and. We are developing automated molecular cytology scanner for augmenting the cytology technique into PoC method in a community (funded by ICMR 2023).

Early detection of oral potentially malignant lesions (OPMLs) is mandatory for down-staging oral cancer and improving survival; understanding the genomic/transcriptomic landscape that governs the oral carcinogenic process is a necessary prerequisite for this process. The study helps to identify relevant candidate molecules in the progression of dysplasia grades and carcinogenesis which would

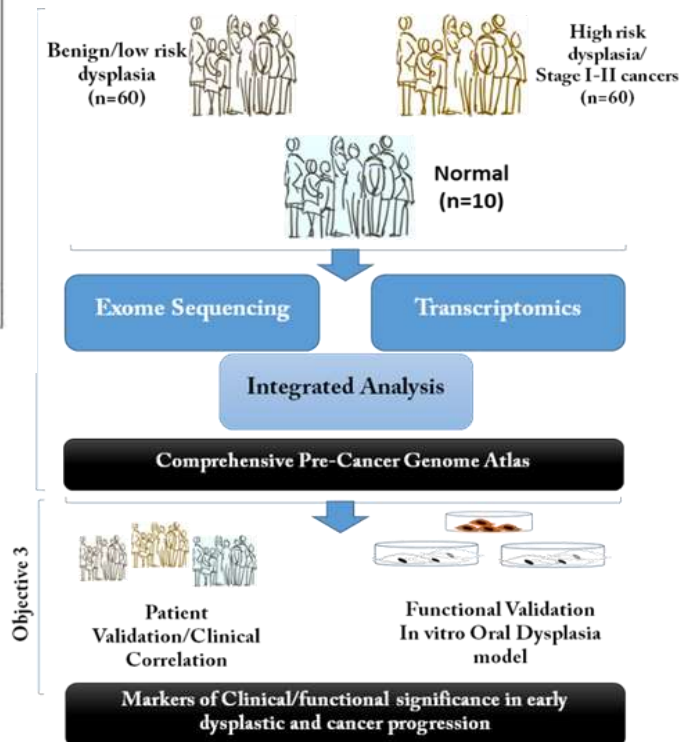
benefit the current population-based oral cancer screening initiatives, especially in distinguishing High-Grade Dysplasia (HGD) from benign/low-grade dysplastic (LGD) lesions.

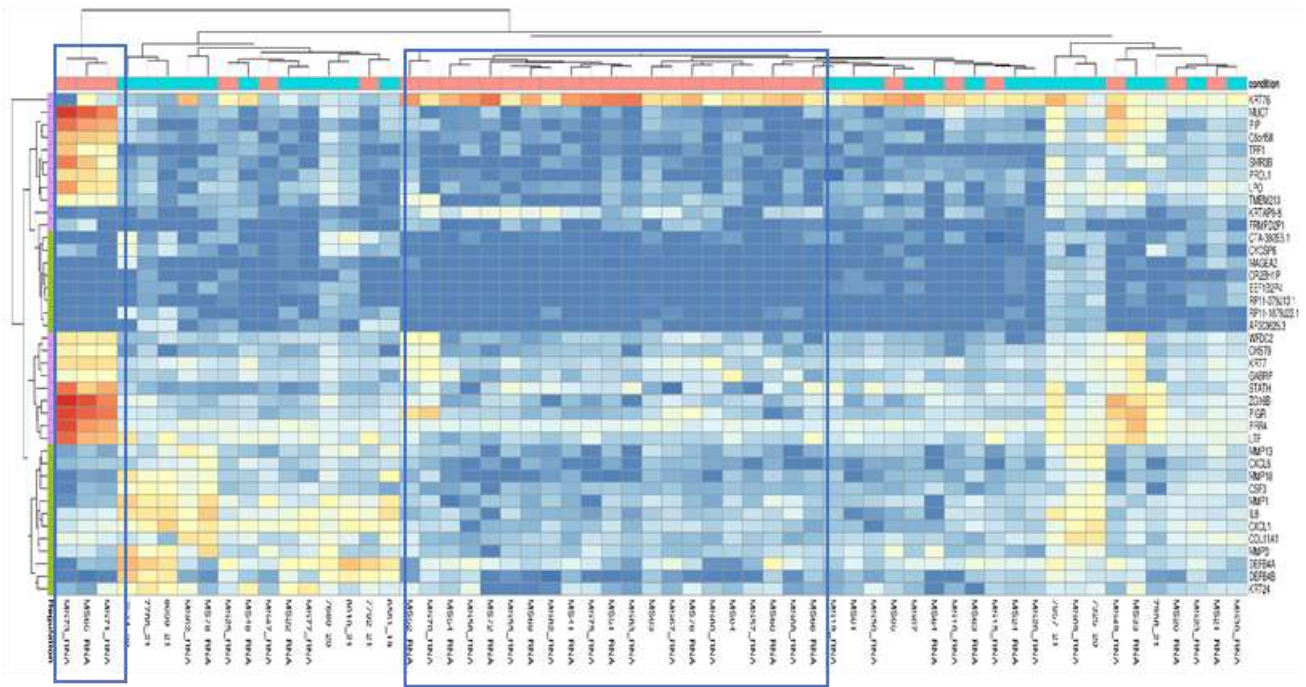
Exome sequencing and transcriptomics (pan-omics) were carried out on patient samples in the low-risk (benign, low-grade dysplasia) and high-risk (high-grade dysplasia) cohorts. The study has completed 105 RNA and 81 DNA samples' sequencing and currently, the analysis of 59 RNA samples (High-Risk; n=33 vs Low-Risk; n=26) has been done to identify the differentially expressed genes (DEGs) and enriched pathways with respect to carcinogenesis. The analysis using a Random Effect Model method to obtain a p-Value and Fold Change for each gene identified 9545 known protein-coding genes as DEGs of which 9449 were upregulated and 96 genes were downregulated. Also, the assessment of the top significant genes indicated the enrichment of genes involved in the pathways:

IL-36 pathway, GPCR ligand binding, IFNA signalling, collagen degradation, and degradation



of ECM. The analysis is currently being expanded to a larger cohort of samples to identify the potential candidate markers





Cellular/ Molecular Prognostication of Head and Neck Cancer

In oral cancer, lymph node metastasis (LNM) is the adverse prognosticator, the 5-year survival rates drop drastically to 30-59% on involvement of nodes and hence it plays a crucial role in treatment decisions. Despite ample studies on LNM with regard to their clinical relevance, a deeper understanding of the mechanistic process, in terms of tumor-lymph node microenvironment that facilitates the cross-talk between lymph node stromal cells (LNSC) and tumor leading to metastasis remains to be explored in oral cancer. Lack of *in vitro* models could be one of the reasons. We are striving to develop *in-vitro* models vital in studying the tumor-LNSC interactions. Fourteen lymph node stromal cells (LNSCs) from metastatic/non-metastatic oral cancer patients (N=7) have been cultured. Two LNSCs, each from non-metastatic, pre-metastatic and metastatic nodes, have been characterized by immunocytochemistry, flow cytometry, H&E staining, and short tandem repeat (STR) profiling. They were found to be heterogenous cell population consisting of fibroblastic reticular cells and double negative cells. The STR profiles of these cells matched perfectly to their parent tumor

tissues. Also, the unique STR profiles identified them to be novel cells. Currently, the comparative molecular profiling for these cells is ongoing.

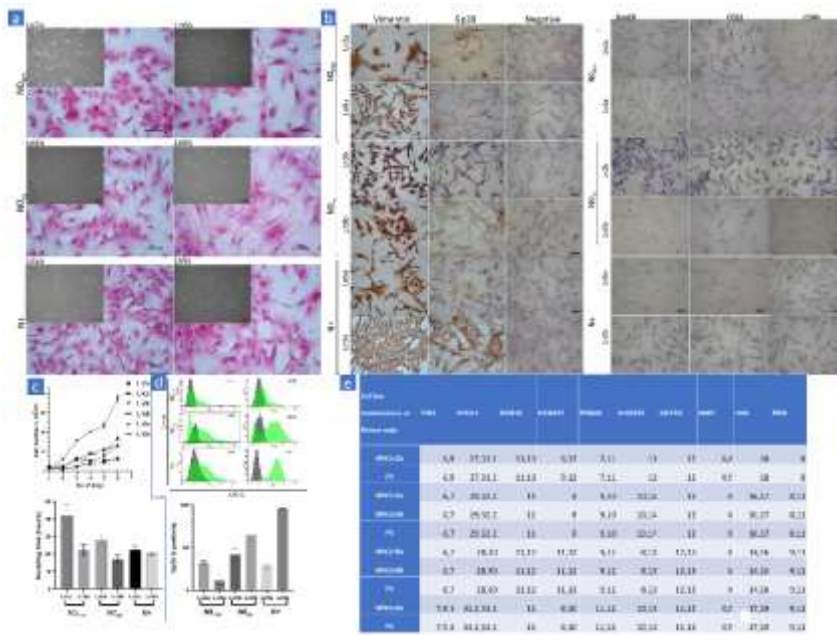


Figure 1: LNSCs development & characterization: (a)The lymph node explants LNSCs from the NO_{nm}(Ln2a, Ln6a), NO_m(Ln5b, Ln9a) N+(Ln5a, Ln9a) groups were cultured for approximately 4-6 weeks, trypsinized, expanded, and frozen at the early passages (passage 1-10). The H&E staining was done on cells cultured on coverslips overnight. The cells showed varied morphology with stellate, reticular and fusiform shaped cells. The images were taken at 10X magnification and the scale bar represents 100µm. (b) The primary cell lines, grouped into NO_{nm}, NO_m and N+, showed positive staining for Vimentin and Gp38. The immunocytochemistry shows lack of CD31, panCK and CD45 expression in NO_{nm}, NO_m and N+ cells indicating the absence of endothelial, epithelial and blood cells respectively. The negative control represents no antibody staining indicating lack of non-specific staining by the secondary detection system. The images were taken at 10X magnification and scale bar represents 100µm. (c) The growth kinetic curve for the cell lines grouped into NO_{nm}, NO_m and N+

showed an initial lag phase followed by exponential phase. The mean cell counts were used for plotting the curve. The doubling time was calculated for the NO_{nm}, NO_m & N+ group of cells for every 24 hours and the mean doubling time was plotted on the graph. (d)Flow cytometry-based detection of Gp38 expression indicated a heterogeneity in the GP38+ cells across the three groups; NO_{nm}, NO_m and N+. The mean percentage positivity was estimated and plotted. (e) STR profile were determined for these six cultures and their corresponding tumor tissues matched perfectly. Abbreviations: H&E: hematoxylin and eosin, LNSCs: Lymph node stromal cells, FRC: Fibroblastic reticular cells, NO_{nm}: Negative node from non-metastatic patient, NO_m: Negative node from metastatic patient, N+: Positive node from a metastatic patient, Ln2a, Ln6a, Ln5b, Ln9b, Ln5a, Ln9a represent the codes of the primary cell lines developed from corresponding patients P2, P6, P5 & P9.

Nodal metastasis is one of the most significant prognostic factor in oral squamous cell carcinoma; with reduced survival rates in patients with nodal metastasis (26-38%) as compared to 80% in early stage patients. Given that currently available imaging techniques have low accuracy to detect occult nodal metastasis or predict the susceptibility to developing metastasis, the use of a marker panel for nodal metastasis prediction/detection independently or as an adjunct to existing methods may prove beneficial. The present study aimed to identify predictive molecular markers in the primary tumor that can predict the occurrence of lymph node metastasis. We screened microarray datasets from ArrayExpress and GEO databases (overall 115 node-positive and 68 node-negative samples), and RNA-seq data from TCGA (146 node-positive and 112 node-negative samples). Differential gene expression (DGE) between the node-positive and node-negative categories in each dataset was enumerated using Limma (microarray) or Limma-Voom (RNA-seq) R packages. Random-Effect model-based gene expression meta-analysis using MetaVolcanoR was applied on individual datasets to obtain significant DGE ($p < 0.05$, Fold-change > 1.25). 82 differentials were identified, 19 genes were up regulated and 63 were down regulated (Figure 1). Further these differentials will be checked for their functional relevance and pathways involved in nodal metastasis. The significant genes will be validated in an in-house cohort of OSCC patients with and without lymph node metastasis (LNM) along with matched LN tissues with pathologic N0 or N+ stages. Significant gene signatures will be correlated with clinical and radiological parameters towards detection two primary outcomes i)

malignant disorders. *Sci Rep.* 2022 Aug 22; 12(1):14283. doi: 10.1038/s41598-022-18249-x. PMID: 35995987; PMCID: PMC9395355.

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4. Mendonca P, Sunny SP, Mohan U, Birur N P, Suresh A, Kuriakose MA. Non- invasive imaging of oral potentially malignant and malignant lesions: A systematic review and meta-analysis. *Oral Oncol.* 2022 Jul;30: 105877. doi: 10.1016/j.oraloncology.2022.105877. Epub 2022 May 23. PMID: 35617750.

Conferences:

American Head and Neck Society 2023 July 8th, Montreal Canada

- Generation of fluorescence images from White light mobile phone image using Cycle Consistent
- Adversarial Network for detection of potentially malignant oral lesions (paper).
- A step-wise risk stratification platform for early detection of oral cancer using artificial intelligence- A multimodal novel approach combining oral imaging and molecular cytology (paper).
- Artificial intelligence reinforced cell-based assay for detection of potentially malignant oral lesions: A Comparison of flow cytometry and immunocytochemistry (poster).
- Molecular Marker Based Approach in delineating Thyroid Nodules (poster).

