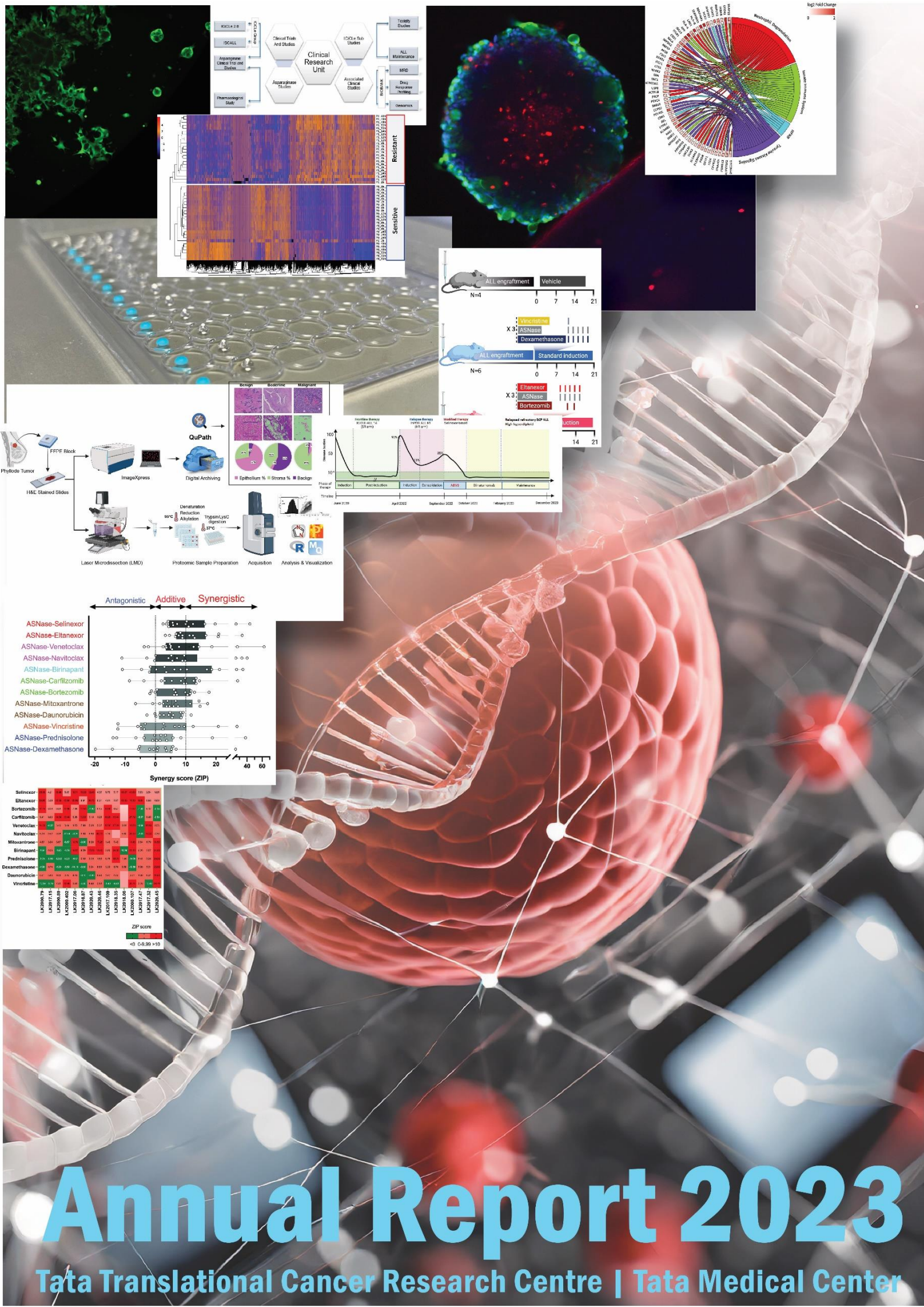


Annual Report 2023

Tata Translational Cancer Research Centre | Tata Medical Center

Key to cover page

Picture Courtesy: Research Team

From Top to bottom:

Organoids in 96 well plate stained with live cell dye Calcein-AM; Trails and studies under CRU; Whole organoid stained with live cell dye (Calcein-AM) dead cell dye (Propidium Iodide) and nuclear dye (Hoechst).Peripheral green cells are live cells (Calcein-AM) and central red cells are dead; Heatmap representing protein profile of PDXs; Chord plot showing enriched pathways their respective important protein; 96 well plate; PDX experiment schema; Study schema for breast phyllode study; Drug response curve of a patient along with progressive time point in path study; Forrest plot & heatmap showing synergy in combinatorial chemotherapy

CONTENT

From Director's Desk

Vaskar Saha

In 2014, Shekhar and I began the concept of TTCRC. 10-years ago from the time this is being written, we still had not finalised the name and there were 3 of us, Mou, Shekhar and myself. We were also located in different parts of the hospital. From that beginning, TTCRC was conceptualised, built and opened in April 2018. This year's annual report demonstrates the coming of age of the centre and the people who work there.

The year began with a visit by Professor's Bourquin and Bornhauser from Zurich. Jasmeet had spent a year with them and they came to follow up. The continuing collaboration took Jasmeet to the American Society of Hematology meeting in San Diego with a travel award. She was subsequently invited to join the I-BFM Resistant Disease Group which met in Bergamo. This was a group that I helped establish many years ago and which I had the privilege of chairing before I came to Kolkata. So her journey has now begun.

Ankita had an abstract accepted for a EMBO meeting in Heidelberg. Hans Clever, the father of organoid biology came to see her poster – a proud moment for the SOLI3D group. Dwijit landed a grant from ICMR adding icing to the cake.

Pritha, the lead of PREDICT (measurable residual disease) also obtained a hard-fought grant from DBT. Aishwarya and Sreyashree spent time in Dresden and Berlin – this was supported by a collaborative grant between PREDICT and Charité University (Dr Eckert). This culminated in a well organised national workshop on MRD which we held in January.

Nandana, the CRU Lead has been pivotal in landing two large multicentre grants from ICMR and as I write is currently training at the Medical Research Council Clinical Trials Unit in London. Parag went there earlier this year. Manas attended the POEM annual research methodology course in Istanbul.

Debdutta, Rubina and Mayur have a well-established collaboration running now with St Jude and University of North Carolina. This includes funding and a RO1 grant application. Mayur also spent a week in Memphis meeting with the teams. Both FORE and SOLI3D teams attended the annual IACR meeting, which they enjoyed and received good feedback.

I had the opportunity to share our decade of work at TTCRC at the national pediatric hematology oncology meeting where I was honoured to give this year's oration. I was also invited to speak on our work at the annual meeting of the ALLTogether European Consortium. It was good to meet many old friends who I have worked with in the past.

Last but not least, I should mention that our women's team are this year's tug-of-war champions! Along with the many successes in grants, training and collaborations, papers are being written and I wish everyone best of luck with getting them into print as quickly as possible.

As usual we have had a turnover of staff. They are listed at the end of this report. Many have left for adventures overseas to further their careers and we wish them every success. Pritha Paul, who has been with us since 2015 left to pursue interests in industry and remains in contact. Arunabha who left the previous year to join SciCom, dropped in to tell us about his adventures. It filled me with pride to listen to how well he is doing in his new role. It is

really nice when those who worked here previously drop by.

It promises to be another busy year as we continue to look for additional funding with our academic and industrial collaborations now entering implementation.

As a youngster on the block, TTCRC has demonstrated how by working together we can create an environment that delivers real benefit for patients and trains the researchers of tomorrow to ask questions. Keep it up.

Administration

Asama Mukherjee

Laboratory Manager

Saheli Biswas

Secretary

Satadru Dey

Administrative Assistant

Nikhilesh Chowdhury

IT Support Assistant

The Research Administration at TTCRC

Journey till date:

The Research Administration has played a pivotal role in shaping the laboratory infrastructure, coordinating logistical aspects of laboratory operations, and supporting academic activities since the inception of TTCRC in 2014. Over the past decade, the team has strategically organized itself and expanded its functioning areas (Figure 1) in a holistic approach to meet the diverse needs of researchers, facilitate seamless integration among research teams (Figure 2) fostering an environment conducive to the development of innovative solutions for cancer patients.

A Reflection of 2023:

In the year 2023 our activities were multifaceted. In addition to supporting routine lab operations, we dedicated efforts to refine admin processes and workflows.

Simultaneously, we took on lab developmental projects to enhance our capabilities, ensuring better service and support for the research team.

Recruitments and Training:

Nikhilesh joined the admin team in March 2023 in the position of IT support assistant.

