

**Indian Council of Medical Research (ICMR)**

Department of Health Research
(Ministry of Health and Family Welfare)

Call for Investigator-Initiated Research Proposals for “ICMR Intermediate Extramural Grants” (“ICMR NISCHAYAK ANVESHAN Extramural Grants”) - 2026

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Proposal ID:	IIRPIG(NAG)-I-2026-01-0073
Title of the proposed research project: should be specific, concise and yet sufficiently informative.:	The PReMQCA-OCS study: Precision drug Repurposing to target tumor-cell Metabolic heterogeneity with a Quantitative Cell-biology Approach, for improving Ovarian Cancer Survival
Proposed Project Duration (in Months)	48 Months
Personal details of Principal Investigator (PI)	
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Date of Birth (DOB) :	30-06-1977
Date of Superannuation :	01-01-2040
Nature of Employment	Permanent
Institute	Ashoka University Type of Institute: Private academic institutions with valid UGC/AICTE/PCI or NMC approved Medical colleges
PART-A	
Summary (up to 500 words): A structured summary should contain the following subheadings: Background, Problem statement and rationale of study, key research question(s), Objectives, Methodology, and Expected outcomes. Background: Meaningful understanding of the profound challenge of cancer heterogeneity needs quantitative approaches tested on real world patient data. Marked heterogeneity in the metabolic rewiring of tumors between mitochondrial-Oxphos and glycolysis has been demonstrated in all major cancer types. Moreover, metabolic state of diabetes and obesity increases the risk of various forms of cancer. Epithelial Ovarian Cancer (EOC) presents a strong case of multilevel cancer heterogeneity, where Mitochondrial-OxPhos-hi and Glycolysis-hi metabolic subtypes have been reported. Although >80% of the EOC patients respond to initial platinum-taxane based chemotherapy and surgery, disease relapse limits the 5-year survival to ~40-50%. Diabetes and obesity is linked to both increased EOC incidence and relapse. Inherently chemo-resistant ovarian-Tumor-Initiating-stem-Cells (ovTICs) are known to be major contributors of relapse, as they proliferate and differentiate into the Bulk-Tumor-Cells (BTCs). Problem statement and rationale: A major focus of current cancer research is to identify metabolic vulnerabilities for building precision therapeutic strategies. Various metabolic inhibitors, like the anti-diabetic drug Metformin, are being tested for their repurposing potential in cancer therapy. Majority of the clinical trials of Metformin failed to demonstrate any significant benefit for all patients, highlighting the critical need of patient selection from a heterogenous patient pool for successful drug repurposing. Certain ovTICs have been reported to be sensitive to Metformin in clinical settings. We reasoned metabolic heterogeneity is the underlying reason of the failure in repurposing clinically relevant metabolic inhibitors like Metformin. Key research question(s): Our preliminary data from cell lines and patient tissues, using single cell, spatial and computational biology approaches, provide proof of concept of the following overarching hypothesis : In comparison to the non-diabetics, prior metformin use in diabetics alters their tumor metabolic landscape established by the ovTICs, understanding of which can be leveraged towards predicting the individual cases where Metformin will improve treatment outcome by eliminating ovTICs. Objectives with methodology: Hypothesis will be tested holistically on EOC patient tumor cells, by a team of biologists, physicists, computer scientists and clinicians, with the following objectives: - Quantitative spatial biology approach for characterization of the Mito-hi and the Glyco-hi ovTICs and ovBTCs, and correlating the findings to prior use of Metformin. - Quantitative single cell and single organelle based elucidation of mechanism of exact metabolic vulnerabilities of Mito-hi and the Glyco-hi ovTICs and ovBTCs isolated from patient groups with different prior use of Metformin. - To develop and deploy classical mathematical and statistical models and simulations, augmented by AI-ML based tools, on the acquired data to predict which patient may benefit with repurposed Metformin or similar metabolic inhibitors. 1. Expected outcomes: With a multidisciplinary approach, we will design a rationalized drug repurposing framework involving cutting edge quantitative models and simulations built from deep scientific understanding of metabolic heterogeneity of EOC patients. Such an approach will allow precise personalized interventions with metabolic inhibitors, like Metformin and others. The collaborative participation of a high volume tertiary care clinical institute and a research oriented university with state of the art facilities promises to bring high translational impact.	
Priority Area	Non-Communicable Diseases (NCD) / Cancer
Keywords Six keywords separated by comma which best describe your project may be provided.	Tumor cell energy-metabolism, mitochondrial biology, cancer metabolic heterogeneity, diabetes and cancer, personalized cancer therapy, quantitative cell biology,
Abbreviations: List of all the abbreviations (maximum of 10) used in the proposal may be provided	
ovTICs: Ovarian Tumor Initiating(stem) Cells	

ovBTCs: Ovarian Bulk Tumor Cells

EOC: Epitheial Ovarian Cancer

Mito-hi: Mitochondria high (more dependent on mitochondrial oxidative phosphorylation)

Glyco-hi: Glycolysis high (more dependent on cytosolic glycolysis)

OxPhos: Oxidative Phosphorylation

WSI: Whole slide imaging

H&E: Hematoxylin Eosin

PFS: Progression Free Survival

FFPE: Formalin Fixed Parafin Embedded

Background, including problem statement, and rationale of study (up to 1000 words)

Problem statement:

Various levels of heterogeneity poses serious challenges in cancer management¹. Therefore, targeted / personalized therapeutic strategies are being developed to overcome such cancer heterogeneity². Attacking the metabolic vulnerability of cancer cells has gained attention worldwide as a way to improve cancer treatment³.

Patients with prior use of the antidiabetic drug Metformin had significantly better treatment outcome than others, which led to investigation of Metformin as an anticancer therapeutics in EOC and other cancers⁴⁻⁶. However, clinical trials, including those in Indian cohorts, have failed to validate the cancer therapeutic benefit of Metformin⁷⁻⁸. Nonetheless, it has been highlighted that Metformin may be beneficial to some patients and approaches are need to identify those patients⁹.

Similar to other cancers, metabolic subtypes of EOC has been identified^{10-12 13 14}. A promising Phase II clinical trial in EOC reported success of Metformin in targeting ovarian tumor initiating stem cell (ovTICs) that differentiate into the bulk tumor cells (BTCs)¹⁵. Based on our preliminary data on EOC cell lines and patient samples, we posit the cancer patients who will benefit from Metformin or similar metabolic inhibitors can be identified and predicted from the metabolic make up of their ovTICs.

Connection of Cancer to Diabetes, and repurposing antidiabetics:

Rewiring of metabolism and energetics is a general hallmark of tumor cells while metabolite dependencies of cancer cells may vary¹⁶. The hyperglycemic and hyperinsulinemic environment of diabetic and obese patients predispose them to cancer¹⁷. Therefore, repurposing blood glucose lowering drugs, like Metformin, GLP1 receptor agonists and others, are being proposed for cancer risk reduction and also cancer therapeutics⁵. However, recent findings ruled out any overall therapeutic benefit of Metformin in cancer therapy. In the MA.32 clinical trial for breast cancer, only some genetically distinct patients were found to have some therapeutic benefit with Metformin⁹. Therefore, repurposing any antidiabetics may eventually face similar hurdles as Metformin.

Metabolic heterogeneity in EOCs and ovTICs:

EOC incidence (%) is significantly higher in India than in Western countries and the EOC cases in India are on the rise according to ICMR registry¹⁸. Although >80% of the EOC patients respond to initial platinum-taxane based treatment, disease relapse limits the 5-year survival to 40-50%. Based on energy metabolism, EOC cells can be Mito-hi (mitochondrial-OxPhos-dependent) or Glyco-hi (Glycolysis-dependent, due to OxPhos-lo status). Distinct ovTICs are vulnerable to inhibition of either OxPhos¹⁹ or glycolysis²⁰. Inhibition of lipid metabolism has also been proposed as a specific treatment option of EOC²¹. High-OxPhos and Low-OxPhos subtypes of EOC can be identified quantitative imaging methods towards predicting treatment response^{10,22}. We reported a metabolic regulator based gene expression signature that partitions EOC patients into two groups of distinct survival²³. Identification of the metabolic subtypes in any Indian cohorts remains an open question, but there is interest²⁴.

Repurposing metabolic inhibitors for ovTIC elimination during EOC treatment:

EOC treatment remains challenged by chemoresistant relapse that happens as inherently resistant ovTICs differentiate to form the ovBTCs. Although various strategies for targeting TICs have demonstrated some promise in preclinical studies, clinical success is limited²⁵. Repurposing potential of various metabolic inhibitors as cancer therapeutics is being widely tested given their prior characterization and available knowledge available. Notably, Metformin, the poster child of drug repurposing for cancer metabolism, has shown some promise for targeting ovTICs in Phase II clinical trial in EOC¹⁵. Although, Metformin (direct inhibitor of mitochondrial Complex I²⁶) and other metabolic inhibitors are being tested in clinical trial for TIC elimination^{3,26}, it is clear they would not benefit all patients⁹. Appropriate patient selection appears to be critical for successful use of metabolic inhibitors in any chemotherapeutic regimen.

Computational modeling in personalized cancer treatment:

Systems biology approaches, including the multidimensional high throughput approaches, have revealed the complexities of cancer as a dynamic disease²⁷. Mathematical modelling, in tune with experimental validation, has been proposed as the major way to tackle such challenges²⁸. Large scale projects have been undertaken towards improving cancer therapy by understanding individualistic behavior of a patient's tumor in the context of the patient's overall health status. The promise of quantitative cell biology in application oriented understanding disease fundamentals is evident in the conceptual framework of various virtual cell projects (ex: The Alpha Cell, State etc), including cell division and metabolism²⁹. More recently, AI driven virtual cell, AIVCs, have been proposed to aid creation of digital twins for ultimate success of personalized therapy³⁰.

Rationale:

Fundamental studies of EOC is scanty compared to the genomically similar breast cancer. Our proposal is rationalized on fundamental science from our lab demonstrating mitochondrial regulation establishes heterogeneity in tumor initiating stem cells^{31 32 33}. The switch between Mito-hi and Glyco-hi energy metabolism can be brought about by interaction of the mitochondrial modulator Dynamin Related Protein 1 (**Drp1**) with core cell cycle regulators towards modulating stemness^{23,34}. Activation of Drp1 driven structural change of mitochondria attenuates mitochondrial OxPhos (and metabolite usage) and enhances glycolysis, which is reversed by Drp1 repression³⁵⁻³⁷. Using our multidisciplinary tools to study properties of Drp1 we have classified EOC cell lines and patients into Mito-hi and Glyco-hi, confirmed their energy metabolism status and demonstrated the vulnerability specific ovTICs to Metformin (**Preliminary data 1 and 2**). More at the fundamental level, we demonstrated how Drp1 could be involved in bringing about the metabolic change by converting one form of mitochondria to a specific form to support TICs, which can be potentially simulated by mathematical modelling and can be predicted by AI-ML tools (**Preliminary data 3**). Therefore, we propose to generate quantitative data to understand the link of Mito-hi and Glyco-hi ovTICs from individual EOC patients with their clinical data. Thereafter, we aim to develop quantitative cell biology based AI augmented mathematical/statistical tools to model their metabolic vulnerability. The proposed retrospective and prospective exploratory study of EOC patients is aimed towards predicting their overall survival improvement by inclusion of precisely tuned levels of metabolic inhibitors, like Metformin or similar, in their chemotherapeutic regimen.

Key Research question(s):

The results of Metformin in various clinical trials clearly demonstrated that metabolic inhibitors cannot be used for all patients, while some clearly have benefits. This trend is also demonstrated in our preliminary data obtained with a holistic multidisciplinary manner from cell lines and patient tissues, using single cell, spatial and computational biology approaches. Our preliminary data provide proof of concept of a rationalized design principle for including metabolic inhibitors to specifically eliminate ovarian tumor initiating cells (ovTICs) in a targeted combinatorial manner. This personalized approach is expected to improve therapeutic outcome of EOC cancer patients. Towards this goal, we specifically aim to ask the following questions:

1. Can prior metformin use in diabetics alter the ovTIC driven metabolic landscape of tumor tissues as compared to the non-diabetics without it, which impacts their treatment outcome?
2. Can the knowledge of the impact of prior metformin use on the tumor metabolic landscape be leveraged towards identifying the right cases where Metformin may be beneficial for eliminating ovTICs?
3. Can quantitative cell biology based computational predictive tools be developed and deployed to repurpose Metformin or similar metabolic inhibitors for eliminating ovTICs in a personalized manner?

Study Objectives: Define the objectives (maximum 4): The objectives should be SMART (Specific, Measurable, Achievable with the project's budget and time, resourced within the project's budget, and time bound)

Study Objective (1): Study Objective 1. We hypothesise that prior metformin use in diabetics alters the ovTIC driven metabolic landscape of tumor tissues as compared to the non-diabetics without it, which impacts their treatment outcome. Our preliminary audit data suggested nearly 20 months of platinum free survival advantage for those who were on metformin compared to those who were not (without adjustment of confounders). This trend has been reported in various other patient cohorts, which led to investigation of Metformin as an anticancer therapeutics. However, researchers have failed to validate the cancer therapeutic benefit of Metformin in clinical trials due to marked heterogeneity in patient groups. Nonetheless, some clinical success has been reported for Metformin's ability of eliminating ovarian tumor initiating stem cell (ovTICs) that differentiate into the bulk tumor cells (BTCs). Therefore, we aim to revisit the impact of Metformin on epithelial ovarian cancer (EOC) in the context of ovTIC driven cancer heterogeneity. Here, the stated hypothesis will be tested by quantitative spatial biology approach to quantify and characterize metabolically heterogeneous ov(TICs) and correlating the findings to prior use of Metformin.