Foundation of Head and Neck Oncology Nov 2-6, 2022, Guwahati, Assam

- A high-confidence biomarker panel for detection of occult lymph node metastasis in Oral Squamous Cell Carcinoma (OSCC)
- Tumor heterogeneity and nodal microenvironment facilitating extracapsular spread in the lymph node in oral cancer
- Flow cytometry-based analysis for risk stratification oral malignant and potentially malignant disorders (poster).
- Artificial intelligence based molecular cytology analysis- A step towards PoC diagnosis of oral cancer (poster)
- Molecular markers in the detection of oral malignant and potentially malignant disorders: A systematic review, meta-analysis and validation (Narendra Desai Award for Best Basic Science Research).

IFHNOS World Congress of the International Federation of Head and Neck Oncologic Societies 2023

- Molecular alterations underlying lymph node metastasis in oral cancer – a mass spectrometry-based approach

Patents

1. System comprising artificial intelligence integrated molecular cytology and radiology for triaging of thyroid nodules [#202341045806]
2. System for *in-vitro* modelling of nodal metastasis in oral squamous cell carcinoma [#202341042308]
3. 3D model for tumour microenvironment analysis [# 2022410474987]

Grants

1. Oral Potentially Malignant Lesion Atlas Project: Validating the efficacy of novel, Point-of-Care diagnostics and developing an integrated multidimensional, prognostic nomogram (ICMR; A multi-centric 5-year grant to develop PoC technologies for Oral Cancer Early Detection)
2. Triaging of indeterminate thyroid nodules combining radiomics, molecular cytology and mutational profiling - a multi-centric study (Endocrine Society of India)

Ongoing Grants

1. Multimodal intraoral imaging system for oral cancer detection and diagnosis in low resource setting (Collaboration project with Univ of Arizona, Narayana Health, NIH)
2. Mobile oral cancer screening system for low-resource settings (Collaboration project with Univ of Arizona, Narayana Health, SBIR grant, NIH)
3. Development of Comprehensive Pre-Cancer Genome/Transcriptome Atlas (CPCGA) of oral cavity, ICMR 2019-2023
4. Biomarkers for Nodal Metastasis in head and neck cancer DHR; 2018-2022
5. PARPCytometry- A quantitative and affordable diagnostic system for head and neck cancer diagnostics (In collaboration with CCRC, Kochi) GCE-BIRAC 2019-2020
6. Phase IIb/III study to determine efficacy of Curcumin and Metformin to reduce the incidence of second primary tumors of aero-digestive tract in patients with history of head and neck squamous cell carcinoma (In collaboration with Narayana Hrudayalaya Foundation, HNCOG) NCG 2018-2024

Team

Principal Investigator: Dr Amritha Suresh

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Intern: Prashasti Shetty, Sukanya, Sushmita,

NEURO-ONCOLOGY RESEARCH PROGRAM

The Neuro-Oncology Program focuses on the study of low and high-grade gliomas, mainly Glioblastoma (WHO Grade 4). Gliomas are one of the most common primary intracranial tumors in adults. Even though brain tumors constitute only 2% of total cancers, they are the second leading cause of death in young males (20-39 years) and the fifth leading cause of death in young females in India. They include different histological types (ependymomas, astrocytomas, and Oligodendro gliomas) and are of different grades – Grades 1, 2, 3, and 4. Grade 3 and Grade 4 (Glioblastoma or GBM) are high-grade tumors. Specifically, Glioblastoma is highly heterogeneous and most lethal of all with median survival in months. The characteristic glioblastoma histological anatomic features reflect specific biological processes, pathways, cell types, and microenvironments, all of which add to the tumor heterogeneity. Gliomas are further grouped into two large groups based on the presence or absence of a mutation in the enzyme, isocitrate dehydrogenase (IDH wild and mutant type) – the mutant having a better prognosis. Most of the GBMs are IDH wild type. The focus of the Neuro-Oncology Research Group is mainly on Glioblastoma – its tumor heterogeneity, microenvironment, and therapy-resistance at cellular, molecular, and histological levels using clinical specimens, established cell lines, tumor-derived cells, 3D culture system, multi-omics analysis, and computational approaches. The broad objectives of our group are described below.

1. Continue a deeper understanding of the pathophysiology of GBM in order to develop newer, clinically feasible biomarker assays for prognosis, treatment surveillance, and other application
2. Understanding tumor heterogeneity and therapy resistance and exploring newer therapeutic strategies including tumor-specific protein variants as targets or immunotherapy
3. Engineer 3D cell culture models with tumor-derived cells to mimic the glioblastoma microenvironment that best reflects the disease and is useful for studying new therapies.

The ongoing efforts, directed towards the above goals are briefly described below. These projects have been undertaken during this academic session.

1: IDENTIFICATION AND DEVELOPMENT OF A MOLECULAR PANEL OF POST-TREATMENT SURVEILLANCE BIOMARKERS BASED ON THE MESENCHYMAL PROPERTY OF GLIOBLASTOMA

Large-scale transcriptomic studies have identified molecular subtypes of GBM, namely proneural (PN), classical (CL), and mesenchymal (MES). Mesenchymal subtype which is one of the more prevalent subtypes has been found associated with more aggressive, invasive, angiogenic, hypoxic, necrotic, inflammatory, and multitherapy-resistant features with the worst prognosis. Although the median survival of GBM is short, there is a need to develop a panel for post-treatment surveillance biomarkers for better GBM management. Importantly, the MES transition of GBM is also associated with the recurrence. Therefore, one aim is to find out a potential panel of MES-associated proteins that can be used as post-treatment surveillance biomarkers.

Earlier we had reported our studies on the role of GPR56 in the MES transition in GBM (1). Starting with MES-associated genes observed in this study and integrating public domain and /or published information on molecules differentially expressed proteins, phosphoproteins, cytokines that are relevant to the MES state of the tumor and are also secretory in nature (Verhaak dataset, 2010; Neftel dataset, 2019; and Vasaikar dataset, 2021 for EMTome), we arrived at a set of over 20 non-redundant genes /proteins. These were further checked for detectability in plasma and are being investigated as post-treatment surveillance markers.

Currently, the top 12 proteins (based on their plasma levels) are taken forward to validate their RNA and protein level expression in GBM cells, tumors, and their appearance in pre-operative and post-operative plasma from GBM patients. Subsequently, we shall check these proteins in longitudinally collected patients' blood samples with follow-up.

2. IDENTIFICATION OF NEW TUMOUR-DERIVED IMMUNOSUPPRESSIVE FACTORS, IF ANY, TO DEVELOP TARGETTED INTERVENTIONS

Multiple cell types make up the tumor microenvironment. Numerous immune cell subsets have been seen in the glioblastoma microenvironment. However, immunosuppressive cells predominate. Gliomas are heavily infiltrated with immune cells of myeloid origin. Past studies have shown that high-grade gliomas have a higher proportion of alternatively activated and suppressive myeloid cells when compared to low-grade gliomas, which correlate with poor prognosis. Even a recent study from our group extensively characterized the myeloid cells from matched blood and GBM tissues (2). We found that CD86 and CD63 were the only cell-surface proteins whose expression levels correlated among myeloid cells in the blood and tumor, suggesting these markers in the blood may be used as prognostic markers for the progression of gliomas.

Further, the GBM microenvironment is comprised of immunomodulatory substances, such as prostaglandin E-2 (PGE2), interleukin 10 (IL-10), and transforming growth factor (TGF). TGF produced from the GBM cells promotes the change of tumor-infiltrating myeloid cells and microglia to an immunosuppressive phenotype, which promotes aggressive tumor growth and progression while suppressing anti-tumour immune responses. But more investigations are needed to explore GBM-derived immunosuppressive factors. There has been no successful immunotherapy for GBM until now.

We considered several publicly available datasets in GBM (secretome, exosome, membrane proteome, and matrisome) and overlapped them with the list of immune suppressive genes in the public database to get GBM-derived immunosuppressive factors. Further, we went ahead to check their secretory potential. After assessing their clinical significance such as survival association using public domain data, we arrived at a panel of 16 genes having strong indications as immunosuppressive factors. Currently, we are checking their mRNA expression in tumour-derived cells vs normal human

astrocytes. We are also detecting them in the conditioned media from tumor-derived cells using a mass spectrometry approach followed by testing their functional roles as immunosuppressive factors.

3. SPLICE VARIANTS AS BIOMARKERS AND TARGETS IN GLIOBLASTOMA

In the earlier report, we discussed the significance of spliced variants of proteins and other novel protein expressions in cells and tissues. Spliced variants, including aberrant forms, may be tissue or physiological or specific to pathological conditions such as malignancy, and may therefore serve as specific biomarkers of cancer. Alternative and/or aberrant splicing has been reported in several cancers including glioblastoma and has been implicated as an important strategy to attack this highly lethal tumor. Alternative splicing of known protein-coding genes and expression of noncoding sequences of the human genome are increasingly expanding the functional diversity of proteins. These events may be specific to cell type or physiological condition of the cells and may be deregulated in cancer.

In last year's annual report (2021-22), we discussed the in-house developed proteogenomic analysis pipeline, and using that pipeline we carried out an analysis of the breast cancer transcriptomic and proteomic data, using public domain CPTAC data, as a model study. We found and discussed some clinically significant leads (3). We have modified the pipeline to incorporate higher rigor and confidence in such identifications and are analyzing in-house generated transcriptomic and proteomics data on GBM. A number of protein variants and novel expressions have been identified. The biochemical and clinical significance of these molecules is under current investigation.

4. RNA BINDING PROTEINS ASSOCIATED WITH HYPOXIA AND CHEMO-RESPONSE IN GLIOBLASTOMA

RNA Binding proteins (RBPs) play a central role in regulating gene expression. More than 1500 RBPs have been discovered so far, accounting for around 7.5% of the protein-coding genes in the human genome. To create ribonucleoprotein (RNP) complexes, RBPs can bind with proteins and numerous kinds of RNAs, including mRNAs, ncRNAs, tRNAs, snRNAs, and snoRNAs. Several studies have linked certain RBPs to cancer, with altered expression and dysregulation of RBPs being linked to various features of carcinogenesis such as tumor growth, invasion, and metastasis. One of the important discoveries in RBPs is hnRNPA1/2 mediated alternative splicing of the pyruvate kinase M (PKM) gene which affects glycolysis and tumor progression. Our earlier studies have reported differential expression of a large number of hnRNPs in GBM as well as LGGs. Although RBPs like HuR, hnRNPs, MSI1, have been shown to play crucial roles in gliomas including GBM progression, migration, invasion, etc, it needs more investigation to find out novel RBP-mediated regulation in GBM.

In this current study, we are investigating RBP's association with tumor microenvironment such as hypoxia and temozolomide (TMZ) treatment response. Using public domain and in-house GBM-

specific multi-omics data, we selected 8 RBPs for the subsequent experiments. We checked their expression in the TCGA glioma dataset. 5 genes showed higher expression in GBM compared to both low-grade glioma and non-tumor samples. We checked their RNA expression in U251 (GBM cell line) and HA (normal human astrocyte cell line). PDIA4, ANG, and SERPINH1 were found to be upregulated in U251 compared to HA. We studied the effect of hypoxia and observed significant upregulation of PDIA4, FLNA, and HMGB2 in hypoxic conditions, indicating their hypoxia-responsive roles such as MES transition and presumably chemo-resistance. Next, in order to identify RBPs associated with TMZ treatment response, we monitored their expression in U251 (TMZ sensitive) and U87MG (TMZ resistant) cell lines after TMZ treatment. Interestingly, HMGB2 and SERPINH1 were exclusively found to be significantly upregulated in U251, whereas HERC5, FLNA, and ANXA2 were found in U87MG. Taken together, our result indicates a strong association between RBPs expression with hypoxia and TMZ treatment response in GBM and its clinical implications.

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2. Immuno-phenotyping of IDH-mutant grade 3 astrocytoma and IDH-wildtype glioblastoma reveals specific differences in cells of myeloid origin. Jayashree V. Raghavan, Raksha A. Ganesh, Pranali Sonpatki, Divya Naik, Arivusudar Everad John, Priyanka Arunachalam, Darshat Shah, Hari P. S., Akhila Lakshmikantha, Shibu Pillai, Komal Prasad Chandrachari, Kiran Mariswamappa, Sathyanarayana Lale, Nameeta Shah, and Siddharth Jhunjunwala. *Oncoimmunology* (2021) 10:e1957215
3. Proteogenomic Analysis of Breast Cancer Transcriptomic and Proteomic Data, Using De Novo Transcript Assembly: Genome-Wide Identification of Novel Peptides and Clinical Implications P. S. Hari, Lavanya Balakrishnan, Chaithanya Kotyada, Arivusudar Everad John, Shivani Tiwary, Nameeta Shah, and Ravi Sirdeshmukh *Mol Cell Proteomics* (2022) 21(4) 100220.