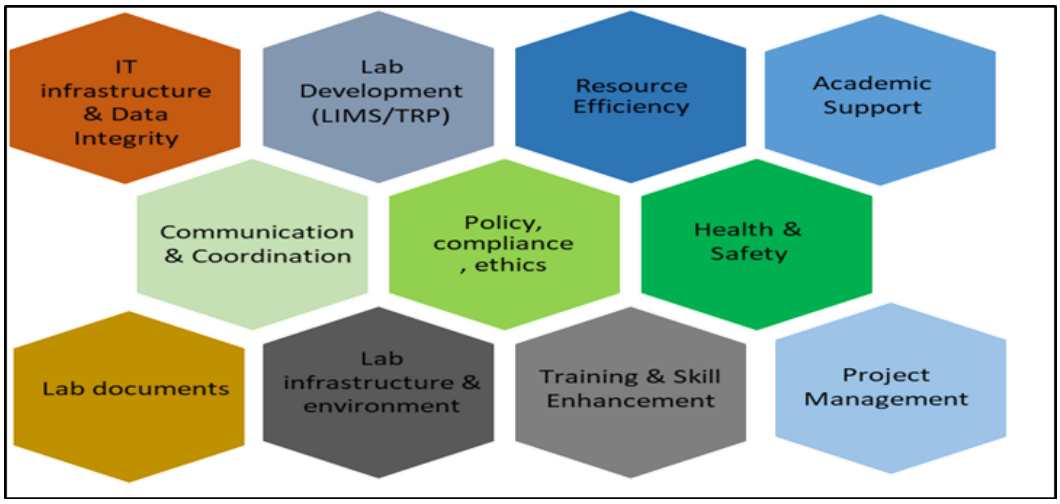


Figure 1. Functioning areas of research administration

The researchers now receive prompt technical support for addressing issues related to softwares, hardwares, or network connectivity.

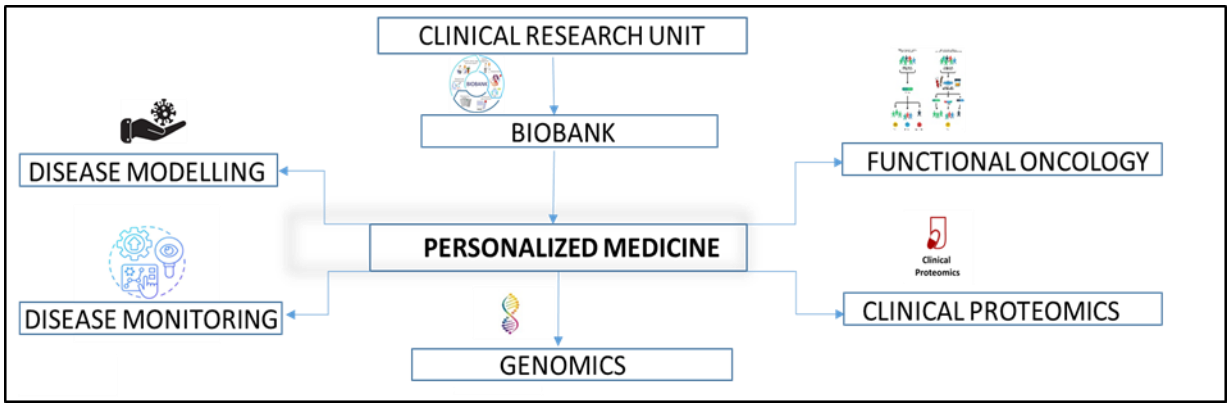


Figure 2. Translational research model at TTCRC

We have recruited 10 new staff in the research team, trained 4 new interns and facilitated observership for one medical student. We have supported training and skill enhancement for several staff within India and abroad. 8 staff were sent overseas for short term training and/or participation in international conferences. Three of them were supported by foreign bursaries. Besides holding regular scientific meetings and journal club we have arranged for invited scientific talks and technical trainings as per Table 1 and 2.

Grant Management:

Currently the team is managing several types of grants each playing a crucial role in supporting our research endeavours. The core funding is derived from TCS-CSR program that provides a stable foundation for our initiatives. Additionally, we manage project-based grants, funded from both government sources, and pharmaceutical companies that help us support the development of companion diagnostic. Notably, this year, we have secured new funding approval from esteemed organizations such as the Department of

Invited Speaker & Talks in 2023 @ TTCRC			
Sl.No	Date	Topic	Speaker
1	25th Jan	Exploring functional dependencies in very high risk acute lymphoblastic Leukemia	Dr. (Prof.) Jean-Pierre Bourquin
2	08th Feb	Bioinformatics at the DKMS Life Science Lab-Structure, Practice and Research	Vineeth Surendranath
3	15th Feb	Update on HemaTrack ALL: A New Technique for MRD detection	Dr. Ulf-Peter Gunther
4	12th Jul	Identification of Somatic Mutations	Dr. Manja Meggendorfer
5	19th Jul	Through the looking glass of single cell	Dr. Ramanuj Dasgupta
6	21st Jul	"PTBP2 promotes cell survival and autophagy in Chronic Myeloid Leukemia by stabilizing BNIP3	Dr. Soumen Chakraborty
7	5th Dec	The role of the protein JMJD6 in the regulation of the endocrine therapy resistance in breast cancer patients	Dr. Kartiki V. Desai

Table 1. Invited scientific talks in 2023

Technical trainings/workshops conducted		
Sl.No	Date	Topic
1	13-14th Feb	Advanced concepts in (a) flow cytometry and (b) multi-colour panel designing
2	18th Oct	The Only Reliable Sample-To-Answer Solution for Single Cell High Dimensional Immune Profiling --- CyTOF Single Tube Cytometry
3	06th Nov	3D-bioprinting of biogels and live cells

Table 2. Technical training arranged in 2023

Biotechnology (DBT), Indian Council of Medical Research (ICMR), St. Jude, and a new industry partner Zydus.

As a part of the grant management process, we have actively supported the principal investigators in budget preparation as required for their grant applications. This has been accomplished through detailed understanding of monetary limit and identification of specific requirements for manpower, consumable, equipment and miscellaneous items related to the project. We have assisted in the grant application process through preparation of pre and post award documentations, grant utilization management, and ensured smooth closure. We have also supported document preparation for the ethical and regulatory processes associated with the project applications ensuring compliance with the highest standards in the conduct of research.

Our focus this year has been supporting the Director with his strategic plan for expansion the of TTCRC laboratories and in strengthening the sustainability of the organization. We engaged actively in budget preparation by understanding new project plans and identification of new resource requirement in terms of manpower, space, equipment. We coordinated meetings with various stakeholders such as the TMC administration, architect, and collaborators to ensure smooth planning for the expansion initiatives. We have hosted several visits from our collaborators like TCS, DKMS and Genova to enhance scientific engagement with the team leads, planning new projects and their execution.

We also managed several high profile institutional review visits that included Members of TMC Trust, CEO Tata Trust and Assistant Vice President Tata Sons.

IT Infrastructure and Data Management:

As a part of our ongoing collaborative studies with DKMS, St. Jude's Hospital and University of Chapelhill we initiated procedures, created management plan and infrastructure for transfer of critical research data between TTCRC and these institutions.

Our current engagement with TCS in setting up the Translational Research Platform aims at revolutionizing research data management and visualization. We coordinated with TCS through several meetings to help them develop utilities for extracting key information from data files of MRD and Asparaginase group. Similar work is underway with Proteomics and Genomics groups. We are also supporting TCS in their endeavours for creating an automated data flow from TTCRC server to TRP with necessary information, software installation and facilitating remote access in TTCRC servers as required by them.

Based on our recent assessment of projected data generation and anticipated project expansion in the upcoming years, it is imperative to enhance our systems and infrastructure for ensuring seamless data transfer, efficient computation, and expanded storage capacity. Planning for these enhancements has already commenced.

Development and Implementation of Laboratory Information Management System (LIMS):

Our plan for procurement of a LIMS that was initiated in the last year was matured and finally executed in this year. We engaged with the LabVantage solutions and worked with them to customize the available module as per our requirement with the aim to achieve the following:

- Track and check item availability in real time.
- Optimize utilisation of consumables
- Maintain accountability of funding.
- Maintain the accuracy and integrity of inventory data by reducing the reliance on paper-

based systems and manual record keeping.
- Avoid duplication and achieve integrity to reduce error.
- Enhance efficiency in lab process
- Timely ordering lab reagents and consumables.

The following steps were performed for the preparation of Data File Definition (DFD) with customised and out of the box entries which were verified by LabVantage team:

A. Preparation of Master Data

- Approximately 627 items were populated in item category in template spreadsheet. 9 such separate spreadsheets were prepared to avoid losing data in a bulk, and for logical segregation of common items and group specific items.
- Corresponding to each item, information was provided for item description, category, manufacturer, vendor, catalogue no, threshold amount with units and container type.
- Precondition elements viz. units, container type, storage environments, vendors were defined and created for above items within the database manually.

B. Data Quality Check

- Rules for validation were defined for each of the fields in the spreadsheet.
- The data was checked manually for completeness, accuracy, consistency and any duplication.

C. Data Testing

- A server was identified and configured for creation of a test environment.
- A Functional Requirement Specification (FRS) document was created and shared by LabVantage team that served as a guide for functionality testing.
- DFDs were individually uploaded. Troubleshooting was done for any issues faced in this step.
- After successful upload of all the DFDs, items lots were created prospectively following their receipt. The following screenshots indicate the creation of several containers for a particular item after their deliveries are received.