Study Objective (2): Study Objective 2. We hypothesize that the knowledge of the impact of prior Metformin use on the tumor metabolic landscape be leveraged towards identifying the right cases where Metformin may be beneficial for eliminating ovTICs. Metabolic subtypes of EOC have been reported in multiple studies. The mitochondrially active (Mito-hi) and glycolytically active (Glyco-hi) groups are differently sensitive to metabolic inhibitors based on our preliminary data. Literature indicates the antidiabetic drug Metformin can eliminate ovarian tumor initiating cell (ovTICs) that differentiate into the bulk tumor cells (ovBTCs). However, researchers have failed to validate the cancer therapeutic benefit of Metformin in clinical trials due to marked heterogeneity in patient groups. Therefore, we aim to identify the right cases where Metformin may be beneficial by untangling cancer heterogeneity that requires approaches to study single units. Here, the stated hypothesis will be tested using single cell and single organelle based characterization of relevant metabolic properties and vulnerability of ovTICs and ovBTCs isolated from the two patient groups with different prior exposure to Metformin.

Study Objective (3): Study Objective 3: We hypothesize that quantitative cell biology based computational predictive tools can be developed and deployed to repurpose Metformin or similar metabolic inhibitors for eliminating ovTICs in a personalized manner. Metabolic subtypes of EOC have been reported in multiple studies. The mitochondrially active (Mito-hi) and glycolytically active (Glyco-hi) groups are differently sensitive to metabolic inhibitors based on our preliminary data. The antidiabetic drug Metformin can eliminate ovarian tumor initiating cell (ovTICs) that differentiate into the bulk tumor cells (ovBTCs). However, researchers have failed to validate the cancer therapeutic benefit of Metformin in clinical trials due to marked heterogeneity in patient groups. Therefore, we aim to identify the right cases where Metformin may be beneficial, by untangling cancer heterogeneity that requires quantitative approaches to study single units. Here, the stated hypothesis will be tested by classical mathematical and statistical modeling, augmented by AI-ML based predictive tools, to be deployed on data from acquired Objective 2 towards identifying patients who may benefit with repurposed Metformin or similar metabolic inhibitors.

Methodology

Describe the research methods that could best achieve the study objectives under the following sub-headings (as applicable):

Study Design :

Study Objective 1: (collaboration between clinicians, clinical labs and quantitaive cell biologists)

The metabolically heterogeneous ovTICs can be classified into mitochondrially active (Mito-hi) and glycolytically active (Glyco-hi), confirmed in our preliminary data. This retrospective study will aim to perform molecular analyses of preserved patient tumor sample blocks to identify and quantify the abundance of Mito-hi and the Glyco-hi ovTICs and correlate the quantitative findings with relevant clinical and health data from prospectively collected electronic medical records (EMR) at Tata Medical Center Kolkata (TMCK) Hospital Management System (HMS) database.

The aims will be executed in the following steps:

SubAim 1.1: Confirmation of difference in treatment outcome (Progression free survival, PFS) in patients with EOC between non-diabetic patients not taking metformin as their regular medication and diabetic patients using regular metformin. The diabetic status and Metformin prescription history will be available in the patients' first consultation document recorded in the electronic medical records (EMR). Almost all of the diabetic patients treated at TMCK are on metformin, therefore the small group diabetic group without Metformin will not be considered. All available patient data (N>1200) from the EMR database will be used. PFS assessments will be done with adjustments of confounding factors (Age, BMI, Race, Disease Stage, Platinum sensitivity, any other medication, molecular markers from biopsy).

SubAim 1.2: Quantify the Mito-hi and the Glyco-hi ovTICs, and their respective ovBTCs, in the patients with prior metformin use (diabetic) and without it (non-diabetic) using Whole Slide Imaging of immunostained FFPE samples of primary tumors.

SubAim 1.3: Detailed spatial characterization of the Mito-hi and the Glyco-hi ovTICs, and their respective BTCs, by studying their abundance and intratumor spatial organization, in FFPE samples of primary tumors of patients with prior metformin use (diabetic) and without it (non-diabetic).

SubAim 1.4: The abundance of Mito-hi and the Glyco-hi ovTICs, and their respective BTCs, will be quantitatively correlated with various relevant demographic and clinical data of the patients and compared between the non-diabetic (without metformin) and diabetic (with metformin) patients.

Study Objective 2: (collaboration between clinicians, clinical labs and quantitaive cell biologists)

This prospective study will aim to quantitatively study multi-level metabolic heterogeneity: inter-patients, inter-cellular and intra-cellular within the metabolic organelles, mitochondria, that has been proven to be the primary site of action of Metformin (also confirmed in our preliminary data, Figure 2). Understanding such metabolic heterogeneity will be useful to identify vulnerability of the tumor cells of each patient to metabolic inhibitors, like Metformin. Thereafter, the overall findings will be correlated with treatment outcome and patient information documented in the electronic medical records (EMR).

Primary ovarian tumor cells will be isolated from fresh tumor tissue obtained from upfront debulking surgery and will be banked thereafter. The following 'live cell' profiling methods will be used on the flowcytometrically isolated ovTICs-Aldh+ and ovBTCs-Aldh:

SubAim 2.1: Each patient will be classified into Mito-hi or Glyco-hi groups by performing bioenergetics profiling of their live cells. To identify the carbon source dependence of each patient's cells, relevant carbon sources, like glucose, chosen fatty acids and amino acids based on our preliminary data on genetically modified cells, will be tested. Similarly, impact of clinically relevant metabolic inhibitors (Metformin, Phenformin and others) on mitochondrial and glycolytic energetics each patient will be tested to. This method will also test the plasticity of tumor cells i.e switch from mitochondrial to glycolytic or vice versa with the metabolic inhibitors.

SubAim 2.2: The heterogeneity of energy metabolism will be profiled using our (published) microscopy based quantitative methods of Mito-SinCe (for single cells) and Mito-SinComp (for single mitochondria) (ref 31 and 33).

Sub Aim2.3: The vulnerability of cells of each patient to Metformin, Phenformin and other clinically relevant metabolic inhibitors (used in SubAim2.1) will be assessed. Two standard cell viability assays, namely crystal violet staining and PI exclusion, will be used for reproducibility.

Sub Aim2.4: Quantitative findings from patient tumor cells will be quantitatively and qualitatively correlated with Progression Free Survival (3 years) and other disease parameters, including any link with prior use of Metformin.

Study Objective 3: (collaboration between quantitaive cell biologists, biophysicists and computer scientists)

This aim will be particularly focused on the Mito-hi cases who are expected to be more sensitive to Metformin and similar metabolic inhibitors (preliminary data, Figure 1 and 2). This study will aim to perform predictive mathematical and statistical modelling and simulation of the microscopy based data on mitochondrial structural and predicted functional forms in classified Mito-hi and Glyco-hi patient samples (Objective 2). Outcome of such modeling will be correlated with collected patient data, including their prior use of Metformin (diabetics) and no use (non-diabetic). The purpose of the modeling would be to precisely identify patients that may benefit from an alternative cancer therapeutic regimen involving inclusion of Metformin or similar metabolic inhibitors, using the follwoing two independent subaims with distinct approaches:

Methodology
Describe the research methods that could best achieve the study objectives under the following sub-headings (as applicable):
SumAim 3.1: (collaboration between quantitaive cell biologists and biophysicists) To model and simulate the impact of metabolic inhibitors, like Metformin, on key subcellular changes that can sustain the Mito-hi status and its vulnerability: Our various published findings imply that the distinction between Mito-hi and Glyco-hi ovTICs result from an altered interaction of mitochondria with cell cycle apparatus that is targeted by the standard chemotherapeutic treatment of platinum-taxane. We also confirmed such finding in ovTICs isolated directly from EOC patients (Fig. 1G). This chemotherapeutic combination disrupts two distinct cell cycle apparatus, namely DNA replication and mitosis. Reorganization of the cellular microtubule networks (disrupted by taxane) leads to the transitioning of DNA replicated cells to mitosis. Importantly, such microtubule reorganization is crucially linked to energy-metabolism requirements of cell division. We found that key mitochondrial changes that underlie the Mito-hi and Glyco-hi status can be potentially simulated and modelled (Fig. 3C-D). Therefore, we will use mathematical modelling to simulate key aspects of interactions of mitochondria and microtubules that could render a cancer cell Mito-hi and Glyco-hi. We will also simulate the disruption of key aspects of such interactions with Metformin and other metabolic inhibitors in various combinatorial possibilities with Platinum-Taxane to predict the dose and window of therapeutic success. We will further match the predictions from this model with data obtained on their vulnerability to metabolic inhibitors with or without platinum-taxane. The model can be potentially refined based on observational data obtained from the patients, and kinetic experiments will be performed in appropriate cell lines as needed. SubAim 3.2: (collaboration between quantitaive cell biologists an computer scientists) To use AI-ML modelling for the development of microscopy based biomarker for identification of Mito-hi cells: The functional ability of mitochondria can be reasonably predicted from using a machine learning regression model (Figure 3B). We will aim to develop a deep learning approach using a publicly available rich microscopy data set that is amenable to our analyses of single mitochondrial components (already downloaded from Allen Institute of Cell Science). The aim of such a deep learning model will be to identify features that can classify the cells based on their mitochondrial states linked to their Mito-hi status (preliminary data). Thereafter, we will use these features on the microscopy data on mitochondria obtained from patients (Objective 2) for training, testing and generalizing the identifiable distinction of the Mito-hi cells from the Glyco-hi cells. This will be validated with WSI based classification of the Mito-hi cases (Objective 1). Study Area or Settings : Study Objective 1: Ashoka University (AU) will access preserved Formalin Fixed Paraffin Embedded (FFPE) samples from Tata Medical Center, Kolkata (TMCK) to perform whole slide imaging (WSI). The partners will work together to obtain the necessary organizational approvals. At TMCK Slides with consecutive tissue sections ((8-10 um thick) will be prepared from preserved FFPE blocks of chosen patients. Thereafter, all slides will be shipped to AU in slide boxes at room temperature. The following steps will be followed at TMCK: 1. Screening to To identify those who will fit the inclusion and exclusion criteria. 2. Quality check of the preserved FFPE blocks in the departmental archive to identify the usable ones 3. Labeling to match the tissues for experimental study with clinical data with unique study ID in REDCap 4. Data tabulation to complete the data collection form. At AU: For each patient, consecutive sections will be processed for : 1. Parallel immunostaining with relavnet molecular markers and H&E staining of consecucitve tissue section, using 2 technical replicates. 2. Whole slide imaging of the slides processed in (1). 3. Quantification of WSI of ovTICs and BTCs (based on our optimized protocol) 4. Relevant personnel from TMCK and AU will work together for integrated analyses and interpretation of the spatial organization of the ovTICS and ovBTCs. Here, molecular and pathological determinants will be matched for interepreation. Study Objective 2: This prospective study will require documentation of patient records, biobanking of tumor cells for downstream analyses. TMCK and AU will work together to obtain the necessary organizational approvals. TMCK: Primary ovarian tumor cells will be isolated from fresh tumor tissue obtained from surgical resection or core biopsy specimen of the tumor patient consenting to participating in the study after elaborate counselling and completing ethical committee mandated guidelines. The tissue will be obtained from patients prior to any treatment with chemotherapeutic or targeted agents and biobanked following standard protocols. In brief, enzymatic digestion of the tumors will be undertaken to obtain tumor cells that will be cryopreserved with labels having deidentified unique identification numbers (UID). The cryopreserved cells will be cryo-shipped to AU in large batches, as requested by AU. The patient data will be recorded following standard approved procedures and shared with deidentified UID with AU as requested. AU: Cryo-preserved cells will be revived into a culture following standard protocols. Thereafter, the cells will be subjected to ALDH staining and flow cytometric sorting to isolate ovTICs(Aldh+) and ovBTCs(Aldh-). Thereafter, proposed experiments will be performed with freshly sorted cells. Such optimized flow cytometric protocol and some downstream analyses has been already successfully tested on ovarian cancer patient cells (Preliminary data, Figure 2). The deidentified patient data will be correlated with the cell biological findings. Study Objective 3: (collaboration between quantitaive cell biologists, biophysicists and computer scientists) Ashoka University personnel will use the microscopy data generated at Ashoka University facility with patient samples obtained from TMCK biobank (see Objective 2). TMCK will share relevant patient information from their health records (in appropriate encrypted format) with Ashoka University personnel.

Methodology

Describe the research methods that could best achieve the study objectives under the following sub-headings (as applicable):

Study Subjects :

Study Objective 1:

The study will include only the diabetic patients with comparable use of Metformin that will be compared with the non-diabetic patients without metformin. Propensity score matching will be undertaken for the non-diabetic patients to appropriately match the demographics and other confounding factors related to treatment with those of the known diabetic patients to minimise any selection bias. From this filtered pool of patients, tissue samples will be chosen from patients to allow technical success of all the molecular techniques to be followed. The most recent samples from the patents will be used for correlation as appropriate.