PATENTS / DELIVERABLES

- CNS cancer repository (Consented- 319 samples, Glioma: 200 samples)
- Panel of mesenchymal biomarkers for post-treatment surveillance of GBM
- Panel of GBM-derived immunosuppressive factors

Team

Principal Investigators: Dr Ravi Sirdeshmukh

Research Scientist: Dr Atanu Ghorai

Ph D Student: Raksha Ganesh

Project Staff: Gouri Raj, Gaurav Sahoo, Athira Kumar

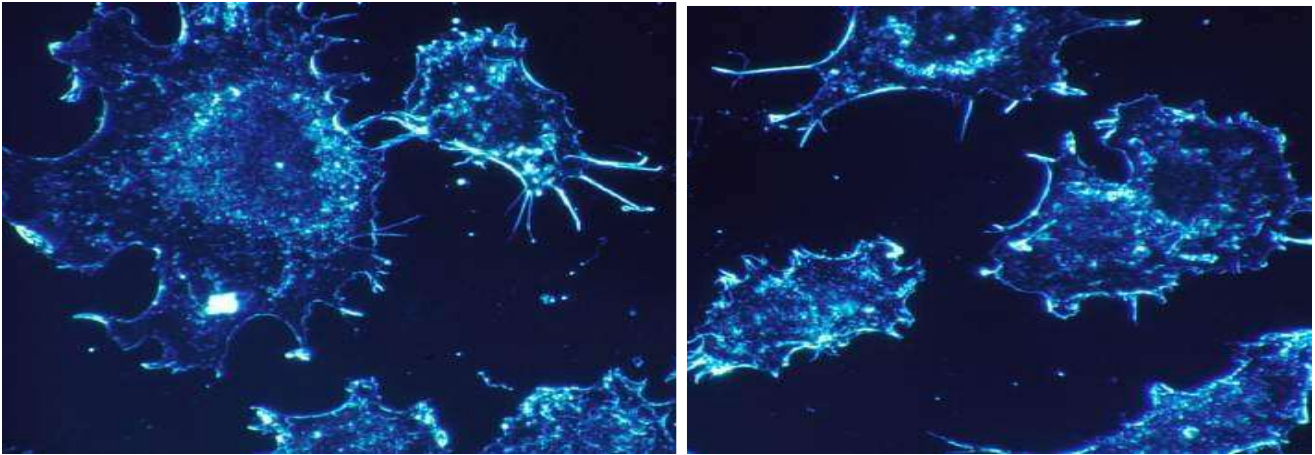
Intern: Suniti Ahuja

Clinical team at MSMC

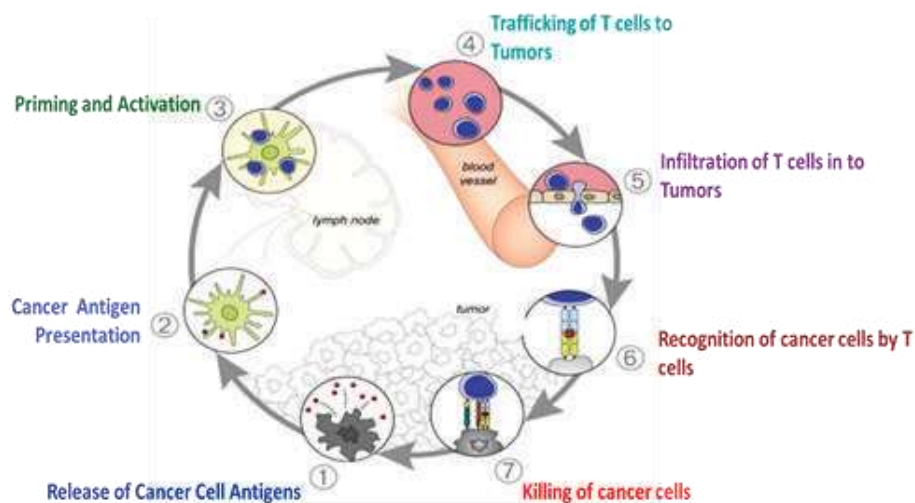
Department of Neurosurgery: Dr. Komal Prasad, Dr. Kiran M., Dr. Shibu Pillai, Dr. Sathyanarayana L D,

Department of Radiation Oncology: Dr. Akhila L., Department of Pathology; Dr. Saurabha Kumar

Molecular Immunology



Immuno Surveillance



Over the last two decades there has been a paradigm shift in our perception of cancer. As we know now, neoplasia contain an abundant and heterogeneous non-transformed component like stromal, endothelial and immune cells. The host immune system can recognize and react against (pre-) malignant cells as they transform, proliferate and evolve. Apoptotic pathways are among the methods of ‘reaction’ where the ‘bad’ cells are made to commit suicide.

The group concentrates on developing anti-cancer therapy by

1. Targeting the suppressors of apoptotic pathways among many suppressor pathways
2. Delineating the role of Cancer Associated Fibroblasts in cancer initiation and progression

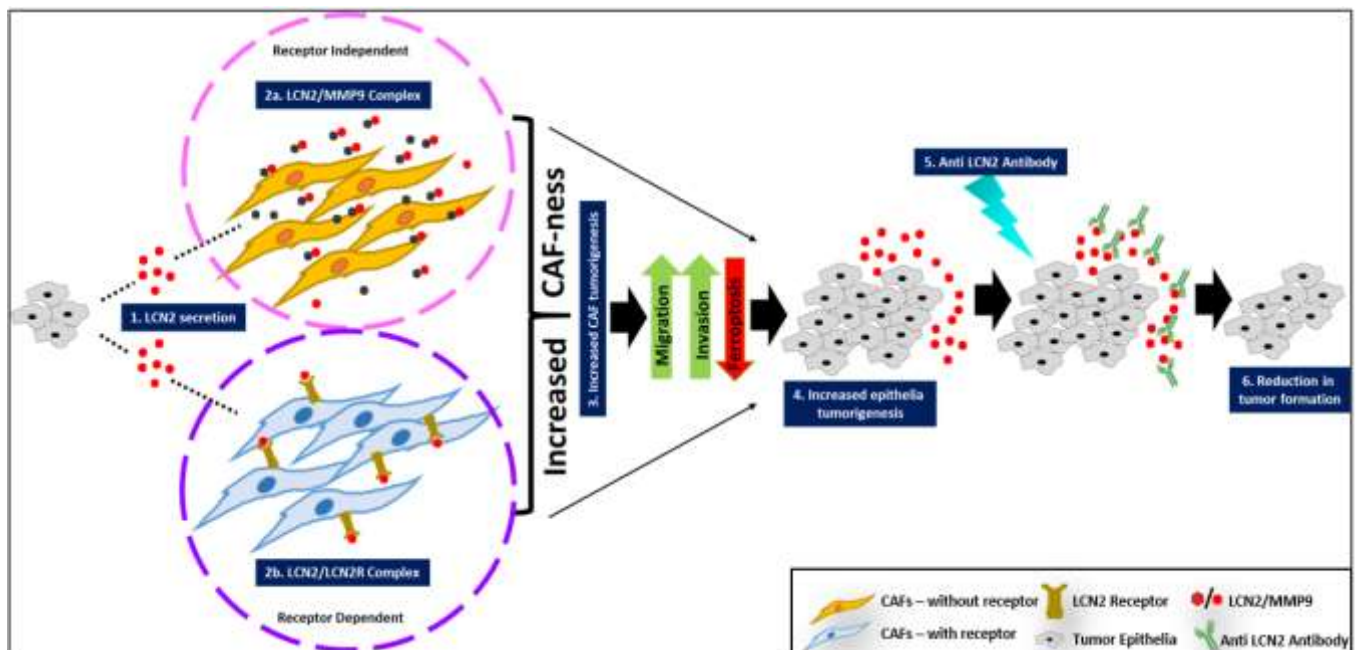
1. Targeted Suppression of Immune Evasion

Cancer cells evade immune surveillance by activating key suppressor molecules of the apoptotic pathways

LCN2: An important molecule in innate immune- pathway has been implicated in various cancers too. Role of LCN2 in inhibition of ferroptosis and therapy resistance through tumor-microenvironment crosstalk has been explored in the following neoplasia in our lab in collaboration with Dr Sorab Dalal (ACTREC) :

- (a) Head and Neck Cancer
- (b) Gastro-intestinal cancers
- (c) Cervical Cancer

i.



inhibition of ferroptosis and therapy resistance through tumor-microenvironment crosstalk has been explored in the following neoplasia in our lab in collaboration with Dr Sorab Dalal (ACTREC) :

- (d) Head and Neck Cancer
- (e) Gastro-intestinal cancers
- (f) Cervical Cancer

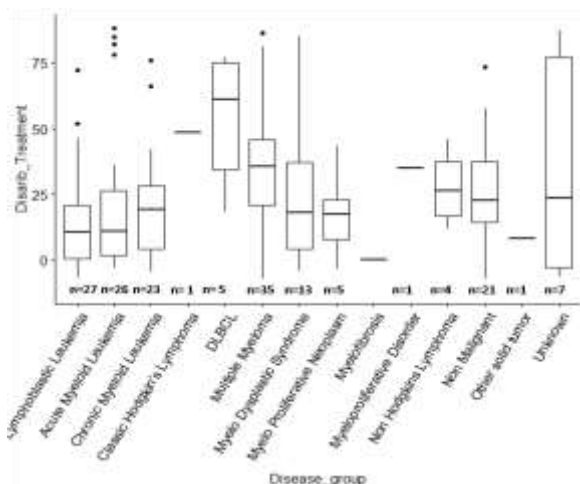
Nehanjali Dwivedi, obtained her PhD deciphering the molecular mechanism of action of LCN2 in Head and Neck and Cervical Cancer. She showed that:

- **LCN2 acts via CAFs to promote tumor progression in cancer.**
- **LCN2 can be a potential immunotherapeutic target (suppression of ferroptosis).**
- **Suggesting CAFness (Importance of CAFs)!**
- **Mouse antiLCN2 hybridoma generated in this project can be developed as a therapeutic MAb for tumor regression.**

Techniques employed are primary mammalian culture, recombinant protein, monoclonal antibody technology, NGS and Immunofluorescence.

- ii. **BCL2:** An anti-apoptotic molecule has emerged as one of the hottest targets in liquid cancer. However, the magic bullet, Venetoclax, often fails despite high expression of the target, ie BCL2. Is it because of co-expression of Myc1, MCL1 or BCL-X? We are trying to find out by checking the efficacy of BCL2inhibitors on patient derived ex-vivo cultures with our clinical collaborator, Dr Sharat Damodar.

Techniques employed are primary mammalian culture, FISH, qPCR and droplet digital PCR.



Publications

- a. Anju Joshi, Anil Vishnu G. K., Smitha P. K, Ashlesh Kadandale¹, Hari R. S, Bidipta Roy, Manjula Das and Hardik J. Pandya: Lab-on-PCB-based Electrical Immunosensing Platform for Point-of-Care Detection of SARS-CoV-2: IEEE Sensor Letters (2023 Jan): 7(1): 1-4: doi.org/10.1109/LSENS.2022.3228306
- b. P.K. Smitha, R.K. Shandil, Pushkarni Suresh, Kunal Biswas, G.R. Rudramurthy, C.N.Naveenkumar, K. Bharathkumar, Naga Puspha Battula, Suprabuddha Datta Chowdhury, Sakshi Sinha , Sarmistha Dutta, Sujan K. Dhar, Shridhar Narayanan, Manjula Das: ACE2-Fc : A promising therapy for SARS-CoV2 infection Medical Research Archives : (2022 Dec): 10(12): https://doi.org/10.18103/mra.v10i12.3322
- c. Gupta U, Ghosh S, Wallace CT, Shang P, Xin Y, Nair AP, Yazdankhah M, Strizhakova A, Ross MA, Liu H, Hose S, Stepicheva NA, Chowdhury O, Nemani M, Maddipatla V, Grebe R, Das M, Lathrop KL, Sahel JA, Zigler JS Jr, Qian J, Ghosh A, Sergeev Y, Handa JT, St Croix CM, Sinha D: Increased LCN2 (lipocalin 2) in the RPE decreases autophagy and activates inflammasome-ferroptosis processes in a mouse model of dry AMD. Autophagy. 2023 Jan;19(1):92-111. doi: 10.1080/15548627.2022.2062887
- d. Nehanjali Dwivedi, Charitha G, Vijay Pillai, Moni A Kuriakose, Amritha Suresh, Manjula Das: Establishment and characterization of novel autologous pair cell lines from two Indian non-habitual tongue carcinoma patients (2022-Sep)150. doi: 10.3892/or.2022.8362
- e. Nehanjali Dwivedi, Sujan K Dhar, Moni Abraham Kuriakose, Amritha Suresh, Manjula Das ; Reference Genes for gene expression analysis in Oral Cancer: a Data Science Driven Approach (2022) Dental Research and Oral Health: 5(2):21-37
- f. Divya CA, Dhar SK, Manjula Shantaram, Manjula Das: Comparison of Anti-diabetic, Anti-oxidant and Anti-lipogenesis activity of Holarrhena antidysenterica plant part: Medicinal Plant - International Journal of Phytomedicines and Related Industries(2022): 14: 240-250