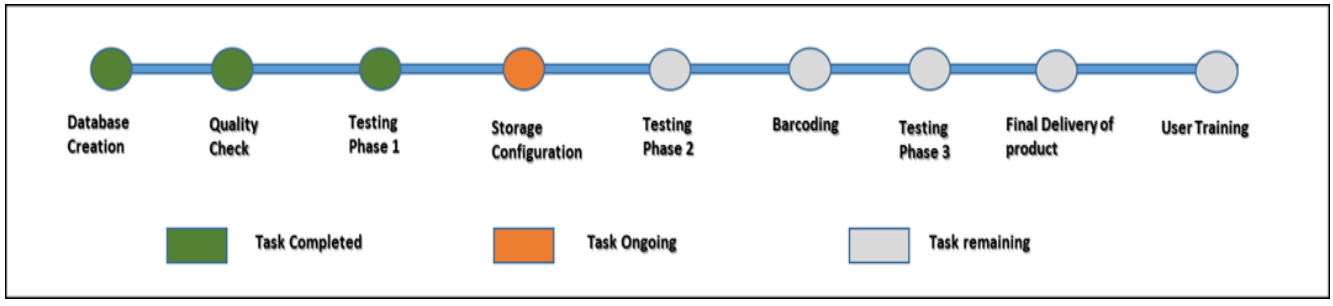


Figure 3. Progress of LIMS execution plan in 2023

Participation in External Lab Quality Management Audit Process:

We have been audited by a three-member team which included representatives from DKMS Life Science GGMBH, Dresden and DKMS BMST Foundation India, Bangalore between November 22nd-23rd 2023. The areas that were covered in the assessment process included laboratory health and safety, equipment management and monitoring, reagent management, facility and environment monitoring, laboratory incident management and reporting, quality controls in biobank, MRD workflows and its documentation, and personnel competency monitoring.
We received positive feedback in the reports with suggestions for some areas of improvement which we have planned for implementation in our processes. This exercise has reassured and reaffirmed that the existing systems and process of laboratory operations that are comparable with any standard research laboratory.

MRD Workshop Organisation

A three-day workshop and conference entitled "Standardising and Optimising MRD and Genetic Diagnostics in ALL" was organised at Tata Medical Center from January 19th to January 21st, 2024 as part of a concerted initiative aiming at standardizing the diagnosis, monitoring, and management of paediatric acute lymphoblastic leukaemia (ALL) across medical centres in India, with the goal of enhancing patient outcomes nationwide. Apart from Tata Medical Center the organizing partners were Indian Council of Medical Research (ICMR), University of Charite, Berlin, Germany, DKMS Life Science Lab, Dresden, Germany and ICiCLe (Indian Collaborative Childhood Leukaemia) group.

The event garnered participants from around the globe, with approximately 100 attendees, including 25 foreign delegates. Forty-two diagnostic laboratory personnel across Indian hospital's ICiCLe network received hands on training on data acquisition and use of software for MRD analysis by flowcytometry method on the first day. There had been keynote speakers in the training and scientific sessions who shared their expertise and insights on the topic in the subsequent days.

The administrative team played a crucial role in supporting the core and peripheral activities related to the planning, conduct and management of the programme. We coordinated with our organising partners for funding arrangement, managed participant registration, made arrangements for transportation and accommodation for invited speakers and trainers, coordinated for ensuring smooth and seamless IT support. We also coordinated with vendors for supplying training equipment and materials, as well as with other internal and external key stakeholders for providing logistics support for the conduct of the workshop. A snapshot of the workshop is represented in Figure 4.

Looking Ahead:

- Continue to enhance systems and processes for efficient lab operations management.
- Upgrade hardware and software systems to ensure compatibility, security, and adherence to evolving research needs.
- Explore and implement cloud-based services to enhance accessibility, collaboration, and scalability of research data and tools.
- Create more synergy and integration among research teams to support the institute's research goals.

Figure 4. Moments from MRD Workshop

Abhirupa Kar

Bio repository Manager

Sayak Manna

Senior Bio repository Officer

Paromita Biswas

Bio repository Officer

Subhajit Kundu

Project Research Officer

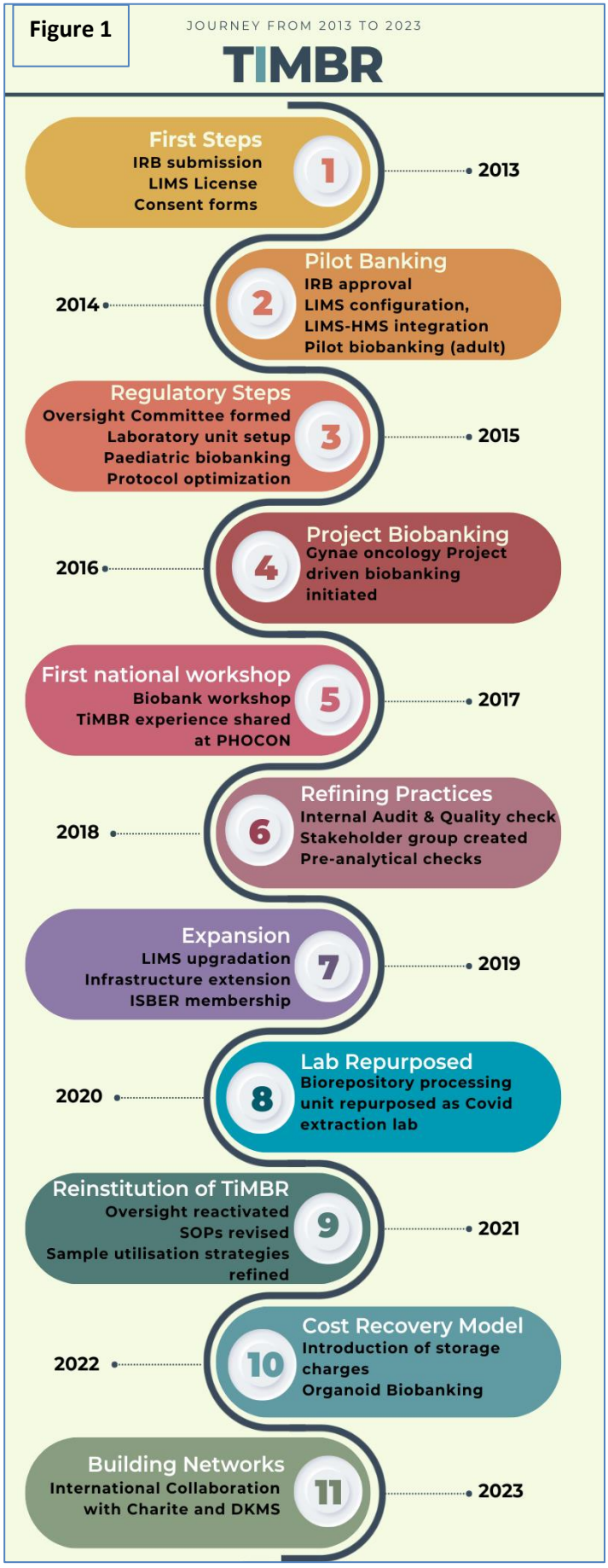
. Introduction:

Since its inception in 2013, the Tata Medical Center's Biorepository (TiMBR) has evolved from a passive sample storage facility to an active contributor in driving evidence-based clinical practice. It transcends the traditional role of biobanking by engaging in comprehensive sample processing, cryopreservation, and long-term storage of blood, tissue, and other critical biospecimens, thereby guaranteeing optimal sample integrity. This meticulously annotated biobank serves as a valuable resource for collaborative research endeavours, facilitating the discovery of crucial clinical insights that translate directly into improved patient outcomes.

Figure 1 depicts the temporal progression of the Tata Medical Center Biorepository (TiMBR), highlighting its continuous learning and unlearning process to propel cancer research forward.

Ms. Abhirupa Kar leads the team responsible for TiMBR's operations, working in close collaboration with a constuctive oversight committee and project investigators to optimize biorepository function. With 9 years of experience in microbiology and biotechnology, Abhirupa oversees the entire functioning of TiMBR, ensuring streamlined operations and the highest achievable standards of sample integrity. Assisting Abhirupa is Mr. Subhajit Kundu, Project Research Officer. Subhajit brings diligence and accountability in the picture honed through his yearlong internship at Biobank (2021-2022), contributing valuable insights and skills to the TiMBR team as well as downstream user groups. Subhajit is currently

Figure 1



working with the genomics group at TTCRC to learn high throughput applications in the downstream RNA based pipelines. In charge of the quality management program Mx Sayak Manna, Senior Biorepository Officer, joined TiMBR in 2022 with 6 years of experience in cancer and stem cell biology. Sayak is on the verge of submitting their Ph.D. thesis, where they investigated the therapeutic potential of cord blood plasma factors in treating leukemia. Their knowledge and research experience has supported shaping scientific quality control approaches in TiMBR to improve cryopreserved cell quality in terms of viability, cell count recovery and nucleic acid yield. Ms. Paromita Biswas, Biorepository Officer, rounds out the team with 2 years of experience in clinical and diagnostic areas in flow-cytometric facility. Paromita's dedication and skill in sample processing and management ensure the smooth operation of the biobank, contributing to the successful execution of critical research projects involving flow-cytometric validations.