Inclusion: All patients of epithelial ovarian cancer who were diagnosed at TMCK and completed first-line treatment including chemotherapy and surgery where the following information are available:

1. Comprehensive documented history of comorbidities and use of metformin
2. Follow up data of at least 12 months
3. Adequately preserved FFPE blocks from tissues obtained during the initial biopsy (Prior to the use of any chemotherapy)

Exclusion

1. Non-epithelial ovarian cancer or patients with previous history of other cancers.
2. Diabetic patients with no use of Metformin.
3. Those who did not receive complete treatment at TMCK
4. Women presented with recurrence having had first line treatment elsewhere
5. Disease progression on initial chemotherapy (Where neoadjuvant chemotherapy [NACT] was used
6. Not fulfilling criteria 1-3 in inclusion section

Study Objective 1:

The following are the inclusion and exclusion criteria for the study population for this prospective study:

Inclusion

1. Patients of epithelial ovarian cancer (EOC) who are diagnosed and being treated at TMCK.
2. Patients with documented history of comorbidities and use of metformin to be included as one experimental group.
3. Patients with no history of diabetes or metformin use will be included in another group.

Exclusion

1. Non-epithelial ovarian cancer or patients with previous or synchronous other cancers.
2. Diabetic patients with no use of Metformin.
3. Those who did not receive complete treatment at TMCK
4. Women presented with recurrence having had first line treatment elsewhere
5. Disease progression on initial chemotherapy (Where neoadjuvant chemotherapy [NACT] was used.
6. Not fulfilling criteria 1-3 in inclusion section
7. Patients under the age of 18.

The diagnosis of epithelial ovarian cancer is confirmed either from the core biopsy of the tissue and/or during upfront surgery. Primarily, abundant fresh tissue from upfront debulking surgery will be used for biobanking. However, core biopsy will remain the only source of tissue for nearly 50% of our patients with advanced (Stages IIIC to IVB) EOC who receive neoadjuvant chemotherapy. Multiple passes core biopsy from metastatic tumour tissue are taken and utilised for histopathology, immunohistochemistry and molecular studies. Fresh tissue from the fifth pass of the biopsy will be used for biobanking, if needed.

Study Objective 3: (collaboration between quantitaive cell biologists, biophysicists and computer scientists)

The study will include patient and analytical data collected in Objective 2. Therefore, the patient groups will be the same as Objective 2.

Interventions :

NA

Comparison :

Study Objective 1:

The multilevel comparisons are as follows:

1. Comparing survival data from the non-diabetic (without metformin) and diabetic (with metformin) patients.
2. Whole slide imaging based quantification and organization of Mito-hi ovTICs and BTCs with Glyco-hi ovTICs and ovBTCs in each patient to categorize them Mito-hi or Glyco-hi tumors. Organization will be studies based on hispathological determitants.
3. Comaring the organization of ovTICs and ovBTCs in each patient cases, based on hispathological determitants.
4. Comapring the histopathology of the Mito-hi and Glyco-hi cases.

Study Objective 2:

The multilevel comparisons are as follows:

1. Comparing the ovTICs and BTCs between patients to confirm their Mito-hi or Glyco-hi bioenergetic profile, carbon source utilization, and their cellular and mitochondrial heterogeneity.
2. Compare the ovTICs and BTCs of each patient to determine whether they have the similar or different properties of their energy metabolism and vulnerability to relevant metabolic

Methodology
Describe the research methods that could best achieve the study objectives under the following sub-headings (as applicable):
inhibitors, including Metformin. - Compare the measured properties of diabetic patients (with comparable prior use of Metformin) and non-diabetic patients (no Metformin). - Compare the concordance of classification of bioenergetic analyses and WSI molecular analyses (Objective 1). - Compare 3 year PFS among those who were on metformin and those who were not based on the biomarkers mentioned above.
Study Objective 3: (collaboration between quantitaive cell biologists, biophysicists and computer scientists) Sub Aim 3.1: (collaboration between quantitaive cell biologists and biophysicists) We will simulate mitochondrial metabolism status of Mito-hi and identify the transition points of conversion to Glyco-hi states, and how microtubule interaction can facilitate or inhibit such transitions. We will also compare the simulations of the impact of different chemotherapeutic combinations with or without mitochondrial inhibitors on the Mito-hi, using Glyco-hi states as negative controls. Each quantitaive outcome of each combination will be studied with statistical approaches to discover regulatory patterns. Sub Aim 3.2: (collaboration between quantitaive cell biologists and computer scientists) We will compare the accuracy of the classification of mitochondria with a model trained on data downloaded from the Allen Institute of Cell Science. The comparison will be done among different deep-learning-based methods. We will compare the accuracy parameters of the prediction of Mito-hi ovTICs by the deep-learning-based setup with the actual ground truth. The comparison is done among feature sets extracted from the best deep-learning-based method of step.
Primary and secondary outcome measures : Study Objective 1: Primary outcome (Quantitative): Median Progression Free Survival difference (Quantitative) between non-diabetics not using Metformin and diabetics using Metformin. Rationale: The premise of our study is progression-free survival difference between non-diabetic (without metformin) and diabetic (with metformin) patients will allow us to investigate the underlying differences of the metabolism of the ovTICs and ovBTCs potentially due to prior Metformin use. Our preliminary audit data suggested nearly 20 months of platinum free survival advantage for those who were on metformin compared to those who were not (without adjustment of confounders) (Figure 1A). In a separate study we reported statistically significant differences in median progression free survival of HGSOc patients that were partitioned based on genomic signatures that are expected to be different between patient groups considered here (Figure 1B). Therefore, will aim for the quantitative measure of Median Progression Free Survival difference of patients between the groups at question. Secondary outcome (Quantitative): Differential abundance and/or spatial organization of Mito-hi and Glyco-hi ovTICs and ovBTCs from each patient under consideration will allow classficiation into Mito-hi and Glyco-hi groups, which will correlate to their prior use of Metformin. Each patient may have both metabplic types of ovTICs with one predomanant type. The ovTICs and ovBTCs of each class may also show metabolic heterogeneity.
Study Objective 2: Primary outcome (semi-quatitative): Difference in bioenergetic profile supports differential lipid and glucose utilization of the sorted ovTICc(Aldh+) and/or ovBTCs(Aldh-), which makes them differentially sensitive to Metformin and similar metabolic inhibitors. Mito-hi ovTICc(Aldh+) are anticipated to be dependent on lipids while Glyco-hi ovTICc(Aldh+) on glucose. Rationale: The premise of our study is progression free survival difference between non-diabetics without metformin use and diabetics using metformin could be due to underlying differences of the metabolism of the ovTICs and ovBTCs potentially due to prior Metformin use. We have provided evidence of classification of EOC cell lines into Mito-hi and Glyco-hi cases and corresponding differences in vulnerability to Metformin and similar metabolic inhibitors. We also demonstrated that inclusion of Metformin makes Glyco-hi cases chemoresistant. Metformin has been shown to induce cell death in ovTICs. Therefore, will aim for the quantitative measure of Median Progression Free Survival difference of patients between the groups at question. Secondary outcome (quantitative): - Quantitative differences in Inter-cellular and intracellular heterogeneity of the ovTICs and/or ovBTCs between Mito-hi and Glyco-hi groups explains the mechanism of their level of vulnerability to combinatorial therapeutics with metabolic inhibitors. - Three year PFS comparison among the Mito-hi and Glyco-hi groups.
Study Objective 3: (collaboration between quantitaive cell biologists, biophysicists and computer scientists) Both modeling and simulation aims are independent, although they will be using the same data sets for validation purposes. Thus the following are primary outcomes of each subaim are as follows: Sub Aim 3.1: (collaboration between quantitaive cell biologists and biophysicists): Computational models and simulations, after experimental validations, will help us understand and predict the outcome of the interaction of chemotherapeutics and metabolic inhibitors on the Mito-hi state, using Glyco-hi state as negative control. Such an approach will help fine tune the combinations of chemotherapy and metabolic inhibitors in each patient, that can be further experimentally validated. Sub Aim 3.2: (collaboration between quantitaive cell biologists and computer scientists): Crossvalidated, generalized and explained deep learning based classification model that will predict the Mito-hi cases by the presence of features of the characteristic mitochondrial form (biomarker) present in the Mito-hi OvTICs of this group. The negative control Glyco-hi group may also reveal distinct characteristics, which can be further evaluated for biomarker potential.
Sample Size estimation and sampling strategy : Study Objective 1: The sample size is calculated based on our previous observation of a median survival difference of 7 months between potential Mito-hi and Glyco-hi TCGA-HGSOc cases (Figure 1B). We calculated the sample size for this cohort as follows : - Median PFS of overall cohort (control): 45 months (TCGA report) - Median PFS of metabolically distinct group (experimental): 35 months (our report) - Type I error : 0.05, Power: 0.75 - Test: 2-sided - Events needed: 125 - Sample size: 284

Methodology
Describe the research methods that could best achieve the study objectives under the following sub-headings (as applicable):
Samples will be chosen from an accrued pool of >1200 patient records, based on inclusion and exclusion criteria mentioned in the study subject section. For WSI study to quantify abundance of Mito-hi and the Glyco-hi ovTICs from FFPE sections, a minimum sample size of 5 is expected to be sufficient for yielding data with 95% confidence, based on the effect size in our preliminary data from from inbred mouse model (not shown). Given the inherent heterogeneity of tumor tissues, we will plan for 20 primary tumors from each metabolic group that will be selected based on best match of health records and maximum survival difference. Study Objective 2: The sample size is calculated based on our previous observation on cell line modes (Figure 2). The difference in bioenergetic profile between the Mito-hi and Glyco-hi EOC cell lines quantitatively matches with their corresponding difference in sensitivity to metabolic inhibitors. We calculated the sample size considering a dichotomous endpoint for two independent study groups : 1. Effect size : 2 fold difference in bioenergetic profile and sensitivity to Metformin 2. Type I error : 0.05 3. Power: 0.75 4. Test: 2-sided 5. Sample size: 100 (50+50) The same statistical power will apply for the secondary outcome given quantitative difference in inter-cellular and intracellular mitochondrial profile is more than double Mito-hi and Glyco-hi cases in EOC cell lines (Preliminary data, Figure 2) Study Objective 3: (collaboration between quantitaive cell biologists, biophysicists and computer scientists) Publicly available data as well as data from Objective 2 will be used. The cross validation of the models will be designed based on data availability. Experimental validations may also be used in certain cases, as needed. Therefore, no sample size calculation is necessary. Data Collection methods : Study Objective 1: Patient data collection methods At TMCK: The secure web based software called Research Electronic Data Capture, REDCap, is used to catalog and manage EMR data at TMCK. This database contains EMR for all patients with epithelial ovarian cancer treated at Tata Medical Center Kolkata (TMCK) between 1st January 2013 and 31st December 2024. A case record form is used to collect patient information. Instruments used at TMCK: Clinical case-record form overview includes the following: Demography (At diagnosis), Disease factors, First Surgery details, Adjuvant Chemo, Follow-up after initial treatment, Repeat Surgery details, Follow-up after treatment of recurrence, Last follow up details Spatial biology data collection methods at AU: Whole slide imaging of immunostained and H&E stained tissue sections: Immunohistochemistry and microscopy will be used to identify the metabolically heterogenous ovTICs and ovBTCs. Fluorescence based immunohistochemistry of pDrp1(S616) and fucntional stem cell marker Aldh1A1 will be performed on FFPE tumor sections following standard methods including use of an antigen retrieval system. The determination is designed based on our previous publications on causative impact of the mitochondrial regulator Drp1 on stemness (see raionale in Bacground section), including Aldh1A1 and Sox2 (Ref 31 and 32). The WSI pipeline has been already optimized and validated in mouse tissues of a different lineage and thus not shown. The corresponding H&E stained sections will also be imaged in brightfield mode to understand histopathological organization of each tissue section. The pipeline is attached as extra document. Instruments used at AU: Fluorescence whole slide imaging will be performed on an automated whole slide scanner at 20X magnification, allowing rapid, high-resolution acquisition of entire tissue sections. High-resolution confocal microscopy with a 63X oil immersion objective, will be performed for chosen regions of interest depending on ovTIC distribution. Measurements: SubAim 1: Median Progression Free Survival between non-diabetic (without metformin) and diabetic (with metformin) patients. This will be calculated from health records using standard methods. SubAim 2: Abundance cells with the the following molecular profile will be recorded: 1. pDrp1(S616)-Aldh1A1+ 2. pDrp1(S616)+Aldh1A1+ 3. pDrp1(S616)-Aldh1A1- 4. pDrp1(S616)+Aldh- Oragnization of the ovTICs and ocBTCs will be qualitatively studied using histopathological identification of tumor core, tumor periphery, angiogenic regions and vasculature, and tumor stromal regions. SubAim 3: Person's correlation between ovTICs and BTCs (as detected in SumAim2) with various clinical information of non-diabetic (without Metformin) and diabetic (with Metformin) patients. Quanlitative information will be confined to scores with well-defiend criteria and further used in this analyses. Study Objective 2: Patient data collection at TMCK: Patient counselling and recruitment formalities will be followed as per Institutional Review Board (IRB) TMCK guidelines. Informed consent will be obtained and an anonymised case record form on the REDCap database will be used to collect relevant patient information, as detailed in Objective 1.

Methodology

Describe the research methods that could best achieve the study objectives under the following sub-headings (as applicable):

Bio-banking at TMCK:

Surgically resected fresh tissue specimens or core biopsy specimens will be transferred to the biobank processing unit on ice cold media within 1 hour. Tissue specimens will be washed in PBS followed by mechanical mincing into smaller fragments and will be subjected to enzymatic digestion using a cocktail mix of enzymes. Thereafter, the digested suspension will be filtered to achieve single cell suspension. Cryopreserved cells will be shipped using certified domestic cold chain courier facility with temperature logger to ensure minimal stress to the samples during transfer.