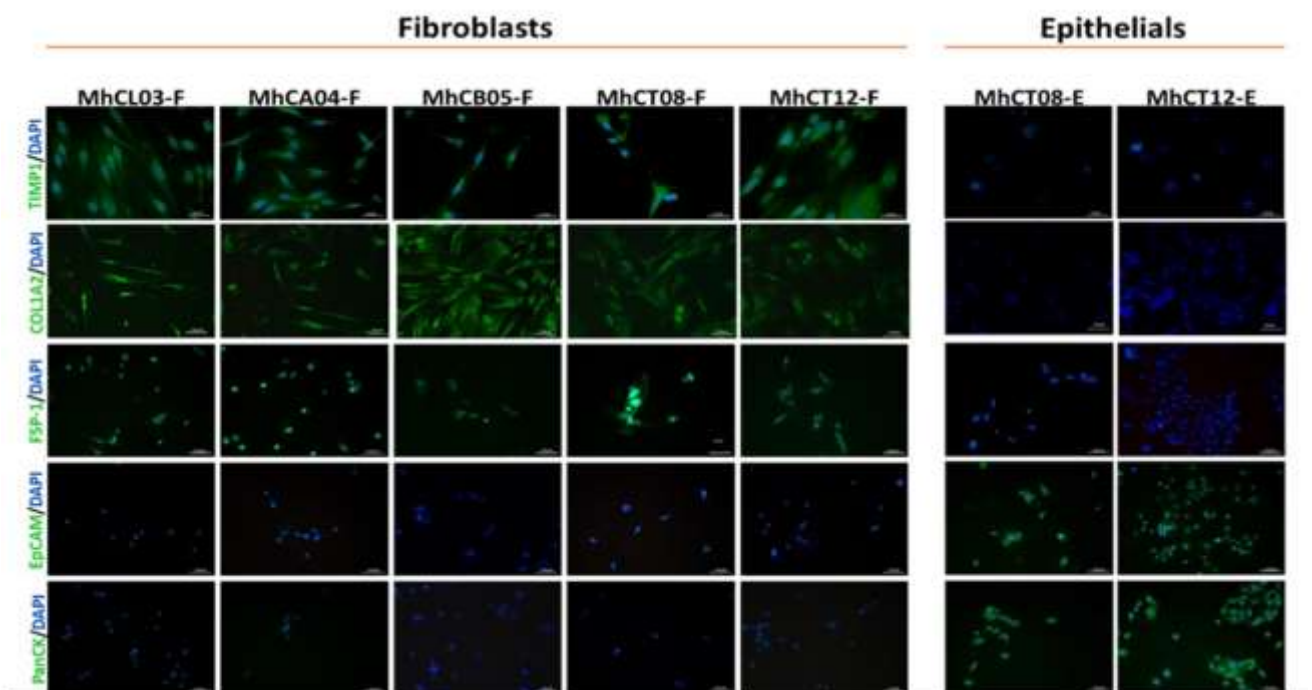
Grants

1. Novel Cov-trap therapy to reduce the intensity of the Covid-19 infection; BT/COVID0072/02/20; BIRAC; 61.74 L; 2020-21
2. Development of A Microfluidics Based Point-Of-Care Device For Intra-Operative Detection Of Metastatic Lymph Nodes In Oral Cancer; DST; 36L; 2019-2022
3. Exploring Novel BCL2-specific Inhibitors against Leukemia and Lymphoma ,DBT; 123L; 2018-2022
4. Biodesign Bioengineering Initiative Phase II (Towards Deciphering the Interaction between Diabetes and Cancer); DBT; 52L; 2018-2022
5. Deciphering the tumor immune heterogeneity of Head and Neck Squamous Cell Carcinoma (HNSCC) in Indian patient population: A pilot Study; BMS; 26L 2016-2018

2. Role of CAFs in tumor initiation and progression

Cross talk between Cancer associated fibroblasts (CAFs) and the tumors are responsible for tumor initiation and progression

We have stabilized several patient-derived epithelial and CAF lines. Exploring the effect of each other, on each other we are in pursuit of the ‘transforming principle’ of CAFs and tumor cells in solid tumors.



Team

Principal Investigator: Dr Manjula Das

Members: Dr Smitha PK, Pushkarni Suresh, Kunal Biswas, Suprabuddha Dutta, B Naga Pushpa

Clinical Collaborators: Dr Rammohan Bhat (NH), Dr RK Prasad (PCMH Restore Health)

Collaboration: Dr. Sharat Damodar, Dr Vijay Pillai, Dr Moni A Kuriakose, Dr Vivek Shetty (MSMC, NH), Dr Sorab Dalal (ACTREC), Dr Sujan K Dhar, Dr Amritha Suresh, Dr Smitha P K (MSMF)

Product Research Group

FOCUS OF PRODUCT RESEARCH GROUP: The focus of this group is to translate potential diagnostic and therapeutic molecules generated as research outcome from the discovery groups (Head and Neck oncology/ Molecular Immunology/Neuro oncology) at MSMF or a product initiated from the product research group. This group was formed in September 2021 and officially started functioning from October 2021.

Group structure: Head of the product research group with two Junior Research Fellows.

Completed projects

- Establishment of three novel CAF cell lines from HNSCC patients:** Project initiated from Molecular Immunology group (Collaborator: Dr. Manjula Das).
AIM: To characterize the cancer associated fibroblast cell lines (MhCL03-F, MhCA04-F and MhCB05-F) isolated from HNSCC patients.

Abstract of the project: In this study, three novel CAF lines, spontaneously immortalized, from Human Papilloma Virus (HPV) negative male patients with habits of tobacco and diagnosed with squamous cell carcinoma of the upper alveolus, larynx and buccal mucosa were characterized for their tumorigenic properties. Negative staining with EpCAM, CD31 and CD45, while positive staining with FSP-1 determined their fibroblast specific lineage. Indirect co-culture experiments with the conditioned media collected from all three CAFs could increase the tumorigenicity of the epithelial cells from tongue. The developed CAF cell lines can act as invaluable tools for learning the site-independent common language between tumor-stroma and tumor in HNSCC.

Results:

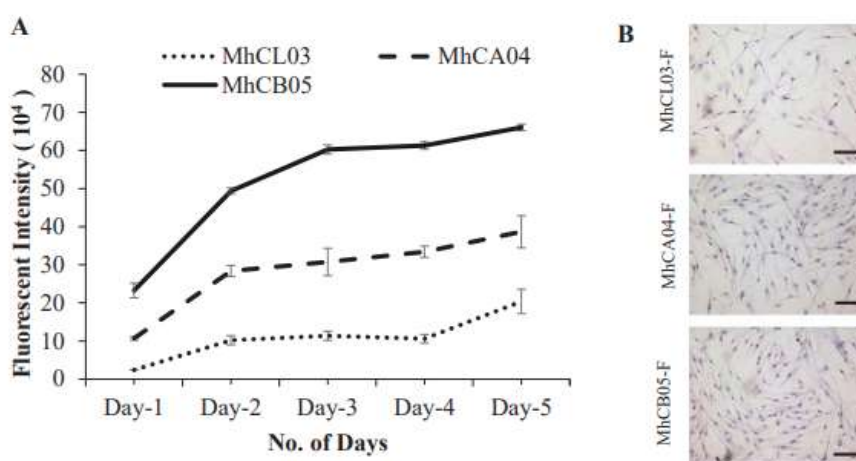


Fig 1A: Growth pattern of MhCL03, MhCA04, and MhCB05 cell lines, B: Microscopic images of Hematoxylin stained cells exhibiting spindle shaped morphology

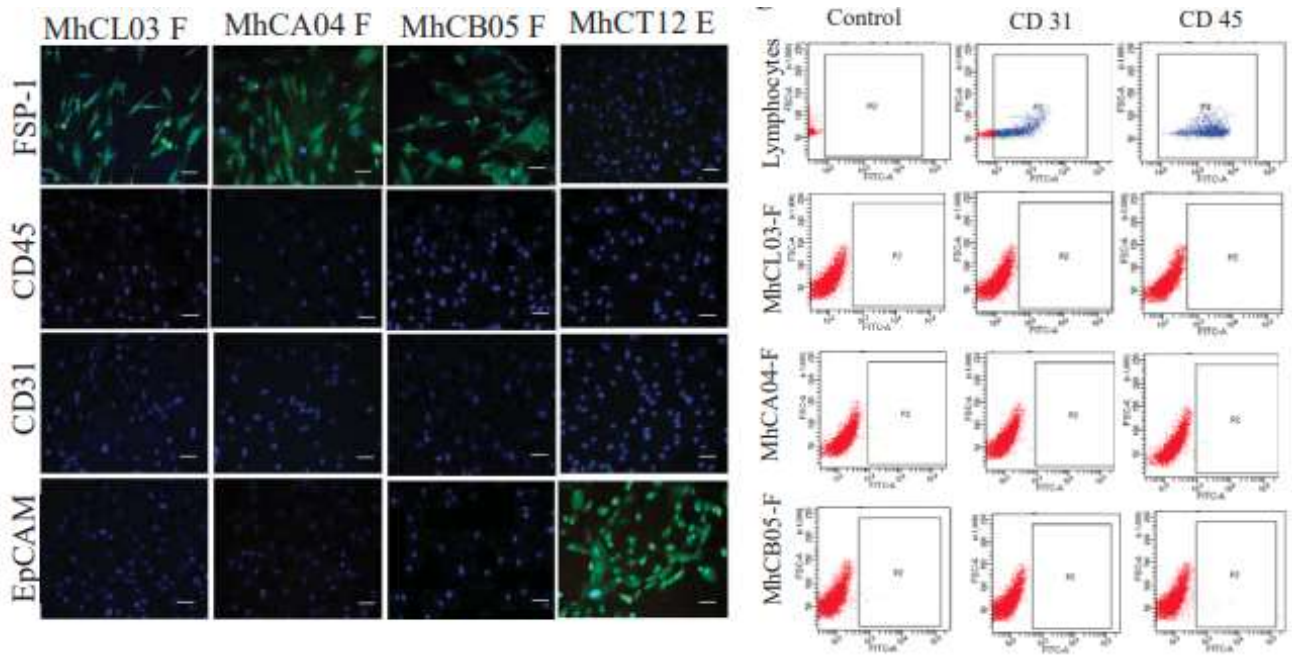


Fig 2: Purity of established cell lines. (A) FACS analysis displaying EpCAM and FSP-1 staining for the established cell lines. (B) Fluorescent images of established cell lines stained with FSP-1, EpCAM, CD31, and CD45 (Magnification 10X, Scale bar- 50µm). (C) FACS analysis displaying CD31 and CD45 staining for the lymphocytes and the established lines as indicated.

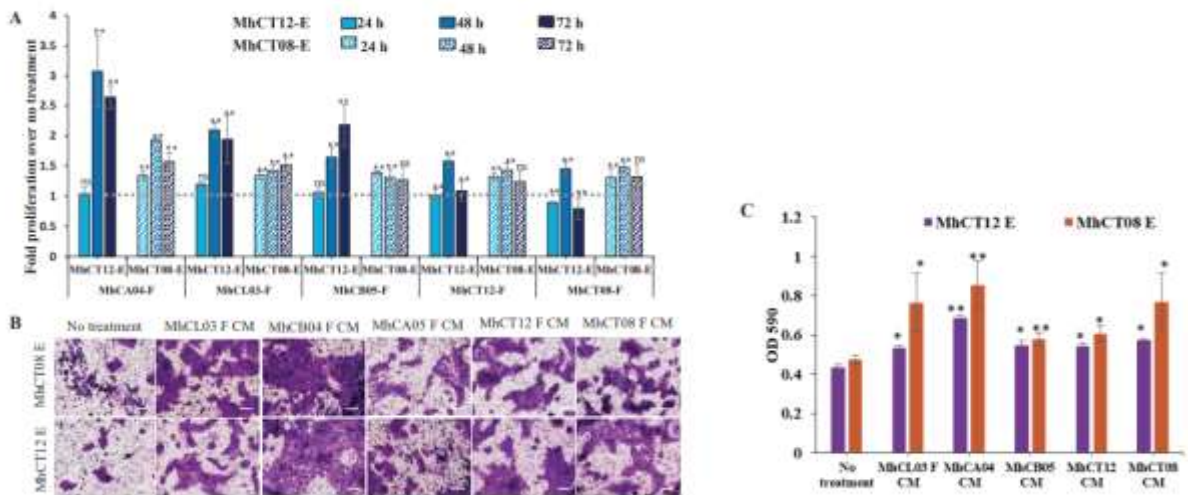


Figure 3 Tumorigenic properties of established CAF cell lines. (A) Proliferative potential of MhCT12-E and MhCT08-E under the influence of indicated conditioned medium. The grey line intersection depicts the no treatment control for both the epithelial cells. Statistical significance over no treatment, where, *p < 0.05

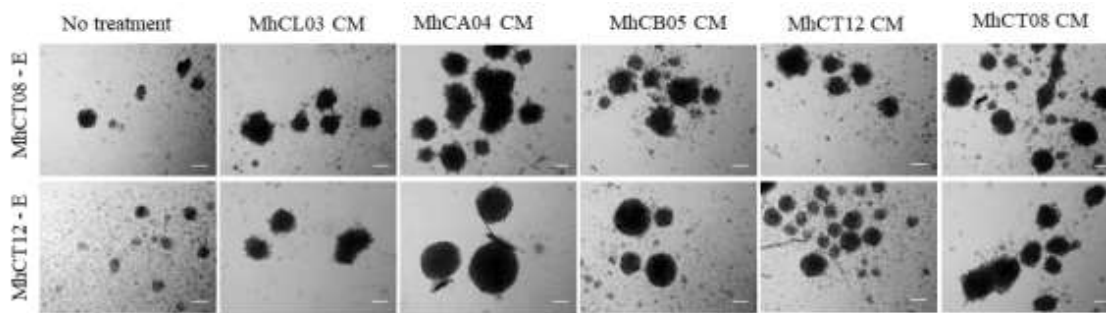


Figure 4: Sphere formation potential of MhCT08-E and MhCT12-E cells under the influence of CAF condition medium. (Magnification 10X, Scale bar-100 μ m), CM-Condition media, NT-NoTreatment, F-Fibroblast, E-Epithelial

Manuscript: Establishment and Characterization of Three Novel CAF Cell Lines from HNSCC Patients, BioRxiv, doi: <https://doi.org/10.1101/2023.01.08.523131>

2. Lab-on-PCB-based Electrical Immunosensing Platform for Point-of-Care Detection of SARS-CoV-2 Virus:

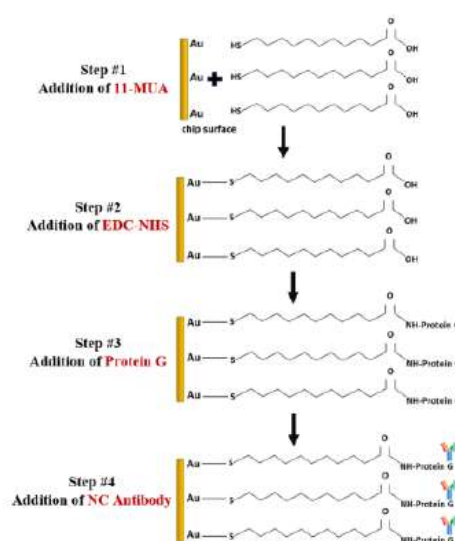
Project initiated from Molecular Immunology group (Collaborator: Dr. Manjula Das and Dr. Hardik J. Pandya, IISc).