The core team of TiMBR is continuously engaged with its stakeholders (downstream users/investigators) and scientific advisors to periodically evaluate its processes for better and cost effective output, which is also at par with the global standards. Our international collaborator and long term advisor, Dr. Cornelia Eckert, from Charite has been instrumental in shaping our biobanking approaches leading to improved sample quality and integrity. Dr. Debdutta Ganguly (Genomics Lead, TTCRC) and Dr. Rizwan Javed (Consultant, Department of Apheresis & Cellular therapy, TMC) has also provided their valuable guidance and inputs to uphold quality biobanking services at TiMBR.

Where we are today:

In the year 2023, TiMBR supported biobanking of around 300 patients. Nearly 50% of these patients are banked at multiple time points i.e., longitudinal sampling during their treatment. From each sample, multiple

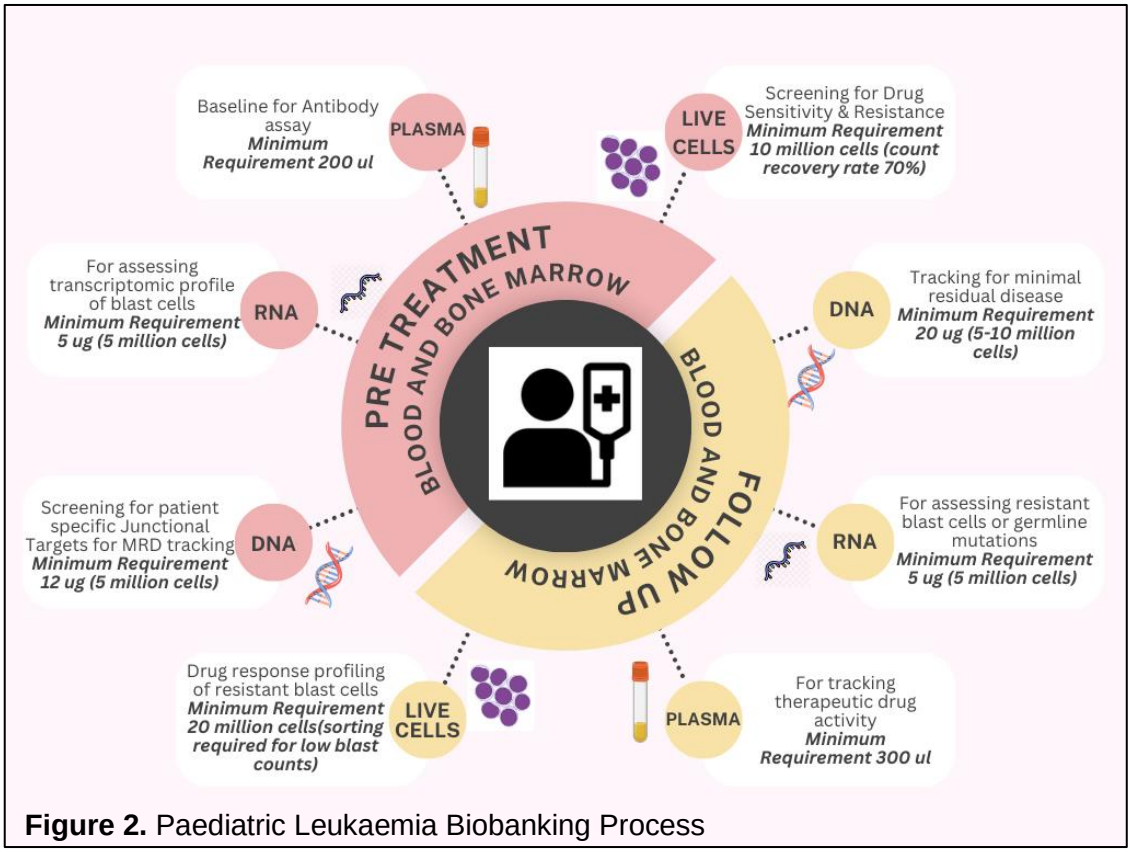


Figure 2. Paediatric Leukaemia Biobanking Process

multiple derivatives are created depending on the downstream application as per the study objectives. TiMBR focuses on opportunistic sampling where only additional samples from diagnostic sampling are biobanked. Figure 2 gives an example of Paediatric Leukaemia Biobanking process. And through these compliances to standard protocols and adherence to international guidelines TiMBR has been able to achieve the following goals:

1. Establishing a comprehensive biobank: Possessing over 60,000 diverse biospecimens from 7,500 consenting patients (both paediatric and adult), TiMBR caters to researchers by offering a substantial resource to investigate a broad spectrum of cancer types and potential therapies.

2. Ensuring sample integrity: Strict protocol adherence guarantee optimal sample preservation conditions, thereby safeguarding the fidelity of data for reliable research outcomes. In November 2023, TiMBR underwent an informal audit by our international collaborator DKMS where gaps in the system were identified and acted upon

Table 1: Groups Biobanking with TiMBR

S. No.	Groups Banking with TiMBR	Project	Principal Investigator (s) /Co-PI (s)	Grant/ Sponsor	Status
1.	Paediatric Oncology	ICiCLe Biomarker Study [Sub-studies: Asparaginase Assay Drug Response Profiling Minimum Residual Disease RNA Seq]	Prof. (Dr.) Vaskar Saha, Dr. Shekhar Krishnan Dr. Jasmeet Sidhu Dr. Pritha Dasgupta Dr. Debdutta Ganguli, Dr. Mayur Parihar	TCS / Gennova / Zydus / DKMS / DBT	Active
2.	Gastrointestinal Oncology	Gallbladder Carcinoma Biomarker Study	Prof. (Dr.) Vaskar Saha, Dr. Manas Roy, Dr. Dwijit Guha Sarkar	TCS	Active
3.	Gynae-Oncology	Cervix (SyMeC)	Dr. Joydeep Bhaumik Dr. Sonia Matthai (In-charge)	DBT	Complete
		Ovary (PROVAT)	Dr. Asima Mukhopadhyay	DBT/DST	Tenure Complete, Samples to be transferred
4.	Adult Haematology	ALTITUDE, PRIME	Dr. Vivek Radhakrishnan Dr. Mayur Parihar	TATA TRUST	Tenure Complete
		Acute Lymphoblastic / Myeloblastic Leukemia Plasma Cell Myeloma	Dr. Arijit Nag Dr. Mayur Parihar Dr. Jeevan Kumar	TATA TRUST/ TCS	Active
5.	Head & Neck Oncology	Oral Carcinoma (SyMeC-GIFT)	Dr. Geetashree Mukherjee	DBT	Tenure Complete
		miRNA Study	Dr. Ruma Dey Dr. P. Arun	DHR	Tenure Complete
		Head and Neck Squamous Cell Carcinoma Biomarker study	Dr. Kapila Manikantan	Rakuten Medical Inc	Active
6.	Breast Oncology	BREXO	Dr. Geetashree Mukherjee	TCS	Tenure Complete
		Breast Cancer Organoid Drug Response Profiling (BCO-DRP)	Dr. Sanjit Agarwal Dr. Dwijit Guha Sarkar	ICMR	Active
7.	Radiation Oncology	INTELHOPE HYPORT	Dr. Sanjay Chatterjee Dr. Rosina Ahmed	Margdarshi/ TCS	Inactive
		KORTUC	Dr. Sanjay Chatterjee	IQVIA	Active
		Astefenia	Dr. Sanjay Chatterjee	Roche	Active
		NATCO	Dr. Moses Arunsingh	Navitas	Active