Quantitative Cell Biological analyses at AU

Flowcytometry based sorting of ovTICs(Aldh+) and ovBTCs(Aldh-) will be performed after Aldefluor staining, as in Figure 2F-G. The following optimized methods will be used for characterization of the sorted patient tumor cells maintained in matrigel in presence of TIC medium or standard RPMI media:

- 1. Cell survival will be assessed with crystal violet staining, as in Figure 2F-G. Dose response curves for the metabolic inhibitors in combination with platinum-taxane (with appropriate controls) will be created for each patient. Thereafter, LD50 will be calculated for each case. This will be confirmed by flow cytometry based PI exclusion assay.
- 2. Seahorse flux analyses based bioenergetic profiling and carbon source utilization, with the metabolic inhibitors in combination with platinum-taxane (with appropriate controls), as in Figure. 2 B-D.
- 3. Our designed Mito-SinCe and Mito-SinComp structural analyses will be performed in live cells, to untangle multilevel mitochondrial heterogeneity, using high-content-confocal microscopy, as in Figure. 2I-J, 3A-C. Time lapse microscopy will be used to study impact of combinatorial therapeutics with metabolic inhibitors. All quatitative structural metrics for Mito-SinCe and Mito-SinComp will be measured (ref 31 and 33). The pipeline is attached as extra document.

Clinical follow up at TMCK

Routine follow up protocol for EOC patients at TMCK will be observed and data entered prospectively in the REDCap database. The date of first proven recurrence will be documented as the patients are on regular clinical, biochemical and if required radiological follow up.

Study Objective 3: (collaboration between quantitaive cell biologists, biophysicists and computer scientists)

Data from Objective 2 will be used. We will only use open source data analyses and management tools as well as algorithms. The source codes are available in GitHub repository and are supported by respective publications.

Data quality assurance methods :

Study Objective 1:

EMR:

The electronic medical records (EMR) in the HMS at TMCK gets input from clinicians and laboratory and radiology services. Once the data is entered in the HMS during clinical interactions or reporting laboratory/radiological results, a competent person authorises it. The data automatically gets transferred to the EMR. This information is permanently stored in the EMR of the HMS with date and time of the authorisation and the name and designation of the person authorising it. Once authorised, the records cannot be amended.

Cellular and molecular data from spatial biology experiments:

Preserved FFPE blocks will undergo standard quality check at TMCK, using H&E staining, before shipping to AU for experimentation. At AU, experimental rigor will be applied for samples processing to obtain high quality data free of experimental artifacts. The data obtained will be passed through internal control and quality checks as per experimental protocols. Technical replicates will be used in every case for reproducibility. Outlier detections will be applied using appropriate statistical tools.

Study Objective 2:

EMR:

The clinical trial coordinator with assistance of the PhD student stationed at TMCK will enter data prospectively into the access secured REDCap database from the time the patients are recruited. The EMR in the HMS at TMCK is permanently stored data with date and time of the authorisation and the name and designation of the person authorising it. Once authorised, the records cannot be amended. The data entered into REDCap will be periodically cross checked with the EMR by the coinvestigators at TMCK who will have the exclusive right to authorise it in the REDCap.

Cell banking: The samples beyond one hour surgical resection will not be processed. Before cryopreservation, cell viability of single cell suspension will be assessed Trypan Blue and Viability >85% and total viable cells >5×10⁶ (for 0.5 g tissue) will be the quality acceptance limit.

Quantitative Cell biological data :

Cells will be revived and viability will be checked with routine tissue culture methods. Samples above 80% viability will be considered. At AU, experimental rigor will be applied for samples processing to obtain high quality data free of experimental artifacts. The data obtained will be passed through internal control and quality checks as per optimized experimental protocols. Technical replicates will be used in every case for reproducibility. Outlier detections will be applied using appropriate statistical tools.

Study Objective 3: (collaboration between quantitaive cell biologists, biophysicists and computer scientists)

Sub Aim 3.1: The simulations will be validated by experimental results in an iterative fashion. New steady state or time lapse microscopy based experiemnts will be performed to validate predictions, necessary.

Sub Aim 3.2: For both deep-learning based tasks (mitochondria classification and Mito-hi ovTIC classification), same data quality assurance methods will be followed. Data will be divided into three disjoint sets: train, validation, and test. Each set will have an equal proportion of Mito-hi datapoints. This ensures that all three sets have similar target distribution. Deep-learning based methods will only be trained on training set, while the best performing model will be picked on the validation set. We will then evaluate the test set to assess the performance quality of the deep-learning based method.

Data management plan :

Methodology

Describe the research methods that could best achieve the study objectives under the following sub-headings (as applicable):

Study Objective 1:

Process of anonymisation followed at TMCK:

1. The initial screening of patients who underwent treatment for epithelial ovarian cancer and are documented to be diabetic, will be identified from HMS records at TMCK from 1st January 2013 till 31st December 2024 constituting the experimental arm. More than 150 patients as per sample size calculation, meeting the inclusion and exclusion criteria will be available in the HMS records. Propensity score matched, a similar number of patients who are not diabetic and treated at TMCK during the same period will be identified and used as a control arm.
2. Each of these patients and their FFP block samples will be labelled with a unique identification (UID) number for the study.
3. The UID number of the study and the medical registration (MR) number of that patient as recorded in the HMS will be listed in a separate secured spreadsheet accessible only to the research team at TMCK.
4. The research team at TMCK will enter the clinical information without any patient identifiable information in the study database with UID. If for any reason, further clinical information will be necessary from the patient record to be added in the study database, the secured spreadsheet will be referred to where the MR number and the UID are matched.

Spatial biology data to be obtained at AU:

Data will be collected from the appropriate platform and will be locally stored in designated storage systems with appropriate encryptions enabled as needed. They will be acquired by and available to only relevant project personnel including the PI and co-PIs, as per need of analyses and discussion. Publication of the findings would require depositing analyzed data to relevant data bases like GEO, and accession number will be included in the publication as per journal requirements.

Study Objective 2:

EMR:

Process of anonymisation is same as Objective 1.

Quantitative cell biological data :

Data will be collected from the appropriate platform and will be locally stored in designated storage systems with appropriate encryptions enabled as needed. They will be acquired by and available to only relevant project personnel including the PI and co-PIs, as per need of analyses and discussion. Publication of the findings would require depositing analyzed data to relevant data bases like Github etc, and accession number will be included in the publication as per journal requirements.

Study Objective 3: (collaboration between quantitaive cell biologists, biophysicists and computer scientists)

Sub Aim 3.1: The simulation data for various tuning optimizations will be compared and saved. This will be crucial for matching the varying possibilities of actual samples.

Sub Aim 3.2: Data from Objective 2 will be used, and no extra management is required for data for training deep-learning based models. For the models, we plan to store the weights of the trained models, which will help in reproducing results at a later stage

Data analysis plan :

Study Objective 1:

EMR data :

Data from the EMR from HMS at TMCK will be exported in appropriate format (*.txt or.csv or *.xcel). All survival analyses will be performed using R package “survival” that includes core survival analysis routines including Kaplan-Meier curves and Cox models for covariate analyses, following our published protocol. Appropriate censorship will be used while following definitions below :

Date of first recurrence: The date when recurrence is confirmed on cross-sectional imaging.

Progression-free interval: PFS is defined as the number of months from the date of last chemotherapy (Not maintenance and/or target therapy) till the date of recurrence or death, whichever is earlier.

Chi square test will be used for statistical analyses for final data interpretation.

Spatial biology data:

WSI images will be analyzed using the optimized pipeline using QuPath software. Identification and quantification of ovTICs and BTCs will be performed were performed with QuPath (version 0.6). First, micrographs will undergo fluorescence signal based thresholding using the open source Fiji / Imaje J platform. The thresholded Images will be further processed on QuPath for background subtraction and normalization to correct for uneven illumination. Thereafter, cell segmentation with nuclei, cytoplasmic boundaries and cell expansion parameter, will be performed Finally, classification and quantification of cell populations will be performed using single measurement classifiers which will be subsequently combined. Finally abundance of 4 populations will be obtained from each WSI analyses and interepreted as follows:

1. pDrp1(s616)-Aldh1A1+ as Mito-hi ovTICs),
2. pDrp1(S616)+Aldh1A1+ as Glyco-hi ovTICs,
3. pDrp1(S616)-Aldh1A1- as Mito-hi BTCs,
4. pDrp1(S616) +Aldh- asGlyco-hi BTCs.

This analysis is based on pur previous findings (ref 32) and is already optimized and validated for Sox2 and pDrp1 for another lineage (thus not shown).

Statistical analyses: The Kolmogorov-Smirnov test, Mann-Whitney U test, Student's t-Test and Chi Square will be used as appropriate. Corresponding p-values will computed and adjusted multiple hypotheses testing using Holm-Bonferroni correction method. Bootstrapping will be used for resampling, as needed.

Methodology

Describe the research methods that could best achieve the study objectives under the following sub-headings (as applicable):

Study Objective 2:

EMR database: Same as in Objective 1

Quantitative cell biological data:

Each quantitative cell biology modality will be analyzed using our optimized and published protocols and reflected in preliminary data (Figures 2 and 3).

a. For bioenergetic profiling, raw OCR and ECAR data will be normalized by the cell content measured by crystal violet staining. Baseline normalization will be used, as necessary for any comparison. The bioenergetic parameters will be calculated from the data of stress test.

b. For mito-SinCe and mito-SinComp assays, confocal micrographs will be processed through in house custom pipelines that include data normalization and statistical evaluations at required steps. Multivariate PLSDA analyses of quatitative structural metrics for Mito-SinCe and Mito-SinComp will be performed (ref 31 and 33) towards identifying any clsutering. Loading weights of the metrics to be determined to understand biological relevance.

Statistical analyses:

All statistical analyses will use appropriate R or Python packages, at AU, as routine done in the lab. The Kolmogorov-Smirnov test, Mann-Whitney U test, Student's t-Test and Chi Square will be used as appropriate. Corresponding p-values will computed and adjusted multiple hypotheses testing using Holm-Bonferroni correction method. Bootstrapping will be used for resampling, as needed.

Study Objective 3: (collaboration between quantitative cell biologists, biophysicists and computer scientists)

SubAim 3.1: (collaboration between quantitaive cell biologists and biophysicists) : We have used a combination of percolation and agent based models state transition models to simulate the interconversion of mitochondrial forms in a manner that matches with experimental data (Figure 3C-D). Therefore, we will refine and tune such an approach to study interaction between mitochondria and microtubules, where each component with be represented on a defined lattice. We will use published parameters and boundary conditions of microtubule dynamics in our simulations, given the richness of biophysical analyses in this area. However, for mitochondrial parameters we will use parameters from few reports with the support of our own data sets and that from Alan Cell Science Center.

SubAim 3.2: (collaboration between quantitative cell biologists and computer scientists): Deep Learning-Based Classification: Using PyTorch on Ashoka University's GPUs, we will employ two strategies: utilizing pre-trained foundations or training networks de novo.

1. Pre-trained Modeling: We will extract features from Cell-DINO (trained on microscopy data) and append a fully connected network to train the final classification model.
2. De Novo Training: We will develop architectures starting with ResNet-50, DenseNet, potentially expanding to transformer-based (ViT, Swin) or iterative inference models (DDPM).

We will incorporate variational components (inspired by Ladder VAEs) to enable sampling-based uncertainty quantification. This allows the model to express confidence levels, a capability absent in non-variational systems. To ensure robustness, we will perform fault-tolerance analysis via synthetic noise injection, simulating real-world imaging variations. Finally, we will use saliency and feature-attribution methods (e.g., Grad-CAM, Layer-CAM) to identify the specific latent channels and spatial regions driving the classification. These features will then be used in Mito-hi ovTIC classification.

Ethical Considerations :

Study Objective 1:

This retrospective study will use de-identified patient data and sampes. All ethical considerations will be made follwing institutionals procedures. We shall apply for a waiver of consent at TMCK as we are handling medical records and tissues only, without any intervention of human subjects. A declaration, however, will have to be given to keep all patient-identifiable information out of our study database, thus de-identifying them. Thereafter, we will apply for approval of the study at AU. Study will commence only after obtaining institutional approvals.

Study Objective 2:

The prospective study will be formed after obatining full scale IRB approval at both the institutions. Approved consent form (in regional languages) and patient informarion form will be used. Th objective will be explained to all women attending the outpatient department of gynaecological oncology with suspected EOC by the treating physicians (PI and co-PIs). The potential participants will be assured that strict confidentiality of the personal identifiable data will be maintained, no additional treatment intervention will be given as a part of this research, and even if the patient declines to participate in the study, her treatment will not by any means be different from those who agreed to participate. Informed consent will be taken from the willing participants adhering to the guideline laid down by the Institutional Review Board (IRB) at TMCK. The clinical trial coordinator will facilitate the process and navigate the participant through different stages of the tissue-collection process and coordinate with biobank for storage and transfer.

Study Objective 3: (collaboration between quantitative cell biologists, biophysicists and computer scientists)

Since we will be primarily using data from Objective 2 and other publicly available data, a declaration to the IRB for the TMCK data will suffice. Also, the consent form will be designed to allow the proposed analyses.

Does you project involve :

Animal Experiments?:

Human as study participants?: Yes

Biosafety concerns e.g. pathogens, recombinant microorganisms, radiations, etc.?:

CDSCO approvals e.g. for investigational new drugs, vaccines, devices, diagnostics?:

Any others (please specify):

Study Objective 1: This retrospective study will use de-identified patient data and sampes.

Study Objective 2: This prospective study will include human participants

Study Objective 3: This computational analyses will use data aquired in Objective 2

Methodology

Describe the research methods that could best achieve the study objectives under the following sub-headings (as applicable):

Project Implementation Plan along with milestone chart (objective wise deliverable & timelines e.g. Gantt/ PERT chart).