AIM: To develop a multiplexed lab-on-PCB platform for label-free immunosensing of SARS-CoV-2 nucleocapsid and spike antigens based on electrical impedance measurements.

Abstract of the project: The present work shows a multiplexed lab-on-PCB platform for label-free immunosensing of SARS-CoV-2 nucleocapsid and spike antigens based on electrical impedance measurements. The sensor consists of an interdigitated electrode of soft gold integrated on a printed circuit board with microwells for sample loading. A mercaptoundecanoic acid-Protein G-based site-specific biofunctionalization strategy is employed to efficiently immobilize dual antibodies on the device surface towards sensitive and rapid antigen test (RAT) of SARS-CoV-2. Electrical impedance measurements carried out in a point-of-care setting using the Palm Sens Sensit Smart system could detect nucleocapsid and spike proteins with a detection limit of 40 pg each. Experiments with nasopharyngeal swab samples from N = 5 healthy and N = 14 SARS-CoV-2 positive subjects showed significantly different electrical responses for subjects with high viral load (Ct < 25) compared to healthy subjects and control..

Results:

Figure 5: Four step immobilization process of the Anti-SARS-CoV-2 NC/Anti-SARS-CoV-2 S on PCB surface.



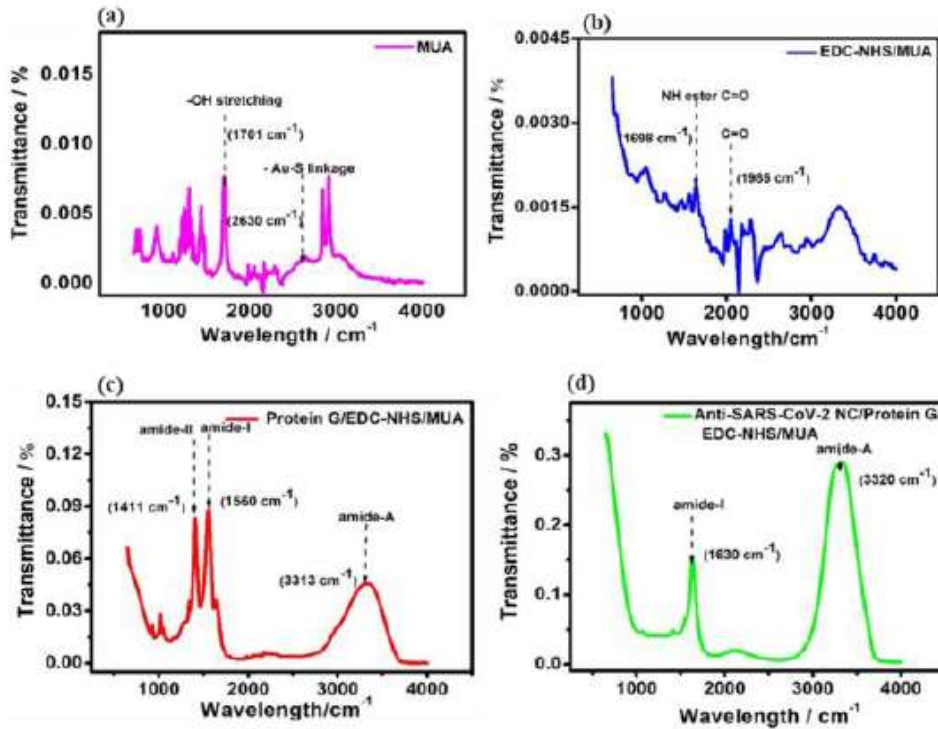


Figure 6: FT-IR studies for the stepwise bioconjugation of the PCB gold surfaces with Anti-SARS-CoV-2 NC and Anti-SARS-CoV-2 S through MUA-Protein G chemistry

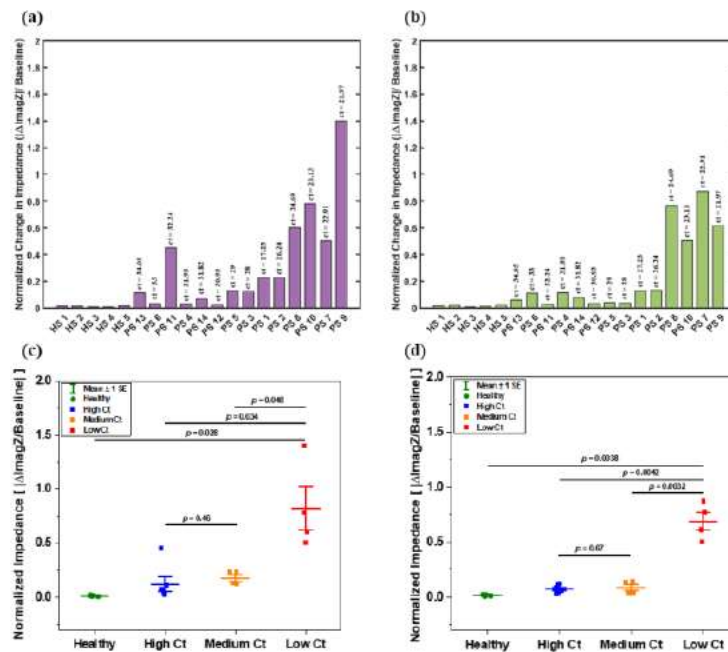


Figure 7: Results from testing with healthy and SARS-CoV-2 positive patient samples showing (a) normalized change in impedance for NC antigen detection, (b) S antigen detection, and scatter plots of samples grouped into healthy, high Ct, medium Ct, and low Ct for (c) NC antigen.

Publication: Joshi, Anju and Vishnu G. K., Anil and P. K, Smitha and Kadandale, Ashlesh and R. S., Hari and Roy, Bidipta and Das, Manjula and Pandya, Hardik J., "Lab-on-PCB-Based Electrical Immunosensing Platform for Point-of-Care Detection of SARS-CoV-2," in *IEEE Sensors Letters*, vol. 7, no. 1, pp. 1-4, Jan. 2023, Art no. 4500204, doi: 10.1109/LSENS.2022.3228306.

Patent: *Device and Method for Rapid Detection of SARS-CoV Antigens and its Variants-of-Interest*, 202241039126, July 07, 2022

3. Expression, purification, and ELISA assay development and validation for S100A7, S100-P and CD44 protein: Project initiated from Head and Neck Oncology group (Collaborator: Dr. Amritha Suresh)

AIM:

1. To make recombinant S100A7, S100P and CD44 protein in *E. coli* expression system
2. To make polyclonal antibodies against S100A7 and S100 P proteins.
3. To make monoclonal antibody against CD44 protein.
4. ELISA Assay development and validation for the detection of S100A7 and S100P-95 proteins from Saliva

Details of work done: (Ms. Gopika, Project Intern, Head and Neck Oncology group)

- Design of recombinant S-100 P protein to be expressed in *E. coli* strain
- Recombinant protein expression and purification: the recombinant pET28a_S100A7, S100-P95 and CD44 constructs were transformed and expressed in *E. coli*. All the recombinant protein expression conditions and purifications have been optimized.

1. Recombinant S100A7, S100P-95, CD44 Protein expression

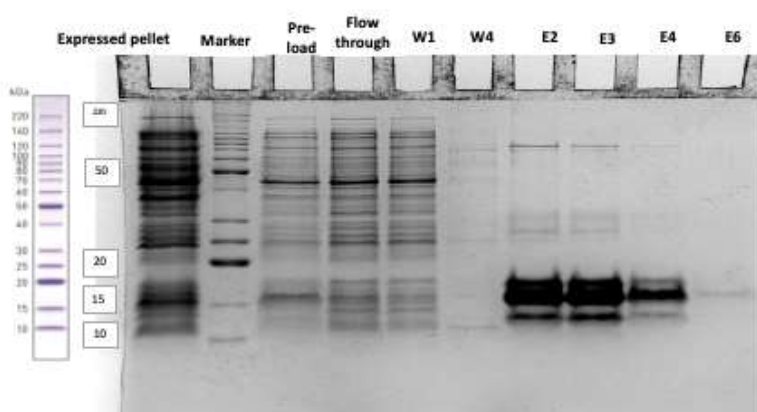


Figure 8: Purification optimization of S-100A7 protein

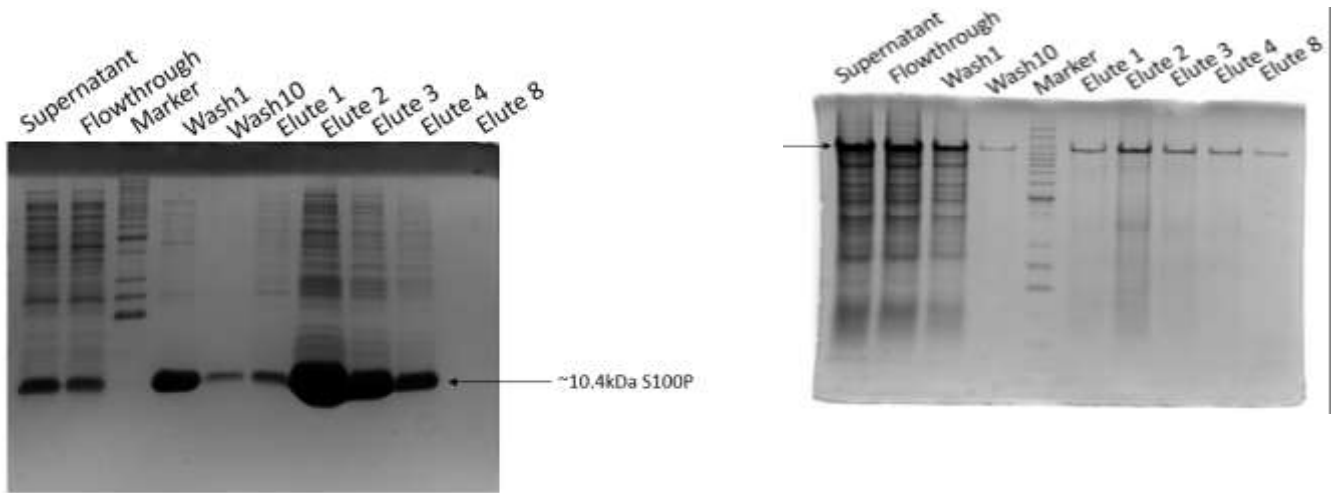
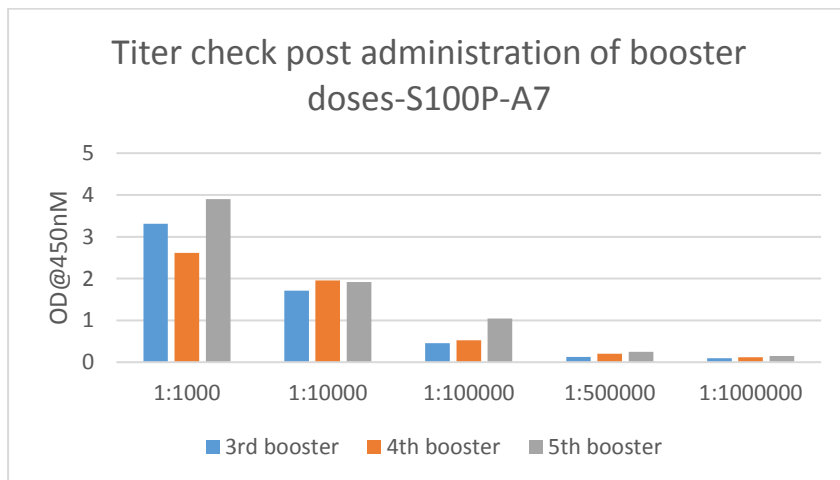
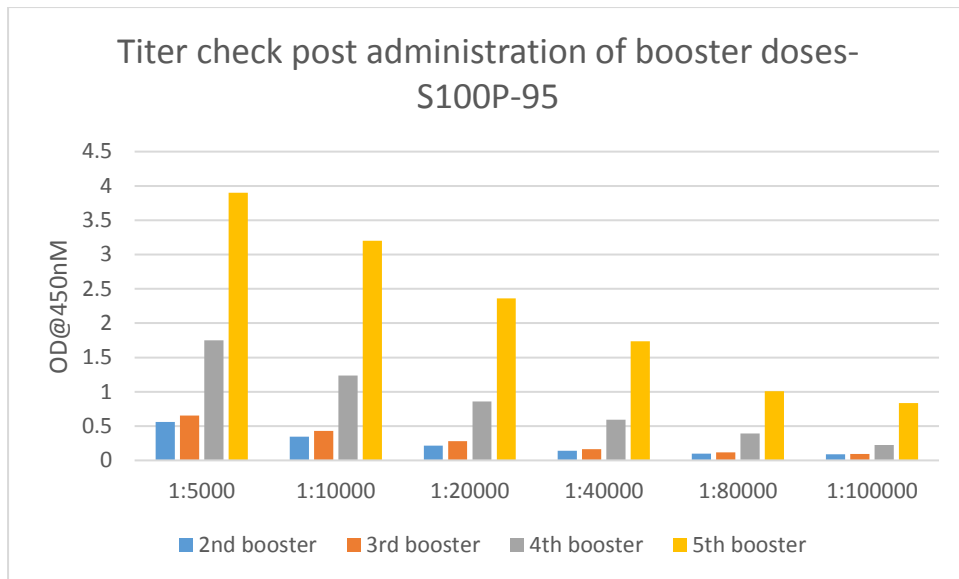


Figure 8: Purification optimization of S100P-95 protein

2. Polyclonal antibody development against S100A7 and S100P-95 in New Zealand white rabbit





3. ELISA assay for S100A7:

Assay development:

- Purification of polyclonal antibody: to be used as capture and detection: completed
- HRP-Conjugation of anti-S100A7 polyclonal antibody to be used as detection antibody for assay development: completed
- Determination of detection antibody concentration: completed
- Determination of capture antibody concentration: completed
- Determination of assay range: completed

Assay validation: on going

- Determination of saliva matrix dilution: completed

4. ELISA assay for S100P-95:

Assay development:

- Purification of polyclonal antibody: to be used as capture and detection: completed
- HRP-Conjugation of anti- S100P-95 polyclonal antibody to be used as detection antibody for assay development: completed

Team

Research Scientist: Dr Smitha PK

Project Staff: Suprabuddha Dutta, Pushkarni Suresh

Intern: B Naga Pushpa

Computational Biology

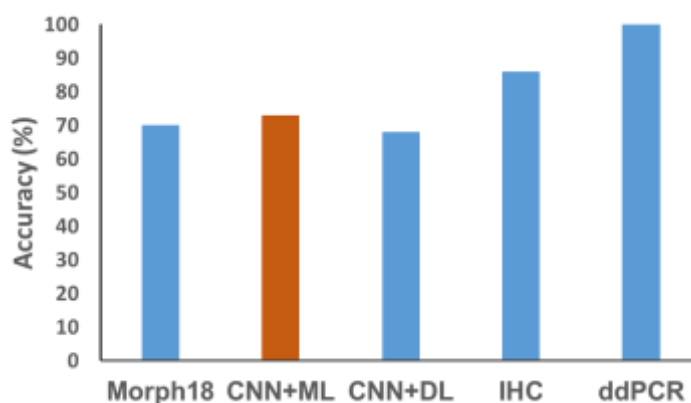
The newly formed Computational Biology group at MSMF aims to employ computational techniques beyond conventional bioinformatics to develop models that can predict clinical endpoints of molecular subtypes of the disease.