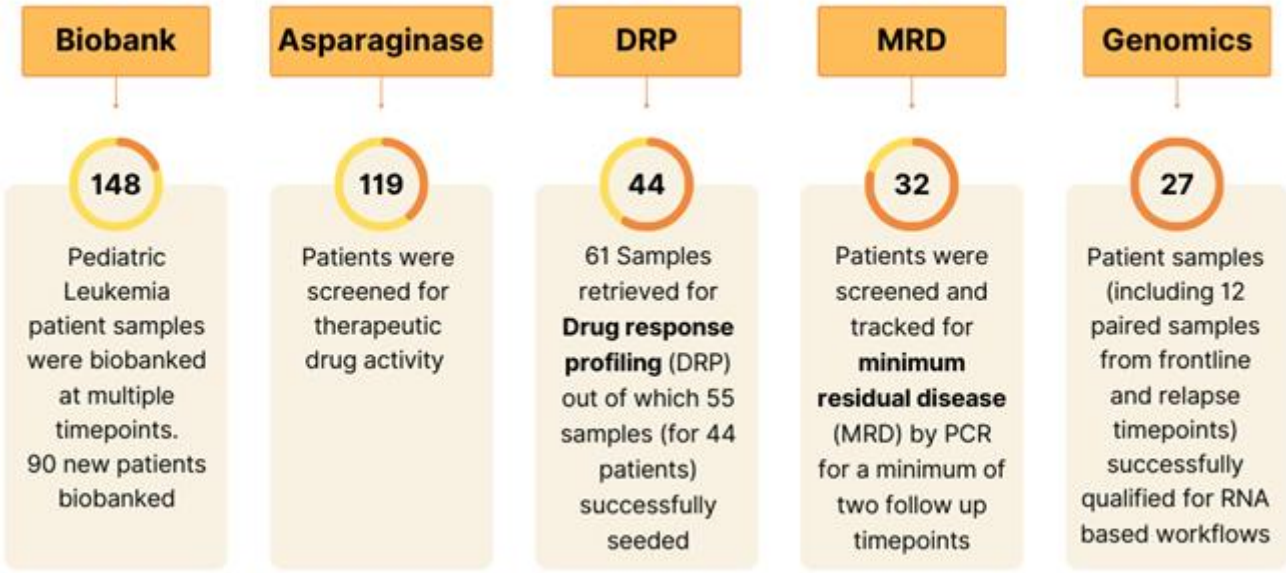


Figure 3. Sample Utilisation Metrics at TiMBR Paediatric Leukaemia Group

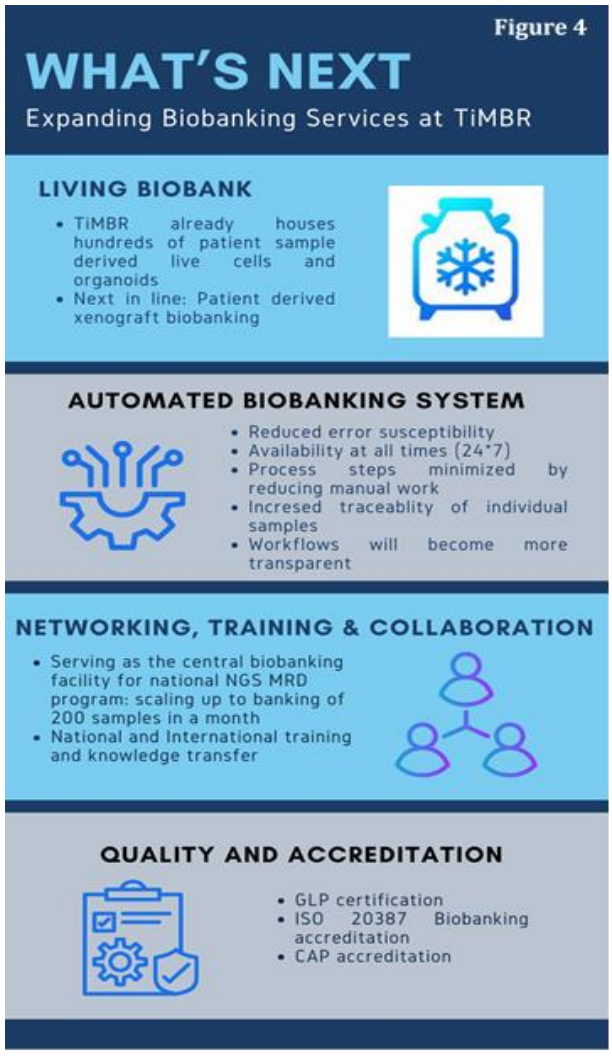
paving the path for quality management system and accreditation.

3. Fostering collaborative research: TiMBR actively engages with diverse stakeholders, including 7 internal departments (Table 1) at Tata Medical Center, along with renowned research institutions, clinicians, and multinational pharmaceutical companies.

4. Translating research into impact: TiMBR's core mission remains translating research insights into tangible improvements in patient care and treatment strategies. Notably, a significant number of biobanked samples were utilized in research projects that directly influenced clinical decisions in the past year. Figure 3 gives an insight to the utilisation metrics of paediatric leukaemia study group in the year 2023.

Future of TiMBR

Figure 4 summarizes the future goals of TiMBR to serve as the central biobank facility in the Hub and Spoke model of Indian Paediatric Leukaemic Clinical Trial Network in the upcoming year.



Clinical Research Unit

Shekhar Krishnan

CRU Lead

Nandana Das

Clinical Trials Administrator

Neerajana Datta

Project Coordinator

Manash P Gogoi

Data Manager

Parag Das

Data Manager

Bishwaranjan Jana

Data Manager

Bony Dasgupta

Data Manager

Tushar Mungle

Post Doc Fellow

Amit K Mehta

Data Manager

Srijani Goswami

Research Assistant

Annwesha Roy

Research Assistant

Imtiyaz Molla

Business Analyst

Clinical Research Unit at TTCRC

Head
Dr Shekhar Krishnan
Team
Dr Nandana Das
Dr Neerajana Datta
Mr Manash Gogoi
Mr Parag Das
Ms Bony Dasgupta
Ms Srijani Goswami
Ms Annwesha Roy
Mr Imtiyaz Molla (secondment from TCS)

The Clinical Research Unit (CRU) at TTCRC leads and participates in the design, development, management, analysis and reporting of investigator-initiated clinical studies in cancer. These studies provide the platform for the translational research programme at TTCRC. CRU's work domains include clinical trials and projects directly managed by the unit and studies that are coordinated by the team (Figure 1).

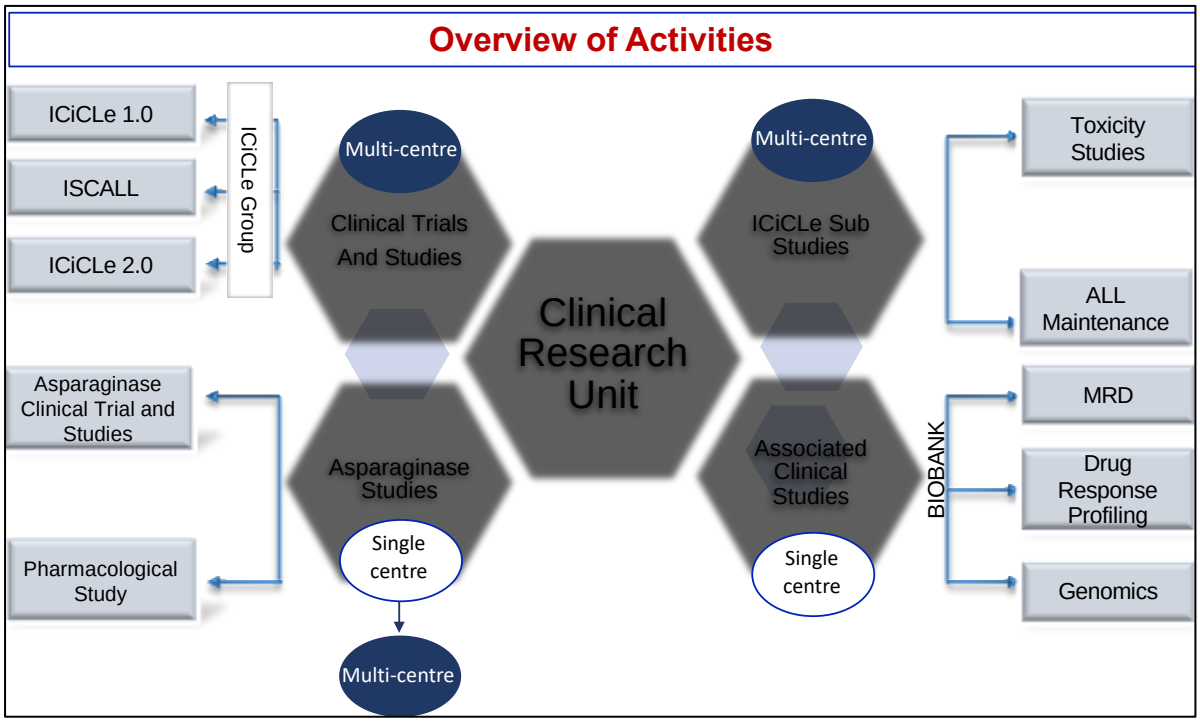


Figure 1. Schematic representation of the domains across which the Clinical Research Unit (CRU) is functional at TTCRC

The ICiCLe Trials and Studies

CRU plays a central role in the conduct and coordination of the multicentre clinical trials and studies under the Indian Childhood Collaborative Acute Lymphoblastic Leukaemia (ICiCLe) group for children diagnosed with acute lymphoblastic leukaemia, aged 1-18 (**1**).
The first ICiCLe ALL protocol was piloted from 2013-2019 and the randomised clinical trial (RCT), ICiCLe-ALL-14 (CTRI/2015/12/006434) from 2016-2022.

Prior to ICiCLe, outcomes at ICiCLe centres were reported at around 65% with treatment-related deaths of 25% and relapse rates of 15-30% [Cancer Institute (WIA) Chennai and PGIMER Chandigarh]. The results of the trial shoes that treatment related deaths and relapses have been reduced with improvements in survival outcomes for all patients (Figure 2). Alongside reducing mortality and toxicity, data from TMC Kolkata centre showed that the risk adapted approach is very cost beneficial (as per WHO criteria).