The project will be implemented according to the following timeline at TMCK (Tata Medical Ceter, Kolakta) and AU (Ashoka University)

0-6 months activities:

1. IRB approval for both prospective and retrospective studies will be obtained from both collaborating institutions.
2. At TMCK, FFPE tissue selection will be done. All Selected tissues will be sectioned and shipped to AU
3. At TMCK, corresponding EMR data of the selected patient ids will be identified and extracted for survival analyses.
4. At AU, the WSI pipeline for immunostained and H&E stained sections will be performed
5. At TMCK, cells will be isolated from fresh tumor tissues and biobanked.
6. First cryo-shipment of cells will be sent from TMCK to Ashoka at the end of 6 months.
7. At AU, mathematical simulation will be optimized to investigate the interaction of lattices of mitochondria and microtubules
8. At AU, the publicly available microscopy data sets will be downloaded and deep learning models will be training to extract features for classification of mitochondrial form.
9. All updates will be discussed in the first joint meeting between all members of the TMCK and AU team.

7 to 12 months activities:

1. TMCK and AU will work together to perform the survival analyses and adjust for all confounding factors.
2. At AU, the WSI pipeline for immunostained and H&E stained sections will be performed
3. At AU, quantitative cell biological analyses and corresponding data analyses will be performed.
4. At TMCK, cells will be isolated from fresh tumor tissues and biobanked.
5. At AU, mathematical simulation will be optimized to investigate the how the interaction of lattices of mitochondria and microtubules can impact mitochondrial metabolic status
6. At AU, features extracted from deep learning training and testing, will be cross-validated on existing data sets of mitochondrial structure-function and also for prediction of mitochondrial structure to function
7. Second cryo-shipment of cells will be sent from TMCK to Ashoka at the end of 6 months
8. All updates will be discussed in the second joint meeting between all members of the TMCK and AU team.

13-24 months activities:

1. TMCK and AU team will work together match molecular and pathological determinants from the WSI data.
2. At AU, the WSI pipeline for immunostained and H&E stained sections will be performed
3. At TMCK, cells will be isolated from fresh tumor tissues and biobanked.
4. At AU, quantitative cell biological analyses and corresponding data analyses will be performed.
5. At AU, mathematical simulation will be fine-tuned to finalize the interpretation of how the interaction of lattices of mitochondria and microtubules can impact mitochondrial metabolic status. This will reveal the expected functional state of metabolically heterogenous state.
6. At AU, the simulations results will be matched with the actual results obtained from cell biological analyses of the patient samples.
7. At AU, more live cell experiments will be done on patient cells to test the prediction from simulations.
8. At AU, features extracted from deep learning training and testing, will be cross-validated on new data sets of mitochondrial structure obtained from patient cells.
9. Second cryo-shipment of cells will be sent from TMCK to Ashoka at the end of 6 months
10. Publication on Objective 3 will be prepared and submitted on the strategy on deep learning based feature extraction for identifying mitochondrial form and predicting mitochondrial and glycolytic metabolic state of patient tumor cells.
11. Publication will be prepared on Objective 3 on the experimentally validated results of simulation of mitochondrial and cytoskeletal interactions to predict metabolic states of patient tumor cells.
12. Third cryo-shipment of cells will be sent from TMCK to Ashoka at the end of 6 months
13. All updates will be discussed in the second joint meeting between all members of the TMCK and AU team every 6 months.

25-36 months activities:

1. TMCK and AU team will work together to finalize the interpretation of the results from WSI experiments
2. At AU, correlation analyses will be performed with the results of WSI and patient data.
3. Publications will be prepared on the findings of Objective 1 as in 2,
4. At TMCK, cells will be isolated from fresh tumor tissues and biobanked.
5. At AU, quantitative cell biological analyses and corresponding data analyses will be performed.
6. At AU, mathematical simulation will investigate the taxane based perturbation of interaction of lattices of mitochondria and microtubules, which can impact mitochondrial metabolic status.
7. At AU, the simulations results will be matched with the actual results obtained from cell biological analyses of the patient samples.
8. At AU, more live cell experiments will be done on patient cells to test the predictions from simulations.
9. At AU, deep learning training and testing, will be initiated for predicting mitochondrial form from patient cells for biomarker development.
10. Last cryo-shipment of cells will be sent from TMCK to Ashoka at the end of 6 months
11. All updates will be discussed in the second joint meeting between all members of the TMCK and AU team every 6 months.

37-48 months activities:

1. At AU, quantitative cell biological analyses and corresponding data analyses will be performed.
2. At AU, mathematical simulation will investigate the impact of taxane and identified metabolic inhibitors on the interaction of lattices of mitochondria and microtubules, which can impact mitochondrial metabolic status and cell vulnerability.
3. At AU, the simulations results will be matched with the actual results obtained from cell biological analyses of the patient samples.
4. Publication on Objective 3 for the result of 3 will be readied.
5. At AU, deep learning training and testing, cross-validation, generalization will be completed for predicting mitochondrial form from patient cells This will lead to a DL based biomarker development identifying cancer metabolic heterogeneity.
6. Publication on Objective 3 for the result of 5 will be readied.
7. At AU, all quantitative cell biological data will be finalized.

8. Correlation with the patient data and the quantitative cell biological data will be tested.
9. All results from 7 and 8 will be finalized to publish a paper from Objective 2.
10. All updates will be discussed in the second joint meeting between all members of the TMCK and AU team every 6 months.

[Download](#)**Any potential risk & challenges to success of this project and how you plan to address them :**

We will apply all due diligence to de-risk the execution of the project. All proposed analyses and methods are optimized. Therefore, we don't expect significant technical hurdles. Any course change necessary will be taken to ensure understanding of the deep science with maximum rigor, which is essential for successful translation. The following challenges and risks may arise, which will be overcome as elaborated in each case.

Enrollment barriers: Any issues of patient enrollment for Objective 2 will be quickly discussed and mitigated. Should there be hurdles of patient enrolment, we will limit our conclusions according to the adjusted statistical power.

Adequacy of tissue sample: Tissue obtained from upfront EOC surgery will always be abundant. However, those who are unsuitable for upfront surgery, will only have diagnosis based on core tissue biopsy taken through multiple passes. The sample obtained from the fourth and the fifth pass will be made available for the research. There is a small possibility that the patient may decline the fourth and fifth passes because of the experience of pain. Also, the cellularity of the tissue obtained from the final passes may not always have adequate cellularity.

Ethnic or regional influence: Our proposal is based on preliminary data on cell lines and published patients cohorts overrepresented by Caucasians. We will unbiasedly follow the observations obtained from the proposed patient group in TMC-Kolkata, which may reflect regional influences. Nonetheless, our study will reveal the energy metabolic make up of this patient group, which has not been previously studied. This information is also expected to be helpful for a wider community of clinicians in India and abroad.

Re-optimization : In some cases our optimizations are based on cell lines. If necessary, we will allow time for re-optimization for patient primary cells and retune our expectations.

Influence of immune cells: Our expected results may suffer from metabolic heterogeneity influenced by immune cells. This is expected to be reflected in differences in the WSI profile. Accordingly, we will include examining the spatial interaction of the metabolically heterogeneous ovTICs with immune cells in our WSI analyses. 2D vs 3D culture: Should there be inconsistencies in results obtained in 2D culture matrigel, we will perform analyses after forming tumorspheres with our published protocol. In this case, only ovTICs will be considered, given the ovBTCs do not form tumorspheres.

Limitation of simplicity in mathematical models: The mathematical models tend to exclude complexities, which may pose a challenge in understanding the proposed key interactions regulating metabolic heterogeneity. Being mindful of such challenges, we will aim to model simpler events that can be experimentally validated.

Limitation of data for AIL-ML model: The accuracy of the AI-ML model may suffer from sample limitations. In that case, we will generate synthetic data of accurate distributions, following standard methods. The proportion of the synthetic data will be appropriately tuned and guided by cross validations

Expected outcome/ Deliverables from the project i.e., what will be known at the end, if the project achieves all the stated objectives (up to 500 words):

The multidisciplinary approach, by a team of biologists, physicists, computer scientists and clinicians, will design a rationalized drug repurposing framework involving cutting edge quantitative models built from deep scientific understanding of metabolic heterogeneity of EOC patients. Such an approach is expected to allow precise personalized interventions with metabolic inhibitors, like Metformin and others. The specific outcomes are as follows:

Objective 1:Primary outcome (Quantitative):

Median Progression Free Survival difference between non-diabetics not using Metformin and diabetics using Metformin. Testing of the premise that progression-free survival difference between non-diabetic (without metformin) and diabetic (with metformin) patients will allow us to investigate the underlying differences of the metabolism of the ovTICs and ovBTCs potentially due to prior Metformin use.

Secondary outcome (Quantitative):

Differential abundance of Mito-hi and Glyco-hi ovTICs from each patient under consideration and any correlation to the prior use of Metformin in the two patient groups.

Objective 2:Primary outcome (Semi-Quantitative):

Difference in bioenergetic profile supports differential lipid and glucose utilization of the sorted ovTICc(Aldh+) and/or ovBTCs(Aldh-), which makes them differentially sensitive to Metformin and similar metabolic inhibitors. Mito-hi ovTICc(Aldh+) are anticipated to be dependent on lipids while Glyco-hi ovTICc(Aldh+) on glucose.

Secondary outcome (Quantitative):

1. Quantitative differences in Inter-cellular and intracellular heterogeneity of the ovTICs and/or ovBTCs between Mito-hi and Glyco-hi groups explains the mechanism of their level of vulnerability to combinatorial therapeutics with metabolic inhibitors.
2. 3 year PFS comparison among the groups mentioned

Objective 3:

Both modeling aims are independent, although they will be using the same data sets for validation purposes. Thus the following are primary outcomes of each subaim.

Objective 3.1: Computational models and simulations, after experimental validations, will help us understand and predict the outcome of the interaction of chemotherapeutics and metabolic inhibitors on the Mito-hi state, using Glyco-hi state as negative control. Such an approach will help fine tune the combinations of chemotherapy and metabolic inhibitors in each patient, that can be further experimentally validated.

Objective 3.2: Validated, generalized and explained deep learning based classification model that will predict the Mito-hi cases by the presence of features of the characteristic mitochondrial form (biomarker) present in the OvTICs of this group.

With the above specific outcomes, we would achieve the following:

1. Develop a rationalized approach of precise use of chosen metabolic inhibitors in a targeted combinatorial manner to improve therapeutic outcome of EOC patients. This will be achieved by using our novel quantitative cell biology based computational approaches to understand, predict and design appropriate therapeutic combinations.
2. Create a deep science foundation for a future clinical trial effort on testing clinically relevant metabolic inhibitors like Metformin and others, towards ensuring success.
3. Build capacity for engaging in the cutting edge AI driven virtual cell efforts towards driving personalized cancer therapy using metabolic inhibitors or interventions.
4. Publish multiple high impact collaborative manuscripts resulting from successful collaborative of a high volume tertiary care clinical institute and a research oriented university with state

of the art facilities.
Describe as applicable, whether the study is going to generate (100 words each):
a. Publications: We aim to publish multiple high impact manuscripts through our multidisciplinary efforts involving biologists, physicists, computer scientists and clinicians. Collaborative publications arising out of the 3 proposed objectives will reveal various quantitative aspects of metabolic heterogeneity of EOC patients, and build tools of translational relevance. The publications will allow mirroring of the successful approaches in other cancer types in India or abroad, and testing of the fundamental findings in other cancer types.
b. Patents: Not applicable
c. Health Technology
i. Proof-of-Concept : Not applicable
ii. Prototype Development: Not applicable
iii. Final Product, or a validated model: Not applicable
d. Public health impact (impact on public health program guidelines/policies): Not applicable
e. Clinical care impact (impact on clinical care guidelines): Not applicable
Please describe what the immediate next steps after the end of the project if study objectives are successfully achieved.: Each objective of the proposed project can be extended further into the following directions: 1. The success of the PReMQCA-OCS study will lead to planning to obtain funding for a PReMQCA-OCS clinical trial with the same partnership. 2. We will plan to obtain further funding to establish our research capacity with organoids to be able to test relevance of nutrition on modulating the ovTIC driven metabolic landscape using quantitative cell biology approach 3. Using the computational biology capacity built through the project, we will further our goals towards investigating the simulating and predicting the impact of carbon source utilization on chemotherapeutic sensitivity.
Have you taken help of Artificial Intelligence tools to write your proposal? (Yes/No) : no
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PART-B

Details of Preliminary work done by the PI including the source of funding (up to 1000 words):Proof of concept (if any)

Our preliminary data provides support for our hypothesis and our experimental methods. Mito-hi and Glyco-hi stem (like) cells have been detected with immunohistochemistry based Whole Slide Imaging (WSI) and standard histopathology based techniques in other lineages (not shown).

Figure 1. Detection of Mito-hi and Glyco-hi groups in EOC cohorts and cell lines, with their stemness correlating with positively lipid utilization only in the Mito-hi group.

A. Audit data for TMCK-EOC patients from the REDCap database suggested ~20 months of platinum free survival advantage for those who were on metformin compared to those who were not. This will be corrected for confounding factors.

B. K-M survival plot of DBGES classified TCGA-EOC patients show ~7 months difference in median-survival between the DBGES classified potential Mito-hi and Glyco-hi groups ²³. DBGES (Drp1-Based-Gene-Expression-Signature) is expected to reflect gene expression difference between the primary tissues of Mito-hi and Glyco-hi groups.