Histopathology images, in particular the haematoxylin and eosin (H&E) stains, are collected as part of routine pathology practice to assist in diagnosis of tumours. Though visual inspection of H&E stains by pathologists reveals important information of cell types, morphology and other events, their molecular subtypes or prognostic indicators are unlikely to be uncovered. However, application of neural network-based algorithms had indicated such correlations in multiple cancers. Computational biology group has initiated a couple of projects in this field in collaboration with other organizations.

The Computational Biology group also works with multiple research groups at MSMF to build disease prognosis models through biomarkers generated from Next Gen Sequencing data generated in-house or acquired from public datasets. We use a blend of conventional functional genomics tools and advanced algorithms to create a functional landscape of the underlying biology captured in the sequence data snapshot.

AI-based Computational Models for Detection of IDH mutation

In tumours formed in glial cells around neurons, mutations observed in the isocitrate dehydrogenase (IDH) family of genes are linked with an improved prognosis. Detection of this mutation, primarily by immunohistochemistry (IHC) using mutation-specific antibodies is common clinical practice to decide the therapeutic course. However, the IHC is

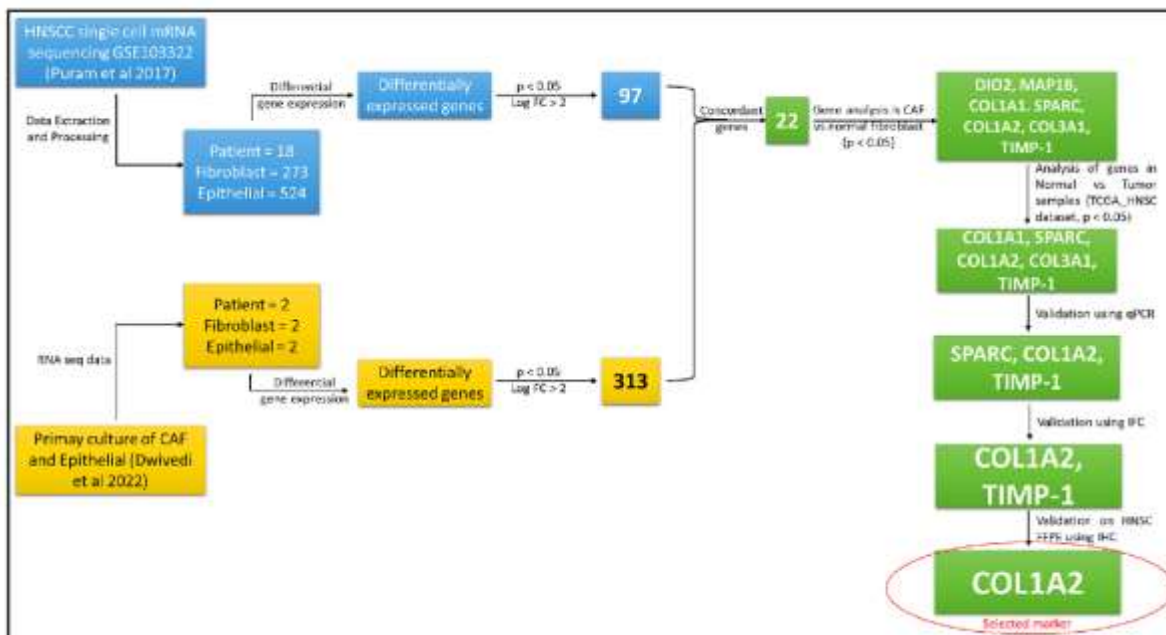


often limited to detect only the most commonly observed mutation whereas the non-canonical mutations are missed out. In this study, in collaboration with pathologists from NH, Bangalore and NIMS, Hyderabad we are developing an AI-based computational model that can categorize the histopathology images into IDH-mutant and IDH-wildtype molecular subtypes. For this, we use both morphological and convolutional features extracted from whole-slide images and classify them with an ensemble of traditional machine learning and deep learning algorithms. Initial results with whole slide images of ~ 60 patients shows a maximum classification accuracy of 75% using droplet Digital PCR (ddPCR) assay as the gold standard for detection of IDH mutation. Currently we are augmenting the number of slides by recruiting more patients and also refining our algorithms to achieve >90% classification accuracy soon.

Biomarkers for Cancer-Associated Fibroblasts

Cancer-associated fibroblasts (CAFs), a prominent component of the tumor microenvironment, play an important role in tumor development, invasion, and drug resistance. The expression of distinct

"CAF-markers" which separates CAFs from normal fibroblasts and epithelial cells, have traditionally been used to identify them. However, these commonly used CAF-markers have been reported to differ greatly across different CAF subpopulations, even within a cancer type.



We

used an unbiased omic approach to identify potential CAF markers using a gene expression meta-analysis of the publicly available dataset GSE103322 and the in-house data to shortlist a set of candidate markers that were further filtered using data from TCGA archive and publicly available dataset GSE135975. Further qPCR and IHC assays performed in collaboration with Molecular Immunology department on head and neck cancer samples identified COL1A2 and TIMP1 as most robust and potent CAF markers to differentiate fibroblasts from epithelia.

Collaborative projects in Integrative and Functional Genomics

In collaboration with the discovery groups at MSMF and other organization, Computational Biology team also worked on translational projects using the expertise in integrative and functional genomics:

- Identification of genomic markers of liquid biopsy for breast cancer patients (initiated by St John’s Research Institute, Bangalore).
- Proteogenomics integration in glioblastoma to discover novel protein variants (initiated by Neuro-Oncology group in collaboration with IOB, Bangalore)
- Genomics and transcriptomic atlas of Oral potentially malignant lesions (initiated by Integrated Head and Neck Oncology group in collaboration with other clinical partners).

Publications

Dwivedi, N., Shukla, N., Prathima, K.M., Das M, Dhar S.K., Novel CAF-identifiers via transcriptomic and protein level analysis in HNSC patients. *Sci Rep* **13**, 13899 (2023)

Nair MG, Ramesh RS, Naidu CM, Mavatkar AD, VP S, Ramamurthy V, Somashekaraiiah VM, CE A, Raghunathan K, Panigrahi A, Das M, Dhar SK and Prabhu JS, Estimation of ALU Repetitive Elements in Plasma as a Cost-Effective Liquid Biopsy Tool for Disease Prognosis in Breast Cancer. *Cancers*, **15**, 1054 (2023)

Smitha PK, Shandil RK, Suresh P, Biswas K, Rudramurthy GR, Naveenkumar CN, Bharathkumar K, Battula NP, Chowdhury SD, Sinha S, Dutta S., Dhar SK and Das M, ACE2Fc: A Promising Therapy for SARS-Cov2 Infection. *Medical Research Archives*, **10**, Dec 31 (2022)

Dwivedi N, Dhar SK, Kuriakose MA, Suresh A, Das M. Reference Genes for gene expression analysis in Head and Neck Squamous Cell Carcinoma: a Data Science Driven Approach. *Dental Research and Oral Health*. **5**, 21-37 (2022)

Grants

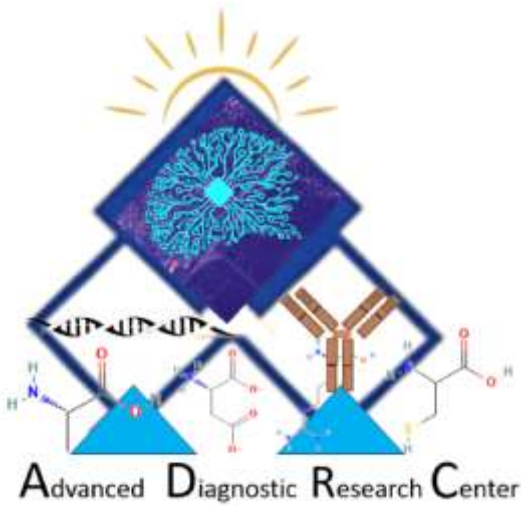
- Development of an AI-enabled computation model for IDH1 mutation detection from H&E-stained glioma histopathology images, in collaboration with NIMS Hyderabad (approved by ICMR), 2023-26
- Near AI: predicting response to save lives in lung carcinoma, in collaboration with 64Codon, Kochi (approved by Kerala Startup Mission), 2023-24

Team

Research Scientist: Dr Sujan K Dhar

Intern: Anuradha Panigrahi, Vijeta D Joshi

Advanced Diagnostic Research Centre (ADRC)



Advanced Diagnostic Research Center (ADRC), started in 2022, got accredited by NABL in 2023, to develop a robust ecosystem geared up to meet the demands of today and anticipate tomorrow's challenges of paradigm shift in the way diagnostic solutions are offered. Research team at ADRC works in tandem with the clinicians to identify unmet diagnostic needs and translate them to diagnostic tests of the present and future, through collaborative research in molecular biology, cell biology and data analytics. Major driver of the research is to establish tests that are reliable to clinicians and affordable to patients. Most of the tests offered by ADRC have been developed or honed in-house and were adopted to clinic after extensive validation.

Where ADRC stands out is in the research performed with the excess sample and the data obtained from the test, with appropriate consent from the patients. The research improves the tests themselves, brings out possibilities of new tests and helps in knowledge creation of the disease.

1. Diagnostic Tests

A correct diagnosis is three-fourths the remedy

– Mahatma Gandhi

Narayana Hrudalaya and Mazumdar Shaw super specialty hospital together handle one of the largest solid organ and bone marrow transplant unit in South East Asia. Thus, advanced transplant diagnostics, capable of supporting emergency cadaver transplants have been made available in the ADRC lab, 24X7.

Molecular diagnostics at an affordable price is another pledge that ADRC has taken to improve patient care.

Currently we offer the following Diagnostic panels:

| Panels | Test |
|------------------------|---|
| Transplant Diagnostics | HLA- high resolution-6 Loci (A, B, C, DPB1, DQB1, DRB1) |
| | HLA typing low resolution - 7 loci (A, B, DPA1, DPB1, DRB1, DQA1, DQB1) |
| | DSA cross match |
| | Single antigen Bead Assay |
| Glioma Panel | IDH1, IDH2, TP53, TERT-promoter |
| Meningioma Panel | TERT-promoter |
| Endometrial Panel | p53, POLE, |
| Beta Thalassemia | Gene mutation |
| Systems Medicine Panel | Mutations and Gene Expression |

Techniques employed are qPCR, ddPCR, NGS and Luminex.

Publications

Keerthi Shetty, Bagirath Raghuraman, Prathip Kumar B.R., Akanksha Sharma, Identification of The Novel HLA-DPB1*1461:01 Allele In Three Individuals From Southern India (2023): HLA: <https://doi.org/10.1111/tan.15097>

Collaboration: Dr. Julius Punnen, Dr Basha J Khan, Dr Aditi Singhvi, Dr R Bagirath, Dr Limesh, Dr Vinod, Dr Pargnya Coca, Dr Sunil Bhat and Dr Sharat Damodar

Team: Dr Arnob Chatterjee, Divakar K, Shashi T, Vijaykumar G, Sowmya C Nandu

Grants

1. Establishment of a NABL Accredited Laboratory for Performing Clinical Immunogenicity Testing BT/CS0056/CS/05/21; BIRAC; 96L; 2022- 2023
2. Engineering conditionally replication-competent SARS-CoV-2 viral molecular clones and evaluation of cross-variant neutralization; BT/TEMP14085/CS/05/21; BIRAC; 96L; 2022- 2023

2. Research

*It is a capital mistake to theorize before you have data
– Sherlock Holmes*

Human leukocyte antigens (HLA) are proteins, displayed on cells of the body, which our immune system uses to differentiate between self and non-self. The HLA genes are known to be the most polymorphic genes in the human genome. The advent of next-generation sequencing has revolutionized the identification of the new HLA allele thereby leading to rapid enrichment of the IPD-IMGT/HLA database. HLA diversity in the South Indian population still remains to be investigated. By analyzing our data we have already started contributing to this ocean of knowledge.