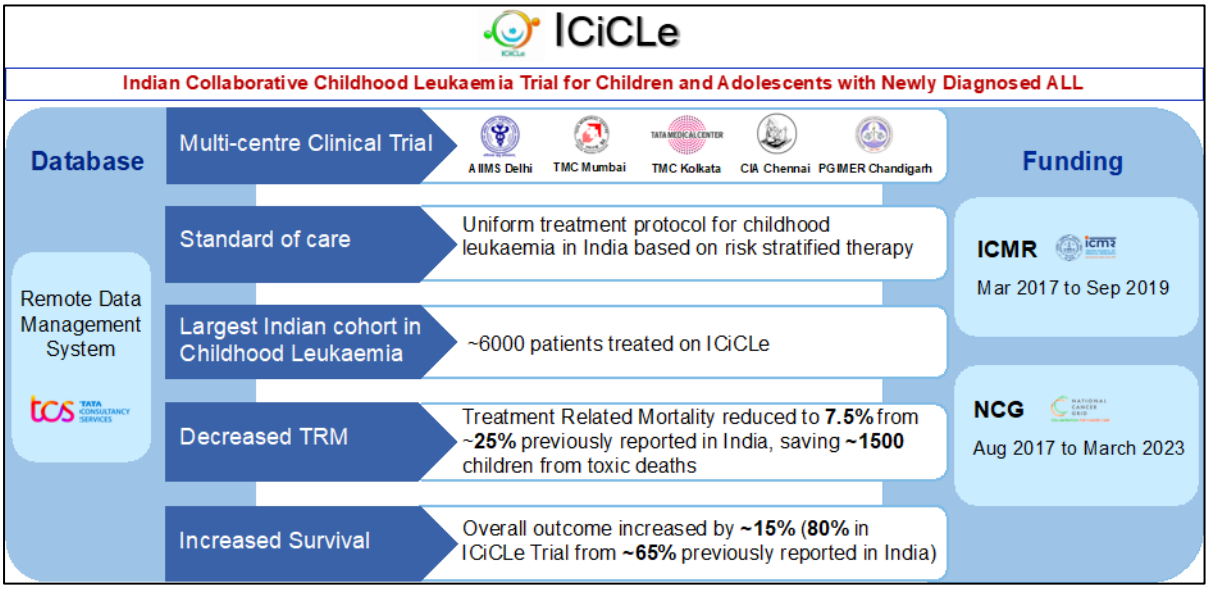


Figure 2. Result snapshot from the ICiCLe multi-centre study

Recognising the ICiCLe results, the Indian Council of Medical Research which has asked us to implement the ICiCLe risk-adapted strategy to publicly-funded hospitals, involving at least one teaching hospital in each state (ISCALL). This study is due to start from March 2024 (CTRI/2023/12/060828) and will be managed by CRU through a database developed by TCS. The original ICiCLe centres (core) will function as hubs for the implementation study, with TTCRC as the innovator hub (Figure 3).

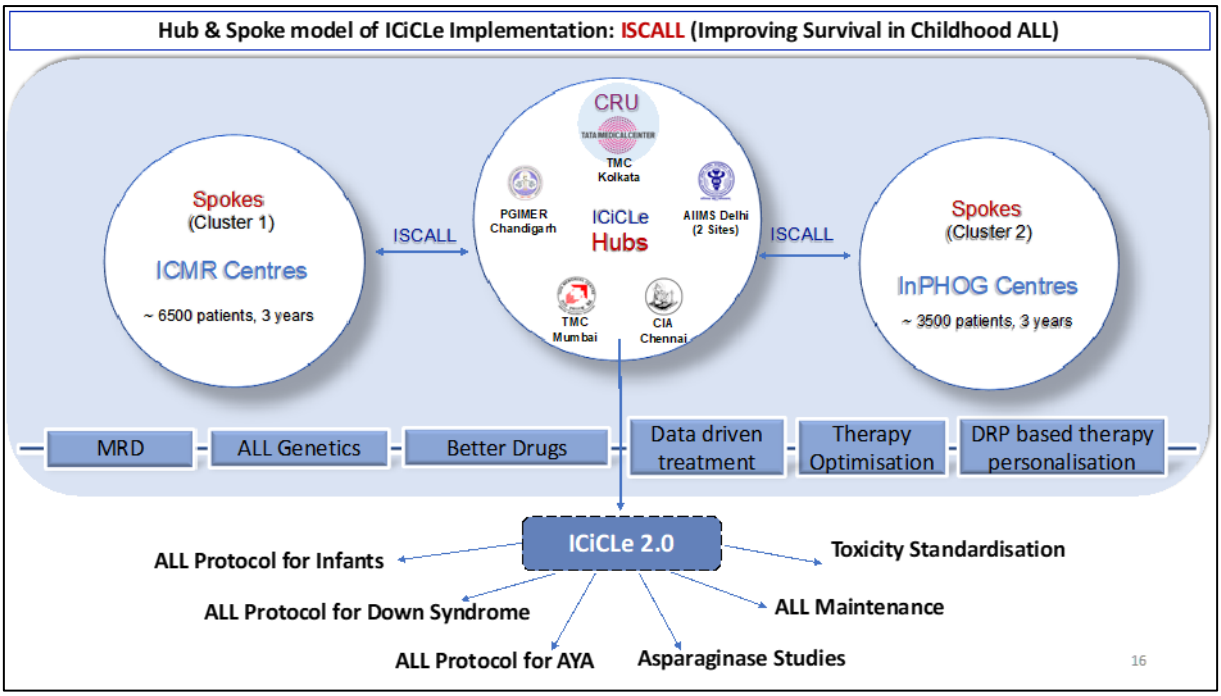


Figure 3. Schematic representation of the Hub and Spoke model of Improving Survival in Childhood Acute Lymphoblastic Leukaemia (ISCALL): Implementation of ICiCLe treatment with CRU at its core, and the successor ICiCLe 2.0 study with sub-studies under the consortium

The core ICiCLe group is also about to launch the successor ICiCLe RCT, ICiCLe 2.0, funded by ICMR and the National Cancer Grid (NCG). The consortium is working on having spin-off studies under the ICiCLe umbrella with CRU as the central clinical trials unit for paediatric oncology rials in India. The unit also coordinates The Indian Paediatric Oncology Group collaborative multicentre treatment protocol for children and adolescents with relapsed acute lymphoblastic leukaemia (InPOG-ALL-19-02-TMC-ALL-R1; CTRI/2019/10/021758).

Asparaginase Studies

CRU conducts clinical research and studies on Asparaginase, a critical chemotherapeutic drug. We demonstrated the poor quality of biogeneric asparaginases (ASNase) available in India. These ASNases have been marketed worldwide making this a global problem. As a result, we developed a systematic clinical and pharmacological monitoring programme to evaluate the therapeutic suitability of generic ASNases marketed in India. As part of the study, quality Asparaginase biogenerics are identified and clinical monitoring of drug toxicity and side effects is carried out. In addition to this, CRU is currently in talk with Gennova Biopharmaceuticals to stat an Asparaginase clinical trial on a recombinant product to determine its safety and therapeutic efficacy (Figure 4).

CRU Sub Studies and Projects

Toxicity Analyses: toxicity studies aim to establish the toxicity profiles of acute and long-term treatment-related toxicities in Indian patients, which are different from that in the west. Working with the Nordic Society of Paediatric Haematology (NOPHO, Prof Kjeld Schmiegelow), we aim to derive commonly agreed parameters and use the data captured in the ICiCLe studies to develop a common toxicity prediction model (Figure 5).