C. Scatter plot shows potentially Mito-hi patients may undergo elevation of lipid metabolism as part of their chemotherapeutic response, while Glyco-hi patients may undergo a downregulation of the same.

Transcriptomic analyses of tumor tissues pre and post neoadjuvant chemotherapy shows heterogeneity of post-chemotherapeutic Drp1 regulation in UAB-EOC patients, identifying the potentially Mito-hi and Glyco-hi groups. Post-chemotherapeutic increase in Drp1 expression negatively correlates with a concomitant increase in expression of lipid metabolism genes.

D. Overall heat map analyses of data from C shows that potentially Mito-hi and Glyco-hi UAB-EOC primary tumors have different profile of mito-active stemness genes ³².

E. Hub gene plot of WGCNA of differentially expressed genes confirms the Mito-hi and Glyco-hi status of the UAB-EOC patients, as the primary tumors of the former harbors mitochondrial OxPhos gene cluster (predominantly mtDNA genes).

F. We successfully used optimized ALDEFLOUR staining ³¹ on UAB-EOC patient cells and isolated ovTICs-Aldh+ to perform microscopy to detect mitochondria.

G. Flow cytometry based cell cycle analyses of isolated Mito-hi and Glyco-hi ovTICs-Aldh+ from UAB-EOC patients shows distinct cell cycle status between the groups.

H. The DBGES (as in B) can partition CCLE-EOC cell-lines into potentially Mito-hi and Glyco-hi groups, similar to TCGA-EOC cohort.

I. Immunoblot analyses validates Mito-hi and Glyco-hi CCLE groups, by demonstrating expected heterogeneity of post-chemotherapeutic Drp1 regulation in representative cell-lines. This correlates with heterogeneity in the functional stemness marker, Aldh1a1, and the oncogenic driver, Cyclin E, as expected ³⁴; Actin is loading control.

- J. Heat map analyses shows that potentially Mito-hi and Glyco-hi cell-lines exhibit distinct differences in the profile of the mito-active stemness genes in the parental cells.
- K. Volcano plot of metabolites from the CCLE metabolomic data identifies 3 significant metabolites (outlined) that are lower in the potentially Mito-hi CCLE group in comparison to the Glyco-hi CCLE group.
- AX. Altered fatty acid utilization between the Mito-hi and Glyco-hi CCLE groups, as observed in the distribution plots of metabolites from K, suggesting fatty acyl carnitine utilization is more in the Mito-hi group.

Figure 2. Mito-hi EOC cells have elevated OxPhos primarily in their mitochondrial networks, are more sensitive to Metformin and other mitochondrial inhibitors that prevents or induces chemoresistance in Mito-hi or Glyco-hi cells, respectively.

- A. The flowcytometry analyses of TMRE stained cells shows distinct profile of mitochondrial-potential (̳V) between the representative Mito-hi and Glyco-hi ovTIC enriched chemoresistant lines.
- B. Bioenergetic profiling of parental-chemosensitive and derived-chemoresistant cell pairs, using extracellular flux analyses (Seahorse), confirms elevated OxPhos (OCR) in Mito-hi pair compared to the Glyco-hi pair.
- C. Confirmation of Glyco-hi status by higher elevation of ECAR in the Glyco-hi pair compared to the Mito-hi pair after inhibition of mito-ATP synthesis by Oligomycin.
- D. Metformin does not induce metabolic switching in Mito-hi cells, as indicated by its inhibition of OCR with no increase in ECAR.
- E. Metformin preferentially kills Mito-hi Aldh+ ovTICs that show overall higher survival, compared to the Glyco-hi Aldh+ ovTICs. Metabolically distinct ovTICs were isolated from ovarian cancer cell line using our optimized method ³¹.
- F. Mito-hi chemosensitive cells are two time more vulnerable to mitochondrial inhibitors in comparison to Glyco-hi chemosensitive cells. We tested sensitivity of 2 representative cell lines from each partitioned group to Oligomycin and Antimycin (pM doses), Metformin and Niclosamide.
- G. Mitochondrial ATP-synthase inhibitor, Oligomycin, induces resistance to Taxane in Glyco-hi cells, but not in Mito-hi cells. Oligomycin can specifically eliminate ovTICs ³¹.
- H. Oligomycin significantly attenuates acquisition of chemo-resistance in Mito-hi cells, thus can potentially prevent relapse in this group.
- I. Mito-hi cells have ~3 folds higher ATP synthesis in the mitochondrial networks compared to the Glyco-hi cells, revealed by high resolution microscopy based Mito-SinComp quantitative analyses of single mitochondrial structure-function (final revision submitted, ³³).
- J. Mito-hi cells harbor longer mitochondrial components than the Glyco-hi cells, as shown by Mito-SinComp analyses, while Oligomycin reduces length mitochondrial components in both cells.

Skill and experience of the research team: Highlight only salient points (along with 5 relevant publications) that provides confidence to reviewers that the team can implement the project with quality.

The rigorous approaches each of the team member practices in their respective field is expected to result in high quality of the research output.

1. Dr. Mitra (PI, Quantitative Cell Biologist) is an expert on various aspects of energy metabolism and focuses on cancer. Discoveries from her team form the fundamental basis of the proposed translational project. She leads deep science multidisciplinary research integrated with translational goals and successfully collaborates with clinicians and scientists from natural sciences, within India and abroad. Her lab takes an approach of developing quantitative tools to address heterogeneity in energy metabolism in stem cells and their progenies (refs a,b). Therefore her quantitative cell biology niche is particularly important for the proposed project.

Saini M..... **Mitra K.** [Quantitative analyses of single mitochondrial components reveal an early role of small-mitochondrial-networks in priming neoplasticity](#), bioRxiv [Preprint]. 2025 (invited final revision submitted)

Spurlock B, **Mitra K.** [Fine-tuned repression of Drp1-driven mitochondrial fission primes a 'stem/progenitor-like state' to support neoplastic transformation](#). Elife. 2021

2. Dr. Bhaumik (co-PI, Gynaecological Oncologist) has been leading the department of gynaecological oncology for the last 15 years at a tertiary level cancer care institute, Tata Medical Center, Kolkata. Among his leadership roles (mentioned in CV), he is currently the VP of Association of Gynaecological Oncologists of India (AGOI) and heads the ovarian cancer guideline development committee at the National Cancer Grid (NCG) of India. He functions as a guide, co-guide and examiner for MCh, DrNB and PhD students. The PI and the co-PI intend to co-mentor a co-PhD project on the proposed research.

[Unfurling the functional association between long intergenic noncoding RNAs \(lincRNAs\) and HPV16-related cervical cancer pathogenesis through weighted gene co-expression network analysis of differentially expressed lincRNAs and coding genes.](#)

Sinha A,.....**Bhaumik J** , ...Sengupta S.[Carcinogenesis](#). 2024

3. Dr. Menon (co-I, Biophysicist). He is internationally reknown for his contributions that spans across the domains of cell biology and epidemiology. He has published multiple research articles on modeling cytoskeletal organization, including dissection of cellular heterogeneity (ref. A, b). He is collaborating with Dr. Mitra to understand the contribution of cytoskeletal organization in determining cellular energetic status. The collaborative team would address the proposed question in the patient samples to elucidate interaction of metabolic inhibitors with paclitaxel (microtubule disruptor) for designing successful therapeutic combinations.

Agrawal A..... **Menon GI.** [Nonequilibrium Biophysical Processes Influence the Large-Scale Architecture of the Cell Nucleus](#). *Biophys J.* 2020

4. Dr. Ashesh (co-I, Computer Scientist) has worked extensively in applying deep learning to fluorecence microscopy data. His research on unmixing superimposed fluorecence microscopy images yielded first-author papers at top international venues of Computer Vision, namely ICCV'23, ECCV'24, NeurIPS'25. His experience in working with deep learning on fluorecence microscopy data will be crucial in Objective 3, where the aim is to train multiple deep-learning algorithms on microscopy data and build a classification model first, and subsequently extract useful features for AI based predictive biomarker development.

[Ashesh, F. Carrara, I. Zubarev, et al., “Microsplit: Semantic unmixing of fluorescent microscopy data”](#) pre-press at Nature Methods, 2026.

Institutional Support/ Facilities: Share a brief note on inter-departmental or inter-institutional collaboration needed for study implementation. Do mention the role and responsibility of the PI and Co-PIs.

Inter-institutional collaboration:

The proposed project will be executed through collaboration of a high volume tertiary care clinical institute (TMCK) and a research university with state of the art facilities (AU), under the already signed institutional MoU framework. The teams are jointly working on obtaining a waiver of regulatory compliance from both the institutions. After the commencement of the project, the dedicated teams will have regular online meetings to ensure timely execution of the goals. Any challenges or course corrections will be discussed on a regular basis to amend the procedures.

Following are the individual responsibilities of the PIs and co-Is:

Dr Mitra (PI, AU): Will be responsible for coordinating the overall efforts between TMCK and AU, as well as within AU. Dr Mitra runs a funded laboratory with UG students, technical assistants, PhD students and postdocs. She will mentor all project personnel and directly train them as necessary. She will recruit personnel jointly with Dr. Bhaumik for TMCK support, Dr. Menon for mathematical modeling and with Dr. Ashesh for computer vision based AI-ML modeling. She will work with each of the 3 teams and coordinate between them. She will also organize online meetings every 6 months with the full team. She will be responsible for finalizing manuscripts with the help of the respective project personnel and co-Is.

Dr. Bhaumik (co-I, TMCK): Will oversee the coordination of projects by the recruited project personnel to ensure seamless coordination between oncologist, pathologist and biobanking team. He will also oversee the data entry from the EMR and the transfer/shipping of the deidentified data and samples from the TMCK team to the AU team. He will ensure his and team's participation in the online meeting of the full team every 6 months. Successful collaboration between the PI and co-I has yielded the preliminary data of this project on survival analyses.

Dr. Menon (co-I, AU): Will be responsible for training and co-mentoring project personnel for the mathematical modeling proposed in Objective 3. He will hold regular meetings with Dr. Mitra and the relevant project personnel for evaluating progress. He will ensure his participation in the online meeting of the full team every 6 months. Successful collaboration between the PI and co-I has yielded the preliminary data of this project on mathematical modeling. The PI and the co-PI are co-mentoring a PhD student in this line of research.

Dr. Ashesh (co-I, AU): Will be responsible for training and co-mentoring project personnel for the deep learning based modeling proposed in Objective 3. He will hold regular meetings with Dr. Mitra and the relevant project personnel for evaluating progress. He will ensure his participation in the online meeting of the full team every 6 months. Being a junior faculty, he will seek mentorship from the PI as needed. He will also seek required advisory help from Dr. Partha Pratim Chakrabarti, Professor, IIT-Kharagpur, with whom the PI collaborated to generate the preliminary data. The PI and the co-PI has already initiated collaboration in this line of research.

Laboratory facilities (in-vitro/ in-silico): Mention the institutional resources (such as animal house, instruments/ equipment etc.) available for use in the proposed project for participating institutes.

TMCK:

The following well equipment at TMCK will be used for execution of the project:

- **Biosafety Cabinet (Class II A2):** Used for the sterile handling, aliquoting, and preparation of tissue and cell samples.
- **Centrifuge (5810R / 5430R):** High-speed centrifuges used for processing samples and separating cellular components; includes rotors for various tube sizes.
- **Vortex Mixers:** Required for homogenizing samples during the preparation process.
- **Precision Pipettes (Gilson, Thermo, Eppendorf):** Essential for accurate sample handling and preparation.
- **4°C Refrigerator (Elanpro):** Used for the short-term storage of samples during processing.
- **Liquid Nitrogen Storage Tanks (Locator 6 plus):** Required for the long-term cryopreservation of cells at approximately -156°C.
- **Ultra-Low Temperature (-80°C) Freezers (Thermo Scientific):** Used for the long-term preservation of associated biobanked samples such as plasma or serum.
- **BD FACSAria Fusion Flow Cytometer (SORP):** Used for multicolor cellular analysis and both bulk and single-cell sorting.
- **Equipment for Block Preparation**

Tissue Processor:

- a. Leica Peloris
- b. Leica ASP 300S
- c. Leica TP1020

Tissue Embedding:

- d. Leica EG1150H Embedding station
- e. EpreDia Histostar Embedding station

Equipment for Tissue Sectioning

Microtomy:

- a. Histocore multicut (Laica Biosystems)
- b. Leica RM2255 (Laica Biosystems)
- c. Leica RM 2245 (Laica Biosystems)
- d. EpreDia HM525NX (EpreDia)

Equipment for Staining & Coverslipping

Autiostainer (Autostainer):

- Leica ST 5020

Coversleeper (Coverslipper):

- Leica cv 5030

Ashoka:

The following well equipped facilities at AU will be used for execution of the project:

1. Microscopy facility (housing proposed Zeiss CD7, LSM900 confocal microscopes, Leica Aperio, others)
2. Tissue culture facility to use both cell lines and patient samples
3. Liquid N2 cell storage facility
4. Bioenergetics assessment system, Seahorse XF-Pro flux analyser,
5. Standard biochemical laboratory
6. Antigen retrieval system, PathnSitu MERS
7. Flow cytometry facility at AU, BD LSR Fortessa X-20
8. Flow cytometry adjacent IIT-D Central Research Facility, BD FACSAria III
9. BMN Labtech plate reader
10. NVIDIA DGX A100 Server (School of Biosciences)
11. NVIDIA DGX B200 (School of Computing)
12. HPC
13. Library with relevant journal access
14. All standard laboratory facilities with standard large and small equipment (including MilliQ water source, autoclave, freezers, cold rooms, bacterial hoods, PCR machines, ice machines, centrifuges, Chemidoc, Nanodrop, DNA and Protein electrophoresis and blotting equipment etc)
15. Workstations for seating as well as for data analysis
16. Dedicated faculty office space