Mazumdar Shaw Cancer Outreach Program

The Philanthropic wing of Mazumdar Shaw Medical Foundation is dedicated to support the underprivileged patients, providing financial, nutritional and psycho-social support thus enabling them to a healthier and happier life. Even though the medical advancements have brought cancer care to better standards, the ability to access this care is still a question for so many. MSCOP is playing its part in addressing this gap in cancer care by supporting families in the successful completion of their treatment. This is facilitated by a two part approach, both treatment and financial support to the sick and needy.

Activities of MSCOP

- Providing financial assistance to underprivileged patients.
- Organizing activities for children in the pediatric oncology wing.
- Daily nutritional supplement support program.
- Drug discounts for needy patients.
- Liaise with various organizations for the benefit of patients
- Providing emotional support to patients and their families
- Counselling patients and caregivers

Financial assistance to underprivileged families

| Category | New Patients | Patients supported |
|-----------|--------------|--------------------|
| Adult | 17 | 21 |
| Pediatric | 33 | 44 |

Patients under treatment at Mazumdar Shaw Cancer Center come from various socioeconomic backgrounds and the treatment costs vary based on their diagnosis and other risk factors present. MSCOP identifies deserving patients with the support of the doctors and social workers at the hospital. MSMF staff assess the financial background of the patient's family and allot funds according to their requirements and support in completing their treatment as per the treatment plan. Drug discounts facilitated through the hospital also make a significant difference in the cost of treatment.

The patients are provided financial aid with the support of generous donors. We often see that patients who are financially capable of completing treatment approach the foundation in offering a helping hand to others undergoing treatment. These gestures of kindness have helped several patients in gaining a new life.

Daily nutritional supplement support program

During chemotherapy patients are given powerful medicines to kill the fast growing cells and along with these medicines high nutritional intake is crucial for their recovery. Most families who come to the daycare for chemotherapy are unable to afford a healthy diet. The nutritional supplement support program initiated by MSCOP ensures that every patient in day care receives nutritional supplements in the form of almonds, cookies and fruits. Around 600 patients benefit from the nutritional support program at MSMF every month.

Emotional support and counselling to patients and their families

Along with the financial burden the diagnosis and the treatment takes the patients and the caregivers through severe stress. Besides financial support, MSMF staff provide them with the required psycho social support. This also provides a safe haven for the caregivers to open up and talk about their stress and anxiety about constantly being with the patients and watching their pain.

Organizing activities for children in pediatric oncology wing

Our team conducts activities in the pediatric oncology wing to lift the mood and to keep the hospitalized patients engaged. This includes fun activities that provide them the opportunities to make new friends and to forget about their painful treatment for a short while. Children undergoing treatment stay away from school for long. The activities during their stay at the hospital helps them stay positive. We celebrate various festivals and arrange small gifts to the children.



Testimonials

Krishnan C.

“We didn’t know what was happening. They did so many tests and informed us that he has blood cancer. He was planned for 12 doses of chemotherapy. After second chemo he had some complications and had to be hospitalized. For this we had to spend all of our savings and I took so many loans. I went to Dr. Pragnya crying and told that we don’t have anything more and I don’t know what to do. It was Dr. Pragnya who sent me to MSMF. From that time MSMF took care of our treatment. It was only because of the support from MSMF that I got my son back. And I am forever grateful for that”, says Krishnan’s mother.

Krishnan is a 21 year old diagnosed with Classical Hodgkin’s Lymphoma. He had to quit his job due to the illness. Weeks before his diagnosis his father fell ill and was unable to

work. The family's expenses are taken care off by Krishnan's mother and brother's daily wages. MSMF has been supporting for Krishnan's treatment. He completed his treatment and is on follow up.

Raima Roy

“When my daughter was diagnosed with brain tumor, I did not know what to do and where to go. I found about Mazumdar Shaw hospital on internet and came here. The doctor here told that she needs a very expensive surgery. We were not financially capable to manage this huge amount and was asked to meet with the MSMF team. MSMF helped us so much and they supported us with my daughter's surgery, radiation and chemo. They helped us in all the ways they could. And now I am happy to say that my daughter has recovered and she has few more follow up visits left. Me and my family are extremely grateful for the support given by MSMF”, say's Raima's father.

Raima is a 2 year old diagnosed with Rhabdomyosarcoma. She was 1 year old when first diagnosed and underwent surgery. The family is from West Bengal and they came to Karnataka looking for better treatment. Her father is a bike mechanic and was unable to manage the treatment expenses. MSMF supported her for her surgery, chemotherapy and radiation. She completed her treatment and is now under follow up.

Samarth

“From the time we reached this hospital MSMF has been helping. With their help we can now complete my child's treatment. A big thanks to MSMF”. These are the words of Samarth's father. Samarth is a 10 year old diagnosed with Acute Lymphoblastic Leukemia. His father is a barber and was finding it very difficult to find money for his son's treatment. There was also delay in getting the government schemes approved. MSMF helped them to continue treatments without any delay. He is currently undergoing his treatments.

In Doctor's Words

“Childhood cancers are highly curable, however biggest challenge in India is abandonment of treatment or inadequate treatment due to economic reasons. Support from MSMF towards the treatment of these children fighting life threatening cancer conditions have helped scores of patients to get cured and live a normal life again. Sincere gratitude from the families and from the medical team towards MSMF.”

Dr. Sunil Bhat
Director and Clinical Lead
Pediatric Hematology,
Oncology and Blood & Marrow Transplantation
Narayana Health Network Hospitals

Technology Business Incubator

Mission

Our mission is to foster innovation and entrepreneurship in the healthcare and technology sectors by providing support, resources, and connections to aspiring entrepreneurs. We aim to create a supportive community that helps turn innovative ideas into successful businesses that improve the healthcare industry and make a positive impact on society.

Vision

Our Vision is to be the leading incubator and technology startup, helping to create a future where everyone has access to high-quality healthcare and innovation.

Objectives

- To foster innovation and entrepreneurship in the healthcare and technology sectors.
- Creating a supportive community that facilitates the transformation of innovative ideas into successful businesses.
- Improving the healthcare industry and positively impacting society.
- contributing to a future where high-quality healthcare and innovation are accessible to all.

Key Achievements of Incubator

COHORT 09

ONBOARDED STARTUPS

With the aim of creating innovative technologies for a better and sustainable future, Sunfox visions to bring the reliable technologies related to Biomedical Instrumentation, IOT and other engineering domains is serving the Indian markets and international clients with fervour.



AWARDS AND RECOGNITION: Shark Tank India 2022 (S-1), Represented India at World Economic Forum- China, Represented India at Grand Challenges Annual Meeting, Extreme Challenge for the World's largest Start-up Competition.

Visbio Technologies Private Limited- VisBio Technologies Private Limited has developed Smart ColpoSpec, a head-mounted colposcope and an operating tool for examination, tele-consultation and surgical procedures in cervical workflow and an assistive tool to detect cervical pre-cancer.



Fitknees is a wearable technology that captures clinically relevant data in chronic knee injuries for assessment and progress monitoring, with potential for remote monitoring in near future.



ANATOMECH has developed Compression Wearable & Portable Medical Device based on shape memory material technology (platform) for lifelong management of secondary lymphedema (medical condition).



Scanbo: Pioneering Diagnostic Evolution! Delivering AI-powered, ultra-portable solutions for point-of-care diagnostics. Swift, precise, and affordable, we empower healthcare providers with faster triage, revolutionizing primary care. Shaping a healthier global future through cutting-edge innovations.

COHORT 10

ONBOARDED STARTUPS



Uncontrolled hemorrhage, a preventable cause, results from torso injuries or tourniquet-impractical areas. Miraqules, a biomedical startup, creates rapid anti-bleeding compounds via patented fabrication, mimicking fibrin but 5x more effective. Since 2018, Miraqules focuses on swift hemostasis for critical bleeding.

AWARDS AND RECOGNITION: Mass Challenge Israel, Defense Research and Development Organization- India, Birla Institute of Management Technology along with Atal Incubation Centre, Y Combinator, start-up school, MedTech Innovator Asia Pacific Accelerator program, RebelBio program, Imperial College London, White City Incubator-UK, Hello Tomorrow, Slingshot, Singapore Government.



EasyHeals is an AI powered online marketplace of healthcare service providers which offer patients the easiest access to best possible healthcare needs at affordable prices. This is a mobile based marketplace which provide location specific search along with comparison & booking system for hospital services.

CysterCare

Cystercare prioritizes collaborative care with a patient-centered approach. Subscribers access diverse medical providers - mental health, gynecology, dermatology, fitness, nutrition, etc. Their holistic model aims to personalize and optimize care delivery for individual patient needs.



Robot-assisted visualization and manipulation platform for Neurosurgery, ENT & Head and Neck surgery. Their vision is to democratise access to precision robotic surgery.



ONI is a connected ecosystem between ObGyns and Mothers. Their focus is to improve Patient Care and Health through Epigenetics ObGyns are fully digitised so that they save on time and operational inefficiencies through a specialised EMR. Mothers are also digitised to provide with all consultations that the ObGyn does, reminders of prenatal visits and test, Education and Consultation on Epigenetics.

GRADUATED STARTUPS



Docturnal's TimBre app: Revolutionizing TB Screening. Record cough sounds, demographics, clinical data; AI-driven real-time analysis for TB detection. retiNNapp: Transforming Diabetic Retinopathy assessment. Capture retina images via 20D lens, ML-based instant grading. Healthcare's future, now.

ONGOING TRIAL



CIMED Labs: Innovating Interceptive Medicine. Pioneer in disease prevention along its continuum, pre-diagnosis. Polymolecular paradigm for advanced therapeutics. Modelling diseases, crafting comprehensive management.

AWARDS AND RECOGNITION: Patent sold to Dr Reddy's Laboratory



A bio-science company catalyzing research with an in-house commercial biobank. They have created a unique network of partner hospitals and laboratories to collect tumor samples.

AWARDS AND RECOGNITION: Idea Grant, Productization Grant & Scale-up Grant from KSUM.



Developing magnetic nanotechnology for radiation free and affordable cancer diagnosis and therapy. SIAMAF brings innovative solutions for cancer staging, screening, localization, imaging, and hyperthermia using advanced magnetic sensing technology and functionalized magnetic nanoparticles.



Unijob Medics aids career decisions, offers affordable learning for healthcare jobs. Assists in course creation, promotion, and hosting on LMS. Tutle Test: Efficient online assessments save time and costs.



Farcast™ Lab: Processed 22K+ live tumor tissues across 17 cancers. Developing Farcast™ TiME ex vivo platform, assessing anti-cancer agent impact on cultured tumors.



India's first Terahertz company, developing compact medical systems using multispectral Terahertz and Fluorescence technologies combined with Artificial Intelligence for accurate diagnosis of cancer margin intraoperatively.

AWARDS AND RECOGNITION: India-Sweden Healthcare Innovation Challenge 2021-22, Wharton India Startup Challenge, Wharton India Startup Challenge, Qualcomm India Design Challenge and Women

Investments, Funding sources, financial performance of the incubator

In 2022, the Startup India initiative extended its support to MSMF-TBI through the Startup India Seed Fund Scheme. This program, dedicated to nurturing early-stage ventures in the healthcare sector, has already facilitated seed funding of up to 1.5 crore INR for three startups. This infusion of financial support empowers startups to transform their innovative concepts into tangible, market-ready products and services. MSMF-TBI plays a vital role in providing financial support to startups in the form of both Seed and Grant funding, with a substantial allocation of up to 50 lakh rupees per startup

Continued Programs Launched

Mentoring Clinic

1. Dr. Aparna Srikantam – October 2022- MSMF collaborated with the Research and Innovation Circle of Hyderabad, and Dr. Aparna Srikantam, the director of Research and Lab Services, joined us to mentor startups, showcasing her remarkable success in the field.



2. Dr. Sujana Dhar – September 2022- Dr. Sujana Dhar, an expert in computational biology and chair of IEEE Engineering in Medicine and Biology Society, provided mentorship on AI techniques, integration, and prognostic biomarkers for various diseases.

MENTORING+ CLINIC

Start-ups focusing on computational biology and AI techniques to integrate multi-omics data towards discovery of diagnostic and prognostic biomarkers in any disease area are invited to attend a one-on-one mentoring session with Dr. Sujan Dhar, who is an expert in computational biology and chairs IEEE Engineering in Medicine and Biology Society.



Dr. Sujan K Dhar
Research Scientist
Mazumdar Shaw Medical Foundation

3. Dr. Pradeep Narayan – August 2022 Dr. Pradeep, a cardiac surgery specialist, mentored entrepreneurs on techniques to develop their medtech startups.

Testimonial on Mentoring clinic.

24th August, 2022 3.00pm



Dr. Pradeep Narayan
Speciality: Cardiac Surgery - Adult

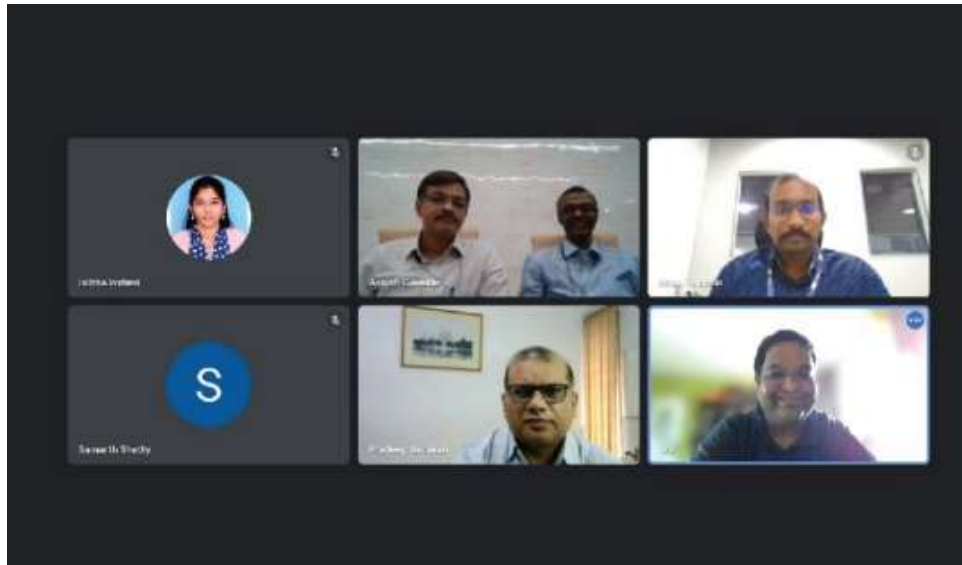


Aniruddha Atre
Co-Founder Jeevtronics



Ashish Gawade
Director and Co-Founder at Jeevtronics

"It was a great mentoring session for us with eminent cardiac surgeon Dr. Pradeep Narayan. We are very thankful for the clinical advise and clarity he provided on the queries we had in defibrillation and cardiac critical care as well as in clinical research."