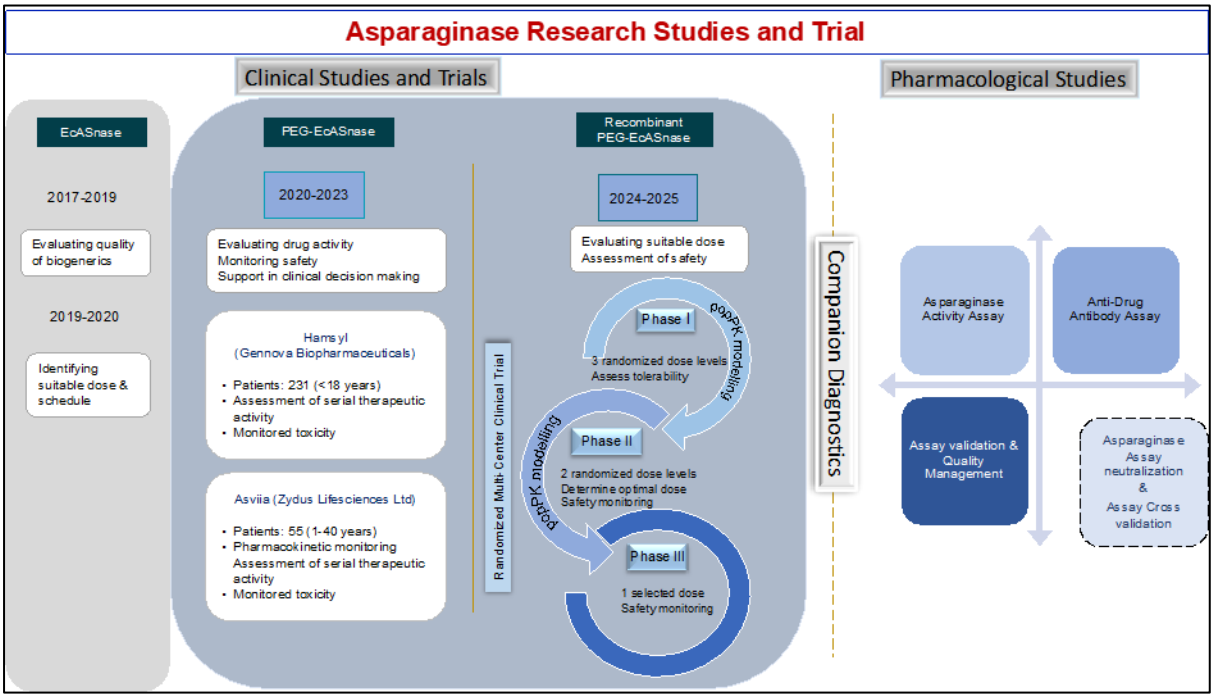


Figure 4. Schematic representation of the Asparaginase Study and research activities at CRU

ALL Maintenance Therapy Optimisation: during the two-year maintenance therapy, the oral antimetabolite drugs administered to patients are 'titrated to tolerance' using the Clinical Decision Support System

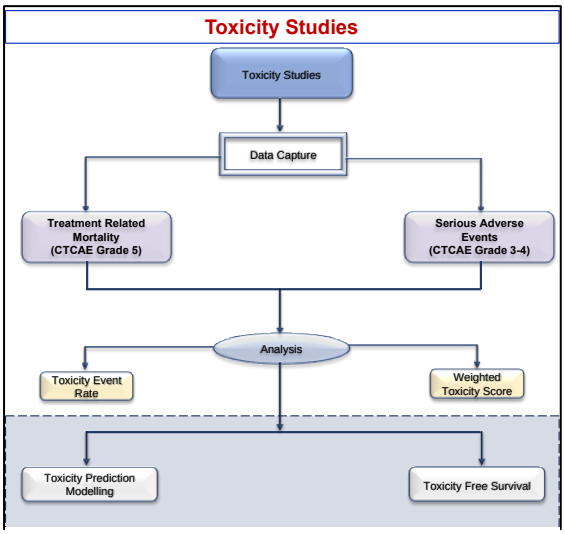


Figure 5. Schematic representation of the Hub and Toxicity study strategies which aims to enhance cure rates while minimizing acute and long-term treatment-related toxicities with developing a toxicity prediction model

(CDSS), currently functional at TATA Medical Centre which aims to aid clinicians in the decision making process and administer the patients with the maximum tolerated dose to ensure durable remission ALL. Intelligent systems for automated dose adaption in maintenance (ADAM) therapy is being developed with Tata Consultancy Services as AI/ML tool for optimising health care delivery (Figure 6).

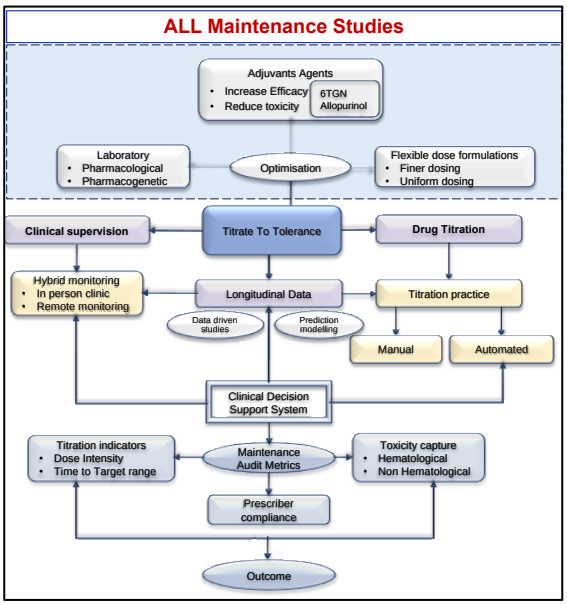


Figure 6. Schematic representation of the strategies to optimise ALL maintenance therapy with development of an automated decision making system for therapeutic dose adaptation and assistance.

In addition, CRU coordinates with and provides data to other research groups like Minimal Residual Diseases (MRD), Drug Response Profiling and Genomics to facilitate associated clinical studies at TTCRC.

Flow Cytometry Facility

Bhaswati Tarafdar

Research Assistant

Flow Cytometry Facility 2023-24

Background:

Flow cytometry is one of the most powerful tools in a researcher's toolkit. Unlike qualitative methods, FACS allows users to get quantitative estimations of their data. It is a type of flow cytometry that sorts different types of cells from a heterogeneous mixture of cells based on a unique fluorescent tags.

In TTCRC researchers try to work with very rare population of patient samples as well as with cell line samples. The Flow Cytometry facility of TTCRC provides technical expertise and training to access state-of-the-art instrumentation, technical and scientific advice to researchers and investigators of TTCRC and other external institutes to develop efficient and reliable flow cytometric assays with high quality control standards and productivity. The facility covers wide range of conventional and advanced flow cytometry applications. The current Flow cytometry personnel cover the entire demand for cell sorting and user assistance needs in experimental design, advice and training.

Instruments in the facility:

1. **Flow Cytometer analyser: BD Accuri™ C6 Plus (2 laser, 4 colour, FL1, FL2, FL3 and FL4)**
   BD Accuri™ C6 Plus can be used to simultaneously analyse multiple physical characteristics of cells like relative size, granularity, internal complexity and fluorescence intensity.
2. **Flow Cytometer sorter: BD FACSARIA Fusion™ (SORP) (5lasers, 18colours, 2B-3R-6V-4YG-3UV)**
   BD FACSARIA Fusion™ (SORP) has cell sorting ability of population of interest from a heterogeneous cell population, based on relative size, granularity, viability and antigen expression using up to eighteen fluorochromes. Sorting up to 4 separate pure populations in tubes and different kind of plates simultaneously at BSL-2 level is possible in this machine.
3. **Flow Cytometry ARCTIC Refrigerated/Heated Bath Circulator by Thermo Scientific**
   This instrument can control the temperature between 5ºC to 40 ºC and circulate it through the sorted collection tubes or collection plate to maintain the viability of post sorted cells during sorting experiment.
4. **Magnetic sorter: Miltenyl Biotec MACS (Magnetic activated cell sorter)**
   MACS is a column-based technology that relies on nano-sized superparamagnetic MACS® MicroBeads. The target cells are magnetically labelled and isolated from the mixed cell suspension.

New developments in the facility in 2023:

Our facility encourages development of new applications based on needs of research groups. New assays standardized this year are:

1. Mitochondrial assays: assessment of mitochondrial mass, activity and mitophagy in ALL cell lines and primary samples.
2. Quality assessment of banked primary samples.
3. Establishment of magnetic bead activated cell sorting (MACS) along with Flow Cytometry sorting.
4. Sorting of blasts from banked primary bone marrow samples.
5. Immunophenotype assessment of up to 12 markers (2 scatter+ 10 color) for patient sample of external users.
6. Sorting of transduced cell lines with up to 98% enrichment and 95% cell viability for external user.
7. Introduced ARCTIC Refrigerated/Heated Bath Circulator facility in BD FACSARIA Fusion™ sorting instrument to increase post sort viability.

8. Use of plate sorting along with tube sorting in BD FACSARIA Fusion™.

Collaborations:

Additionally, this year we were able to cater external research institutes for immuno phenotyping and cell sorting.