Additional supplementary information including figures, tables, flow diagrams, data collection tool etc. can be shared as PDF (Please see in Document Consideration section)

List two non-preferred reviewers assessing your project:

S.No.	Name	Designation	Affiliation
1	Ramray Bhat	Associate Professor	Indian Institute of Science
2	Manisha Inamdar	Professor and Director	Institute of Stem Cell Science and Regenerative Medicine

Investigator (PI/Co-PI) Details

#	Name	Institute	Designation	Division /Section / Department	Email	Contact No.	Nature of Employment	Role in Proposal
1	Dr Kasturi Mitra	Ashoka University	Associate Professor	Biology	kasturi.mitra@ashoka.edu.in	9773917406	Permanent	PI
2	Dr ashesh	Ashoka University	Assistant Professor	Computer Science	ashesh@ashoka.edu.in	8800984154	Permanent	Co-PI
3	Prof Gautam Iqbal Menon	Ashoka University	Professor	Biology	gautam.menon@ashoka.edu.in	9444032134	Permanent	Co-PI
4	Dr Jaydip Bhaumik	Tata Medical Center	Surgical Oncologist	Gynaecological Oncology	jaydip.bhaumik@tmckolkata.com	9748181117	Permanent	Co-PI

Documents consideration

#	Document Name	Uploaded Document	Remarks
1	Short Resume format (PI and Co-PI) (Maximum two pages) in merge in single pdf	View	All 3 brief CVs have been included in the attached document, with the best possible formatting done.
2	Declaration & Attestation Form(duly signed by Head of Department/ Director)	View	The declarations of the two institutes are included separately.
3	Additional supplementary information including figures tables flow diagrams etc can be shared as PDF	View	This file has the Mito-SinComp and The WSI pipeline proposed for Objective 1 and Objective 2, respectively.

Proposed Budget Details

Institute	Budget Year	Manpower Budget (Rs.)	Contingency	Consumables	Equipment	Travel	Overhead	Total(Rs)
Ashoka University	1	4113200.00	1950000.00	11100000.00	1600000.00	1500000.00	478464	20741664
Tata Medical Center	1	873600.00	50000.00	925000.00	0	100000.00	55458	2004058
Ashoka University	2	4804800.00	1450000.00	10100000.00	800000.00	2000000.00	490644	19645444
Tata Medical Center	2	873600.00	0	925000.00	0	100000.00	53958	1952558
Ashoka University	3	7054740.00	1550000.00	7600000.00	0	2000000.00	486141.4	18690881.4
Tata Medical Center	3	917280.00	0	925000.00	0	100000.00	55268.4	1997548.4
Ashoka University	4	2661120.00	1850000.00	6100000.00	0	2000000.00	318332.8	12929452.8

Proposed Budget Details								
Institute	Budget Year	Manpower Budget (Rs.)	Contingency	Consumables	Equipment	Travel	Overhead	Total(Rs)
Tata Medical Center	4	1375920.00	0	125000.00	0	100000.00	45027.6	1645947.6
Total in (Rs.):		22674260	6850000	37800000	2400000	7900000	1983294.2	79,607,554.20

Budget Breakup Details (Staff/Manpower)								
#	Budget Year	Institute	Designation	No. of Person(nos)	Require Month(nos)	Cost Per Person(Rs.)	Overhead(Rs.)	Total Cost(Rs.)
1	Year: 1	Ashoka University	Project Research Scientist - II (Medical)	1	12	96,000	0.00	1,152,000.00
Justification of Staff/Manpower :Required for providing the administrative and logistic support for all project PIs, Co-Is and personnel								
2	Year: 1	Ashoka University	Project Research Scientist - I (Non Medical)	2	1	61,600	3,696.00	123,200.00
Justification of Staff/Manpower :Required for performing computational analyses proposed in Objective 3								
3	Year: 1	Tata Medical Center	Project Technical Support - III	1	12	36,400	13,104.00	436,800.00
Justification of Staff/Manpower :Required to manage patient consent and overall coordination between labs and clinicians								
4	Year: 1	Tata Medical Center	Project Technical Support - III	1	12	36,400	13,104.00	436,800.00
Justification of Staff/Manpower :Required to help with tissue collection and other technical work								
5	Year: 1	Ashoka University	Project Technical Support - I	1	12	19,800	7,128.00	237,600.00
Justification of Staff/Manpower :Required for dedicated tissue slide processing proposed in Objective 1								
6	Year: 1	Ashoka University	Project Technical Support - I	1	12	19,800	14,256.00	237,600.00
Justification of Staff/Manpower :Required for supporting all laboratory maintenance during the course of the project								
7	Year: 1	Ashoka University	Project Research Scientist - I (Medical)	1	12	73,700	26,532.00	884,400.00
Justification of Staff/Manpower :Required for performing medical record analyses proposed in Objective 1 and 2								
8	Year: 1	Ashoka University	Project Research Scientist - I (Non Medical)	2	12	61,600	44,352.00	1,478,400.00
Justification of Staff/Manpower :Required for experiments proposed in Objective 2								
9	Year: 2	Ashoka University	Project Research Scientist - I (Non Medical)	2	12	61,600	44,352.00	1,478,400.00
Justification of Staff/Manpower :Required for performing computational analyses proposed in Objective 3								
10	Year: 2	Tata Medical Center	Project Technical Support - III	1	12	36,400	13,104.00	436,800.00
Justification of Staff/Manpower :Required to manage patient consent and overall coordination between labs and clinicians								
11	Year: 2	Tata Medical Center	Project Technical Support - III	1	12	36,400	13,104.00	436,800.00
Justification of Staff/Manpower :Required to help with tissue collection and other technical work								
12	Year: 2	Ashoka University	Project Technical Support - I	1	12	19,800	7,128.00	237,600.00
Justification of Staff/Manpower :Required for dedicated tissue slide processing proposed in Objective 1								
13	Year: 2	Ashoka University	Project Technical Support - II	1	12	22,000	7,920.00	264,000.00
Justification of Staff/Manpower :Required for supporting all laboratory maintenance during the course of the project								
14	Year: 2	Ashoka University	Project Research Scientist - II (Medical)	1	12	88,000	31,680.00	1,056,000.00
Justification of Staff/Manpower :Required for performing medical record analyses proposed in Objective 1 and 2								
15	Year: 2	Ashoka University	Project Research Scientist - II (Non Medical)	2	12	73,700	53,064.00	1,768,800.00
Justification of Staff/Manpower :Required for performing experiments proposed in Objective 2								
16	Year: 3	Ashoka University	Project Research Scientist - I (Non Medical)	2	12	64,680	46,569.60	1,552,320.00
Justification of Staff/Manpower :Required for performing computational analyses proposed in Objective 3								
Total Cost (Rs.) including overhead							23,327,054.20	

Budget Breakup Details (Staff/Manpower)								
#	Budget Year	Institute	Designation	No. of Person(nos)	Require Month(nos)	Cost Per Person(Rs.)	Overhead(Rs.)	Total Cost(Rs.)
17	Year: 3	Ashoka University	Project Research Scientist - I (Non Medical)	2	12	64,680	46,569.60	1,552,320.00
Justification of Staff/Manpower :Required for performing computational analyses proposed in Objective 3								
18	Year: 3	Tata Medical Center	Project Technical Support - III	1	12	38,220	13,759.20	458,640.00
Justification of Staff/Manpower :Required to manange patient consent and overall corodination between labs and clinicians								
19	Year: 3	Tata Medical Center	Project Technical Support - III	1	12	38,220	13,759.20	458,640.00
Justification of Staff/Manpower :Required to help with tissue collection and other technical work								
20	Year: 3	Ashoka University	Project Technical Support - I	1	12	20,790	7,484.40	249,480.00
Justification of Staff/Manpower :Required for dedicated tissue slide processing proposed in Objective 1								
21	Year: 3	Ashoka University	Project Technical Support - I	1	12	20,790	7,484.40	249,480.00
Justification of Staff/Manpower :Required for supporting all laboratory maintenance during the course of the project								
22	Year: 3	Ashoka University	Project Research Scientist - III (Medical)	1	12	107,415	38,669.40	1,288,980.00
Justification of Staff/Manpower :Required for performing medical record analyses proposed in Objective 1 and 2								
23	Year: 3	Ashoka University	Project Research Scientist - III (Non Medical)	2	12	90,090	64,864.00	2,162,160.00
Justification of Staff/Manpower :Required for performing experiments proposed in Objective 2								
24	Year: 4	Tata Medical Center	Project Technical Support - III	1	12	38,220	13,759.20	458,640.00
Justification of Staff/Manpower :Required to manange patient consent and overall corodination between labs and clinicians								
25	Year: 4	Tata Medical Center	Project Technical Support - III	1	12	38,220	13,759.20	458,640.00
Justification of Staff/Manpower :Required to help with tissue collection and other technical work								
26	Year: 4	Tata Medical Center	Project Technical Support - III	1	12	38,220	13,759.20	458,640.00
Justification of Staff/Manpower :Required to help with tissue collection and other technical work								
27	Year: 4	Ashoka University	Project Technical Support - I	1	12	20,790	7,484.40	249,480.00
Justification of Staff/Manpower :Required for dedicated tissue slide processing proposed in Objective 1								
28	Year: 4	Ashoka University	Project Technical Support - I	1	12	20,790	7,484.40	249,480.00
Justification of Staff/Manpower :Required for supporting all laboratory maintenance during the course of the project								
29	Year: 4	Ashoka University	Project Research Scientist - III (Non Medical)	2	12	90,090	64,864.00	2,162,160.00
Justification of Staff/Manpower :Required for performing experiments proposed in Objective 2								
Total Cost (Rs.) including overhead							23,327,054.20	

Contingency Budget Breakup Details				
#	Budget Year	Institute	Overhead Charges (Rs.)	Total Cost(Rs.)
1	Year: 1	Tata Medical Center	1,500.00	50,000.00
Contingency Name :Regulatory process				
Justification :For obtaining regulatory clearance				
2	Year: 1	Ashoka University	4,500.00	150,000.00
Contingency Name :Amazon Web Services subscription				
Justification :Required for use exceeding 24 GB vRAM GPUs.				
3	Year: 1	Ashoka University	0.00	200,000.00
Contingency Name :Software subscriptions (Adobe CS2, SAS, SPSS etc)				
Justification :Required for image postprocessing, specialized statistical analyses for all the proposed Objectives				
4	Year: 1	Ashoka University	15,000.00	500,000.00
Contingency Name :Publication charges				
Justification :Required for publication of the research findings				
5	Year: 1	Ashoka University	0.00	100,000.00
Total Cost (Rs.) including overhead			7,046,500.00	

Contingency Budget Breakup Details				
#	Budget Year	Institute	Overhead Charges (Rs.)	Total Cost(Rs.)
Contingency Name : General data storage devices				
Justification : Required primarily for storing spatial biology raw related raw and analyzed data for Objective 1				
6	Year: 1	Ashoka University	30,000.00	1,000,000.00
Contingency Name : High volume data storage devices				
Justification : Required primarily for storing microscopy raw and analyzed data from Objective 2 to be used in Objective 3, and also downloading publicly available data for deep learning tasks				
7	Year: 2	Ashoka University	4,500.00	150,000.00
Contingency Name : Amazon web services				
Justification : Required for uses that exceed 24 GB vRAM GPUs				
8	Year: 2	Ashoka University	6,000.00	200,000.00
Contingency Name : Software subscriptions (Adobe CS2, SAS, SPSS etc)				
Justification : Required for image analyses, specialized statistical analyses for all the proposed Objectives				
9	Year: 2	Ashoka University	30,000.00	1,000,000.00
Contingency Name : Publication charges				
Justification : Required for publication of the research findings				
10	Year: 2	Ashoka University	3,000.00	100,000.00
Contingency Name : General data storage devices				
Justification : Required primarily for storing spatial biology raw related raw and analyzed data for Objective 1				
11	Year: 3	Ashoka University	4,500.00	150,000.00
Contingency Name : Amazon Web services				
Justification : Required for uses that exceed 24 GB vRAM GPUs				
12	Year: 3	Ashoka University	6,000.00	200,000.00
Contingency Name : Software subscriptions (Adobe CS2, SAS, SPSS etc)				
Justification : Required for image postprocessing,, specialized statistical analyses for all the proposed Objectives				
13	Year: 3	Ashoka University	30,000.00	1,000,000.00
Contingency Name : Publication charges				
Justification : Required for publication of the research findings				
14	Year: 3	Ashoka University	6,000.00	200,000.00
Contingency Name : General data storage devices				
Justification : Required primarily for storing all microscopy, flow cytometry, seahorse raw related raw and analyzed data for Objective 1 and 2				
15	Year: 4	Ashoka University	4,500.00	150,000.00
Contingency Name : Amazon web services				
Justification : Required for uses that exceed 24 GB vRAM GPUs				
16	Year: 4	Ashoka University	6,000.00	200,000.00
Contingency Name : Software subscriptions (Adobe CS2, SAS, SPSS etc)				
Justification : Required for image postprocessing, specialized statistical analyses for all the proposed Objectives				
17	Year: 4	Ashoka University	45,000.00	1,500,000.00
Contingency Name : Publication charges				
Justification : Required for publication of the research findings				
Total Cost (Rs.) including overhead			7,046,500.00	