4. Dr. Mayur Shetty – July 2022- Dr. Mayur Shetty, a renowned Plastic and Reconstructive surgeon, imparted his valuable tips for development to the Medtech entrepreneurs.

Mentoring Clinic

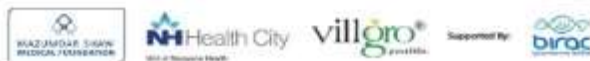


Dr. Mayur R Shetty

Speciality: Plastic Surgery, Reconstructive Microsurgery



July 2022

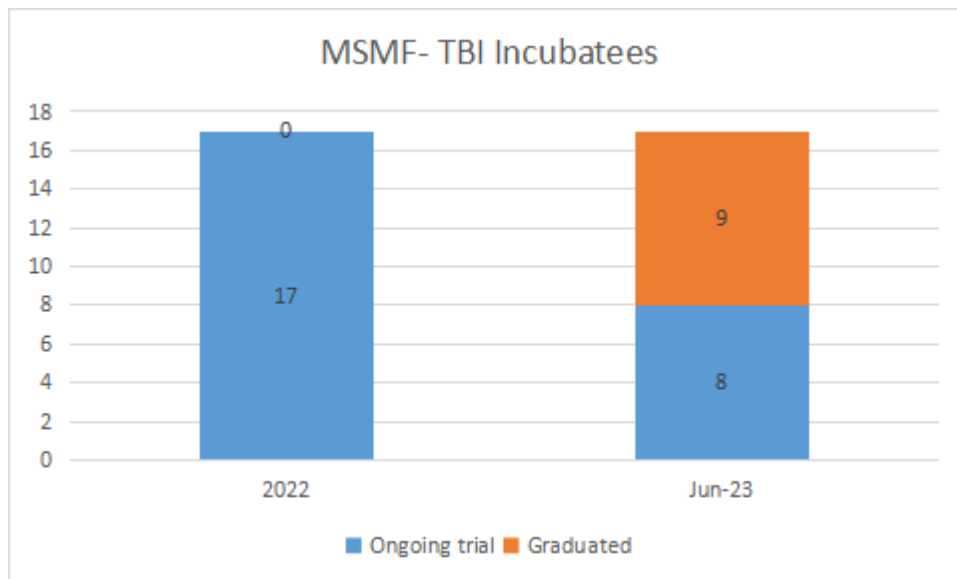


SISFS Startup India Seed Fund Scheme-

Starting from December 2022, MSMF TBI operates as an incubator providing support to healthcare startups as part of the Startup India Seed Fund scheme.



MSMF-TBI Incubation Program



Collaborative Programs

1. Wadwani LIFT-OFF, May 2023-The program is designed to uplift early-stage startups in the life science and healthcare space, helping them become investment-ready through a structured 14-week program.



2. ISB I-Heal- The accelerator program empowering innovators to learn, grow, and scale by providing the right resources and support

ISB | aic | I-Venture@ISB | AIM

I+JEAL @ ISB Healthcare Entrepreneurship and Acceleration Lab

In association with ISB | Max Institute of Healthcare Management

Sectors

- Digital Health
- Women & Child Healthcare
- Disease Diagnostics
- Healthcare Management & Infrastructure
- Geriatric Population

APPLY NOW

Application deadline: January 30, 2023

OUR PARTNERS

Accelerator Partners: SOCIAL alpha, etc.

Investor Partners: USAID, SAMRIDH, Endlyx, W HEALTH VENTURES, MENTERRA, enzia

- Impact conclave, Feb 2023 – MSMF-TBI served as the ecosystem partner for the HeadStart networking and pitching event, Impact Conclave, held in KIMS, with Mr. Kiran Vuppala, HOO, representing MSMF-TBI at the event



4. HSX, Feb 2023, MSMF-TBI played a pivotal role as the ecosystem partner for the HeadStart networking and pitching event, HSX, held at IITB. The event featured MSMF and TBI stalls showcasing their research and development, while TBI startups actively participated
5. Bharat CSR Summit, Dec 2022 - MSMF TBI is announced as an ecosystem partner for the largest Indian Ecosystem gathering in Mumbai, offering incubators diverse opportunities to learn and explore the corporate social responsibility (CSR) divisions of corporates.



MSMF-TBI @ Bharat CSR Summit



6. Aadyaa 2.0 Weneurs Sep 2022- The initiative aims to foster girl entrepreneurship development, supporting young Indian girl entrepreneurs, with MSMF TBI as an ecosystem partner for the launchpad



7. Manterra Impact Challenge, June 2022- The platform's initiative is to assist early-stage startups in developing technologies in the field of Cancer Care, with MSMF TBI supporting as the innovative partner.



BIRAC-BIG Awareness sessions

1. SIIC, IITK

Aditi Kumar, a member of SIIC-IITK, engaged in a comprehensive discussion at Harald Varmus auditorium with NH doctors, MSMF Researchers, and fellow innovators. The focus of the discussion revolved around innovation, translation, and capitalization of clinical ideas, with the backing of BIRAC-BIG Grant and various other grant schemes. The event witnessed a significant gathering, with Dr. Paul delivering the welcome note, followed by SIIC-IITK providing insights into BIRAC-BIG and other Grant schemes.



2. C-CAMP, Bangalore

Dr. Nitya Raman and Dr. Sowmya, representing C-CAMP, engaged in an extensive discussion with NH doctors regarding innovation, translation, and capitalization of clinical ideas, all made possible through the support of BIRAC-BIG Grant.



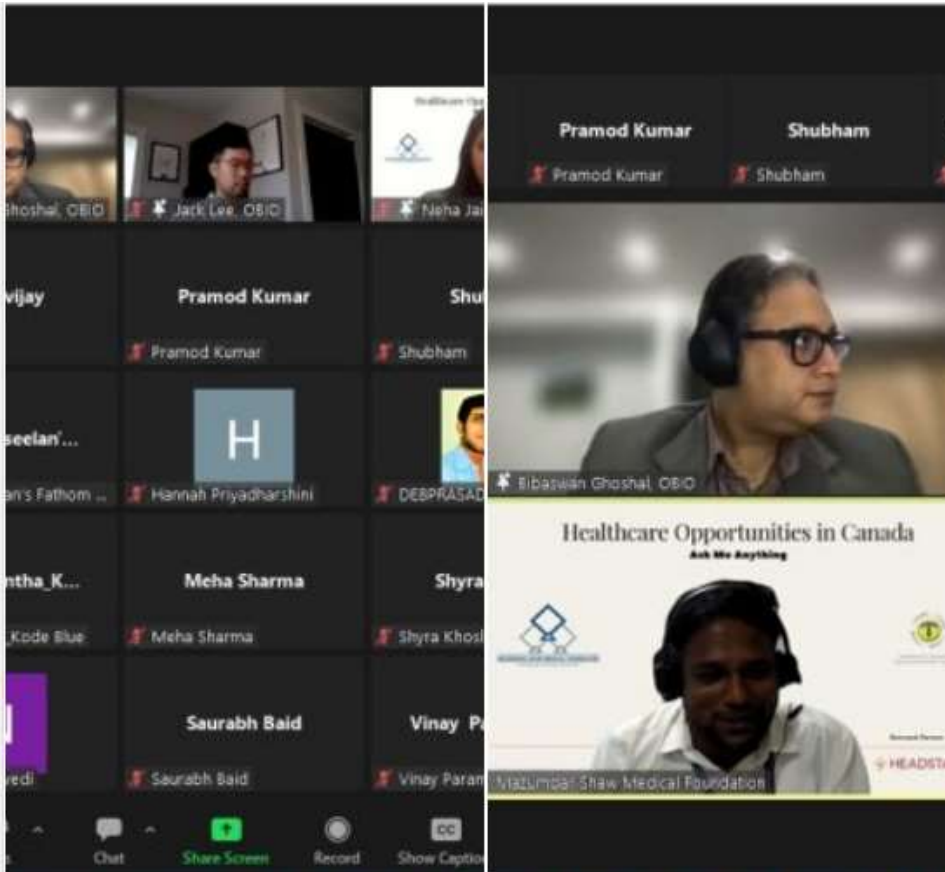
Webinar / Workshops / Live Sessions

1. Raghul Bagga – Patent Filing framework for startups – Dec 2022 Rahul Bagga founder of Amuirah shared his extensive knowledge and experience in intellectual property law, particularly in the context of startups and innovative ventures. He briefed the participants through the essential components of the patent filing process, offering practical tips on how startups can maximize their chances of success.



2. TBDC – Healthcare Opportunities in Canada- June 2023 The speakers from Toronto Business Development Centre and Headstart Network Foundation shed light on market opportunities and growth prospects that will greatly benefit the attendees in their pursuit of business growth.





3. Headstart – Plan to scale first 100 days of startup. Nov- 2022 Mr. Kiran Vuppala and Mr. Saurabh Pandey provided enlightening guidance to startups on formulating a comprehensive business scaling plan within the initial 100 days.



4. Beating the Pandemic Blues – September 2022- Headstart Network Foundation organized a platform for engaging with experts, fostering knowledge gain, and enabling

numerous startups to overcome the challenges of the pandemic while maintaining successful business operations.



5. Scaling a Healthcare Startup – Gaurav Agarwal (1mg) – August 2022 – During the webinar, Mr. Gaurav Agarwal, the co-founder of 1mg, shared his remarkable journey, encompassing insights into scaling enterprises, achieving a successful acquisition by TATA, and many other invaluable experiences.



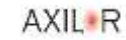
6. IKMC2022-July 2022 - Mr. Salai Jeyaseelan A and Mr. Ajay Nithish M presented their innovative concept for a one-step diagnostic device at IKMC2022 Hyderabad .



7. It was great to host Dr. Danda, Dr. Vinod, Dr. Sandhya at MSMF-TBI for interaction with the team, where we had interesting conversations about the Indian startup ecosystem and HOW MSMF-TBI will play an important role in it through its unique interventions and programs.



Funding / Investment



Academic Institutions



Hospital





Outreach / Ecosystem





Corporate Partners



Government Agencies



Services Partner



Projects undertaken

1. RW Therapies- Prototype development of a pulse lavage device for wound care management.
2. Vimaya3D and Ola Electric - Providing on-demand 3D printing of components and designs for the company as needed.

Team MSMF

Board of Directors



Kiran Mazumdar Shaw

Chairperson, MSMF



Dr Paul C Salins

Managing Director, MSMF



Dr Devi Prasad Shetty

Chairman and Founder, NH



Kunal Kashyap

MD & Chairman, Allegro Capital

Executive Management



Dr Paul Salins
Managing Director



Dr Amritha Suresh
Head of Operations

Scientific Core



Dr Amritha Suresh
*PI – Integrated Head and Neck
Oncology*



Dr Ravi Sirdeshmukh
PI – Neuro Oncology



Dr Manjula Das
*PI – Molecular Immunology &
Lab Director, ADRC*



Dr Atanu Ghorai *Research
Scientist,
Neuro Oncology*

Dr Smitha PK
*Research Scientist,
Product Research*

Dr Sujan K Dhar,
*Research Scientist,
Computational Biology*

TBI Team



Dr Samarth Shetty
BioNest-in-Charge



Dr Sherin James
Sr Program Manager



Dr S Jeyaseelan
Chief Engineer

Administration

| | |
|---|--|
|  |  |
| <p>Archana Ann J Lab Administrator, Manager - MSCOP</p> | <p>Preethu BU Manager – Finance</p> |



Blessy Rosewin

*Executive – Philanthropy,
MSCOP*



Abhilash J

Executive – Facility Maintenance



Raghavendra Rao,

Executive –IT

Team Members

Post-Doctoral Scientists

Dr. Arivusudar M
Dr. Chandrashekhar GK
Dr. Ritu Raj
Dr. Safeena Kulsum
Dr. Ram Bhupal Reddy
Dr. Prabhuraj

PhD Students

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Gangotri Siddappa
C A Divya
Nehanjali Dwivedi
Dr. Sumsum Sunny
Dr. Narayanan Subramanian
Dr. Vijay Kumar S
Raksha Ganesh

Project Staff

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Yogesh
Hari P. S.
Divya Naik
Vaishnav Vasudevan
Pranali Y S
Pavan Hallur
Darshat Shah
Rathijit Maliick
Aditi Hariharan
Pushkarni Suresh
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Kunal Biswas
Suprabuddha Dutta
Nidhi Shukla
Gouri Raj
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Dr. Pramila M
Dr. Shruti Nambiar
Dr. Sumsum Sunny
Ashwini Murali

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Mohit Santosh
Ajay Nitesh
Kartheek SA

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Sreeraman Deepika
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Aishwarya Chitoor
Anuradha Panigrahi
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Shashikumar T
Neha N Damodar
Vijeta D Joshi
B Naga Pushpa