Consumables Budget Breakup Details					
#	Budget Year	Institute	Consumables Name	Overhead	Total Cost(Rs.)
1	Year: 1	Ashoka University	Misellaneous	3,000.00	100,000.00
Justification : For stationary and such smaller but necessary items					
2	Year: 1	Tata Medical Center	Miscellaneous	750.00	25,000.00
Justification : For stationary and such smaller but necessary items					
3	Year: 1	Tata Medical Center	Tissue Biobanking Charges Tissue Dissociation Reagents Cryopreservation Media Cryovials (2 mL) General Lab Consumables Viability QC Reagents Biobank Storage Fee Domestic Shipment (-80°C) Section Preparation shipping	27,000.00	900,000.00
Justification : For isolation of tumor cells from fresh frozen tissue, cryofreezing them and shipping them as needed					
4	Year: 1	Ashoka University	Metabolic inhibitors	30,000.00	1,000,000.00
Justification : Required for all proposed experiments in Objective 2					
5	Year: 1	Ashoka University	Ovarian cancer cell lines	60,000.00	2,000,000.00
Total Cost (Rs.) including overhead				38,934,000.00	

Consumables Budget Breakup Details					
#	Budget Year	Institute	Consumables Name	Overhead	Total Cost(Rs.)
Justification : Will be need for certain optimization work for Objective 2					
6	Year: 1	Ashoka University	For flow cytometry, including aldefluor kit, test tubes with filters, sheath fluid chemicals, flow sorting service fee etc	60,000.00	2,000,000.00
Justification : Required for stem cell sorting proposed in Objective 2					
7	Year: 1	Ashoka University	Related to immunohistochemistry, including antibodies, biochemicals, stains, staining jars, diamond marker etc	60,000.00	2,000,000.00
Justification : Required for spatial biology experiments proposed in Objective Aim 1b					
8	Year: 1	Ashoka University	This budget will cover all expenses for microscope usage including slides, coverslips live cell chambers, mounting medium,etc	30,000.00	1,000,000.00
Justification : Required for experiments proposed in Objective 1 and 2					
9	Year: 1	Ashoka University	This budget will be used for consumables related to bioenergetics analyses,including bio-chemicals,standards, flux packs etc	45,000.00	1,500,000.00
Justification : Required for experiment proposed in Aim 2					
10	Year: 1	Ashoka University	Tissue culture ware	45,000.00	1,500,000.00
Justification : For performing experiments on tumor cells in Objective 2					
11	Year: 2	Ashoka University	Miscellaneous	3,000.00	100,000.00
Justification : For stationary and such smaller but necessary items					
12	Year: 2	Tata Medical Center	Miscellaneous	750.00	25,000.00
Justification : For stationary and such smaller but necessary items					
13	Year: 2	Ashoka University	This budget will be used for consumables related to bioenergetics analyses,including bio-chemicals,standards, flux packs etc	75,000.00	2,500,000.00
Justification : Required for experiment proposed in Objective 2					
14	Year: 2	Tata Medical Center	Tissue Biobanking Charges Tissue Dissociation Reagents Cryopreservation Media Cryovials (2 mL) General Lab Consumables Viability QC Reagents Biobank Storage Fee Domestic Shipment (-80°C) Section Preparation shipping	27,000.00	900,000.00
Justification : For isolation of tumor cells from fresh frozen tissue, cryofreezing them and shipping them as needed					
15	Year: 2	Ashoka University	Metabolic inhibitors	30,000.00	1,000,000.00
Justification : Required for all proposed experiments in Objective 2					
16	Year: 2	Ashoka University	For flow cytometry, including aldefluor kit, test tubes with filters, sheath fluid chemicals, flow sorting service fee etc	60,000.00	2,000,000.00
Justification : Required for TIC and BTC sorting proposed in Objective 2					
17	Year: 2	Ashoka University	Related to immunohistochemistry, including antibodies, biochemicals, stains, diamond markers etc	60,000.00	2,000,000.00
Justification : Required for spatial biology experiments proposed in Objective Aim 1b					
18	Year: 2	Ashoka University	This budget will cover all expenses for microscope usage including slides, coverslips live cell chambers, mounting medium,etc	30,000.00	1,000,000.00
Justification : Required experiments proposed in Objective 1 and 2					
19	Year: 2	Ashoka University	Tissue culture consumables	45,000.00	1,500,000.00
Total Cost (Rs.) including overhead					38,934,000.00

Consumables Budget Breakup Details					
#	Budget Year	Institute	Consumables Name	Overhead	Total Cost(Rs.)
Justification : For performing experiments on tumor cells in Objective 2					
20	Year: 3	Ashoka University	Miscellaneous	3,000.00	100,000.00
Justification : For stationary and such smaller but necessary items					
21	Year: 3	Tata Medical Center	Miscellaneous	750.00	25,000.00
Justification : For stationary and such smaller but necessary items					
22	Year: 3	Ashoka University	This budget will be used for consumables related to bioenergetics analyses,including bio-chemicals,standards, flux packs etc	75,000.00	2,500,000.00
Justification : Required for experiment proposed in Objective 2					
23	Year: 3	Ashoka University	Tissue culture consumables	45,000.00	1,500,000.00
Justification : For performing experiments on tumor cells in Objective 2					
24	Year: 3	Tata Medical Center	Tissue Biobanking Charges Tissue Dissociation Reagents Cryopreservation Media Cryovials (2 mL) General Lab Consumables Viability QC Reagents Biobank Storage Fee Domestic Shipment (-80°C) Section Preparation shipping	27,000.00	900,000.00
Justification : For isolation of tumor cells from fresh frozen tissue, cryofreezing them and shipping them as needed					
25	Year: 3	Ashoka University	Metabolic inhibitors	15,000.00	500,000.00
Justification : Required for all proposed experiments in Objective 2					
26	Year: 3	Ashoka University	For flow cytometry, including aldefluor kit, test tubes with filters, sheath fluid chemicals, flow sorting service fee etc	60,000.00	2,000,000.00
Justification : Required for TIC and BTC sorting proposed in Objective 2					
27	Year: 3	Ashoka University	This budget will cover all expenses for microscope usage including slides, coverslips live cell chambers, mounting medium,etc	30,000.00	1,000,000.00
Justification : Required for experiments proposed in Objective 1 and 2					
28	Year: 4	Ashoka University	Miscellaneous	3,000.00	100,000.00
Justification : For stationary and such smaller but necessary items					
29	Year: 4	Tata Medical Center	Miscellaneous	750.00	25,000.00
Justification : For stationary and such smaller but necessary items					
30	Year: 4	Ashoka University	Tissue culture consumables	45,000.00	1,500,000.00
Justification : For performing experiments on tumor cells in Objective 2					
31	Year: 4	Tata Medical Center	Storage and shipping of samples	3,000.00	100,000.00
Justification : For storage and shipping of remaining samples					
32	Year: 4	Ashoka University	For flow cytometry, including aldefluor kit, test tubes with filters, sheath fluid chemicals, flow sorting service fee etc	60,000.00	2,000,000.00
Justification : Required for TIC and BTC sorting proposed in Objective 2					
33	Year: 4	Ashoka University	This budget will cover all expenses for microscope usage including slides, coverslips, live cell chambers, mounting medium,etc	30,000.00	1,000,000.00
Justification : Required for experiments proposed in Objective 1 and 2					
34	Year: 4	Ashoka University	This budget will be used for consumables related to bioenergetics analyses,including bio-chemicals,standards, flux packs etc	45,000.00	1,500,000.00
Justification : Required for experiments proposed in Objective 2					
Total Cost (Rs.) including overhead				38,934,000.00	

Equipment Budget Breakup Details					

#	Budget Year	Institute	Equipment Name	Equipment Model	Equipment Manufacturer	Equipment Type	Total Cost(Rs.)
1	Year: 1	Ashoka University	Workstation	Pro Maven GS I MM (4 in number)	Pro X PC	Domestic	1,600,000.00
Justification : Assembled product required to train deep-learning models and also to evaluate them.							
2	Year: 2	Ashoka University	Workstation	Pro Maven GS I MM (2 in number)	Pro X PC	Domestic	800,000.00
Justification : Assembled product required to train deep-learning models and also to evaluate them.							
Total (Rs.):							2,400,000.00

Travel Justification		
#	Year	Amount(Rs.)
1	1	100000.00
Justification : Required for domestic travels for Co-I and project personnel to travel between Delhi and Kolkata, as needed		
2	1	1500000.00
Justification : Required for domestic travels for the PI, Co-Is and project personnel to travel between Delhi and Kolkata, as needed.		
3	2	100000.00
Justification : Required for domestic travels for the Co-I and project personnel to travel between Delhi and Kolkata, as needed.		
4	2	2000000.00
Justification : Required for domestic travels for the PI, Co-Is and project personnel to travel between Delhi and Kolkata, as needed. Also, for travels to domestic and international meetings/ workshops.		
5	3	100000.00
Justification : Required for domestic travels for the Co-I and project personnel to travel between Delhi and Kolkata, as needed.		
6	3	2000000.00
Justification : Required for domestic travels for the PI, Co-Is and project personnel to travel between Delhi and Kolkata, as needed. Also for domestic and international meetings/workshops.		
7	4	100000.00
Justification : Required for domestic travels for the Co-I and project personnel to travel between Delhi and Kolkata, as needed.		
8	4	2000000.00
Justification : Required for domestic travels for the PI, Co-Is and project personnel to travel between Delhi and Kolkata, as needed. Also for domestic and international meetings/workshops.		
Total:		7,900,000.00

Short resume PI/Co-PI						
Name of PI/Co-Pi	DOB	Domain Expertise	Number of articles in Pub Med (Past 10 years)	h-index	Fellow of Academies	Role in Proposal
Dr ashesh	1993-02-15	Deep learning based Computer Vision applied to Fluorescence microscopy data.	0	5	None	Co-PI
Maximum of 10 primary research publications related to the proposal						
Publication details in AMA style		Impact factor of journal		Author type (first, corresponding, coauthor)		Name of policy/programme/ protocol document or patent/commercialization of products where cited.
Experience as Investigator (completed projects)						
Title of the project		Role	Funding Agency		Amount of Funding	Reference of main publications
All Ongoing research projects						
Project Id	Title	Grant Amount	Funding Agency	Start Date	End Date	Duration
Name of PI/Co-Pi	DOB	Domain Expertise	Number of articles in Pub Med (Past 10 years)	h-index	Fellow of Academies	Role in Proposal
Dr Jaydip Bhaumik	1963-12-06	Leading the department of gynaecological oncology at a tertiary level cancer care institute as a clinician, surgeon, teacher and researcher	29	3	2) Vice president of the Association of Gynaecological Oncologists of India (AGOI)	Co-PI
Maximum of 10 primary research publications related to the proposal						
Publication details in AMA style		Impact factor of journal		Author type (first, corresponding, coauthor)		Name of policy/programme/ protocol document or patent/commercialization of products where cited.
Experience as Investigator (completed projects)						
Title of the project		Role	Funding Agency		Amount of Funding	Reference of main publications
All Ongoing research projects						
Project Id	Title	Grant Amount	Funding Agency	Start Date	End Date	Duration
Name of PI/Co-Pi	DOB	Domain Expertise	Number of articles in Pub Med (Past 10 years)	h-index	Fellow of Academies	Role in Proposal
Prof Gautam Iqbal Menon	1966-06-16	Biophysics,	29	28	National Academy of	Co-PI

Short resume PI/Co-PI						
Computational Biology, Epidemiology				Sciences		
Maximum of 10 primary research publications related to the proposal						
Publication details in AMA style	Impact factor of journal		Author type (first, corresponding, coauthor)		Name of policy/programme/ protocol document or patent/commercialization of products where cited.	
Experience as Investigator (completed projects)						
Title of the project	Role	Funding Agency		Amount of Funding	Reference of main publications	
All Ongoing research projects						
Project Id	Title	Grant Amount	Funding Agency	Start Date	End Date	Duration
Name of PI/Co-Pi	DOB	Domain Expertise	Number of articles in Pub Med (Past 10 years)	h-index	Fellow of Academies	Role in Proposal
Dr Kasturi Mitra	1977-06-30	Cancer metabolism, Mitochondria, Stem Cells, Microsocopy	20	19	None	PI
Maximum of 10 primary research publications related to the proposal						
Publication details in AMA style	Impact factor of journal		Author type (first, corresponding, coauthor)		Name of policy/programme/ protocol document or patent/commercialization of products where cited.	
Experience as Investigator (completed projects)						
Title of the project	Role	Funding Agency		Amount of Funding	Reference of main publications	
All Ongoing research projects						
Project Id	Title	Grant Amount	Funding Agency	Start Date	End Date	Duration

Declaration
I hereby declare that the entries in this form and the additional particulars, if any, furnished herewith are true to the best of my knowledge and belief. I understand that in the event of my information being found false or incorrect at any stage, my project/proposal shall be liable to cancellation / termination without notice or any compensation in lieu thereof.
I hereby certify that the research proposal I have submitted to ICMR, New Delhi, for potential funding is entirely my original idea and has not been copied or replicated from any other source. Furthermore, I confirm that this proposal has undergone scrutiny using a standard plagiarism detection tool, verifying its originality and confirming that its contents have not been directly taken from any other sources. Additionally, I declare that there have been no established or pending plagiarism charges against me in the last five years. In the event that the funding agency identifies any form of plagiarism or inconsistencies in the aforementioned proposal, I acknowledge and agree to comply with any actions deemed necessary by ICMR. I take full responsibility for any such discrepancies and will adhere to the consequences as required.

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