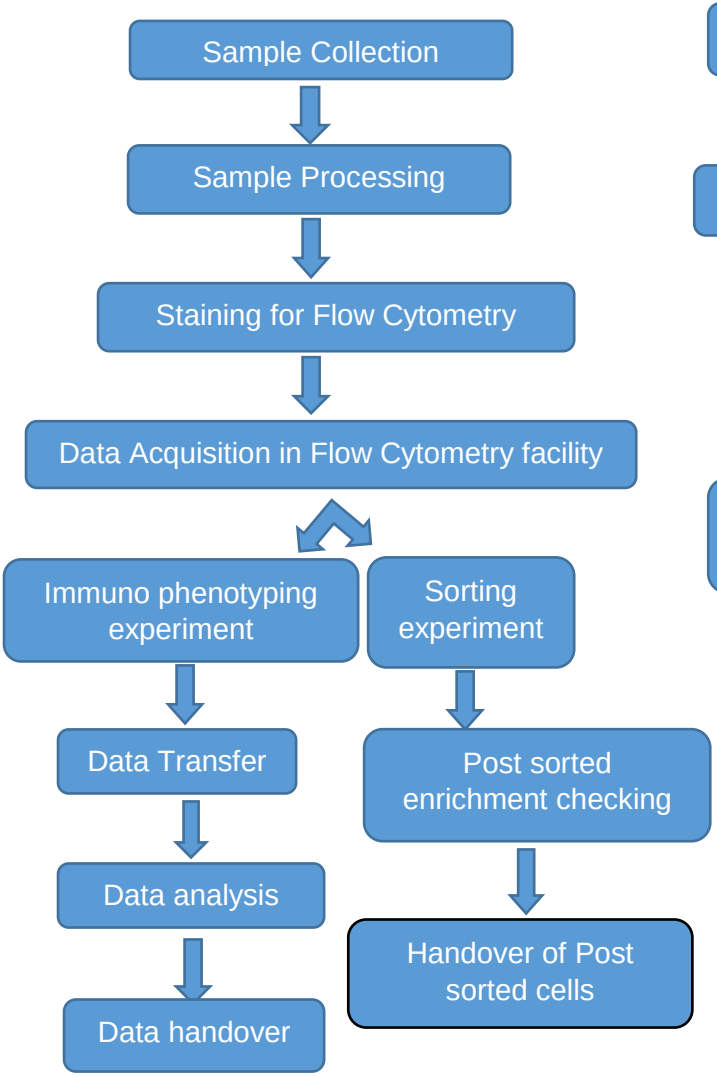
- National Institute of Cholera and Enteric Diseases
- Indian Institute of Chemical Biology

Activity:

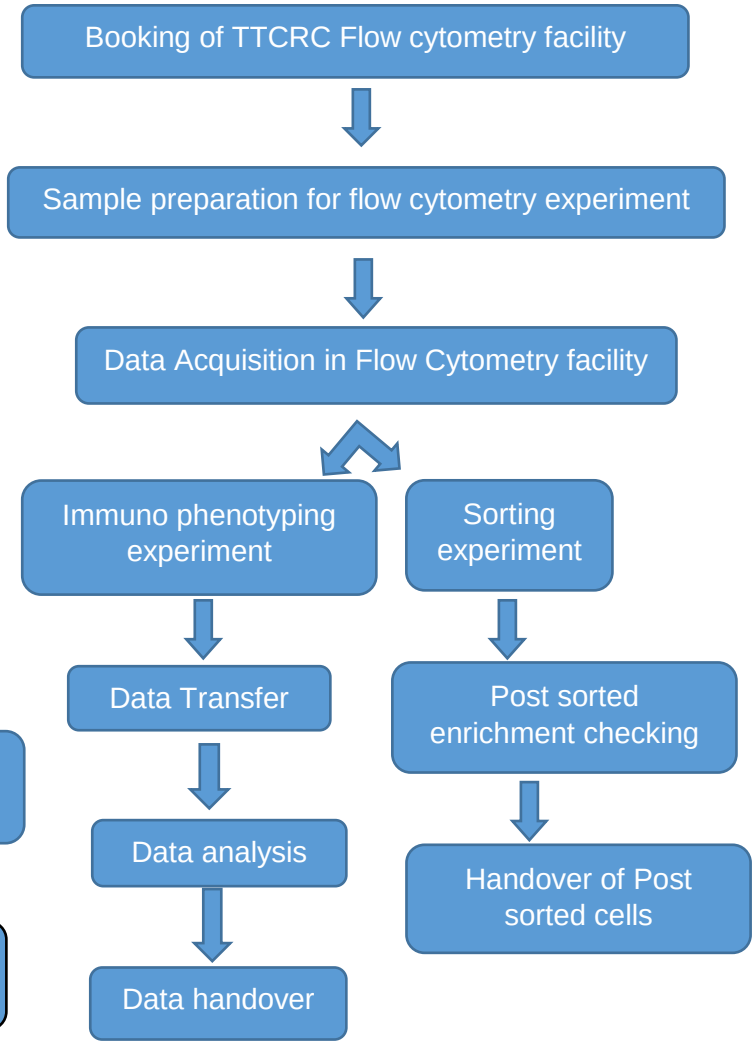
Instrument	Number of experiments
BD Accuri™ C6 Plus	20
BD FACSARIA Fusion™	Immuno phenotyping: 55 Sorting: 7
Miltenyl Biotec MACS	5
Total	**87**

Process Flow:

For internal (TTCRC) Samples:



For external Samples (outside of TTCRC):



FORE Group

Jasmeet Sidhu

DBT Welcome IA Early Career Fellow, Lead-DRP

Arijit Chakraborty

Research Assistant

Tanima Dey

Research Assistant

Bhaswati Tarafdar

Research Assistant

Ankita Das

Intern

Developing precision oncology platform for patients with very high risk acute lymphoblastic leukaemia

Team Members

Current:

Jasmeet Sidhu	Team lead
Arijit Chakraborty	Research assistant
Tanima Dey	Research assistant
Bhaswati Tarafdar	Research assistant
Ankita Das	Intern

Previous:

Arko Bhowal	Research assistant
Priyanka Bose	Research assistant

Background

In India, early results of the ICiCLe-ALL - 14[1], risk stratified randomised clinical trial for childhood ALL, suggests survival of nearly 75% in the last 5-years compared to ~65% in the previous two decades. Around 15% of ALL patients have either very high levels of minimal residual disease (MRD) at the end of induction therapy or persistent MRD is detected at subsequent time points despite intensified therapy (very high risk, VHR). Outcomes for these patients remain poor even with stem cell transplantation and therapeutic strategies remain to be defined. The solution lies in using high quality drugs and sensitive synergistic chemotherapy combinations for patients with poor response to standard protocols.

Aim: To identify strategies to decrease MRD in acute lymphoblastic leukaemia (ALL)

Research objectives:

1. High-throughput imaging-based *ex vivo* drug screening to identify alternate sensitive agents in real time for patients with poor response ALL

2. Identify drug resistance mechanisms using an *ex vivo* model of MRD

Ongoing work

Use of high-throughput drug profiling identifies potential alternate chemotherapeutic agents for relapsed/refractory ALL patients.

We, and others, have shown that the cellular and soluble components of the bone marrow microenvironment protect leukaemic cells from chemotherapy [5,6]. Leukemic cells in the marrow niche adapt to cytotoxic stress through exchange of nutrients and redox adaptation [5]. To mimic this marrow microenvironment *ex vivo*., we established an experimental workflow using a co-culture system (Figure 1). Primary ALL cells are cultured along with bone marrow mesenchymal cells (BMSCs) prior to drug exposure [7,8]. A panel of 14 chemotherapeutic drugs are then added in the cell suspension in serial dilutions and in triplicates, based on number of cells available for assay. Remaining live leukemic cells are counted after 72 hours of drug treatment. Drug response is then quantified to assess sensitivity profiles of the primary sample (**Figure 2**).

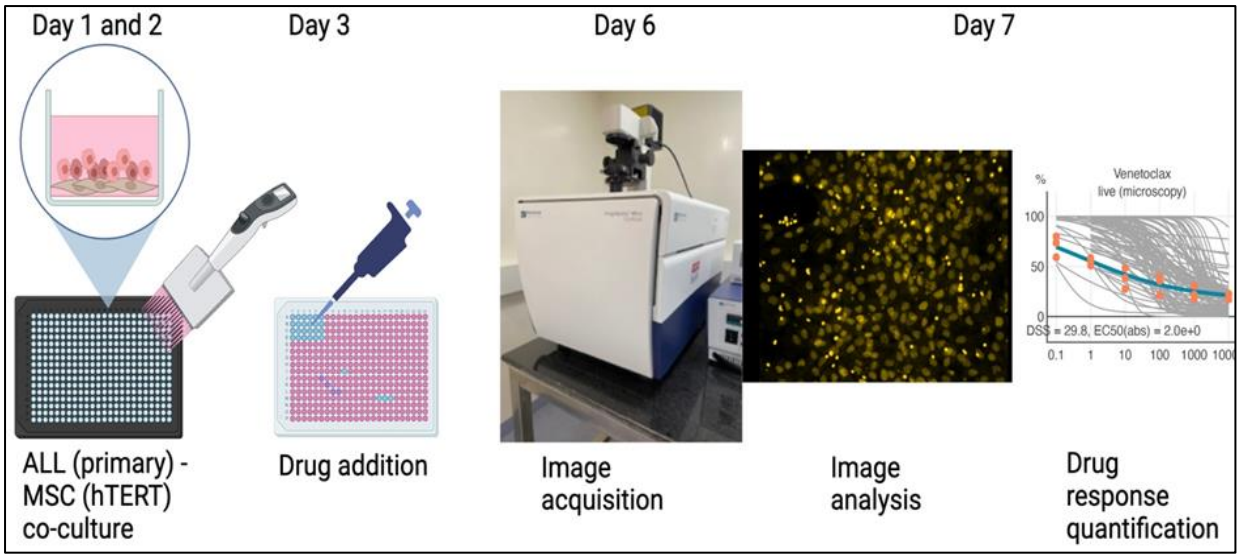


Figure 2. Schematic representation of high-throughput drug screening pipeline

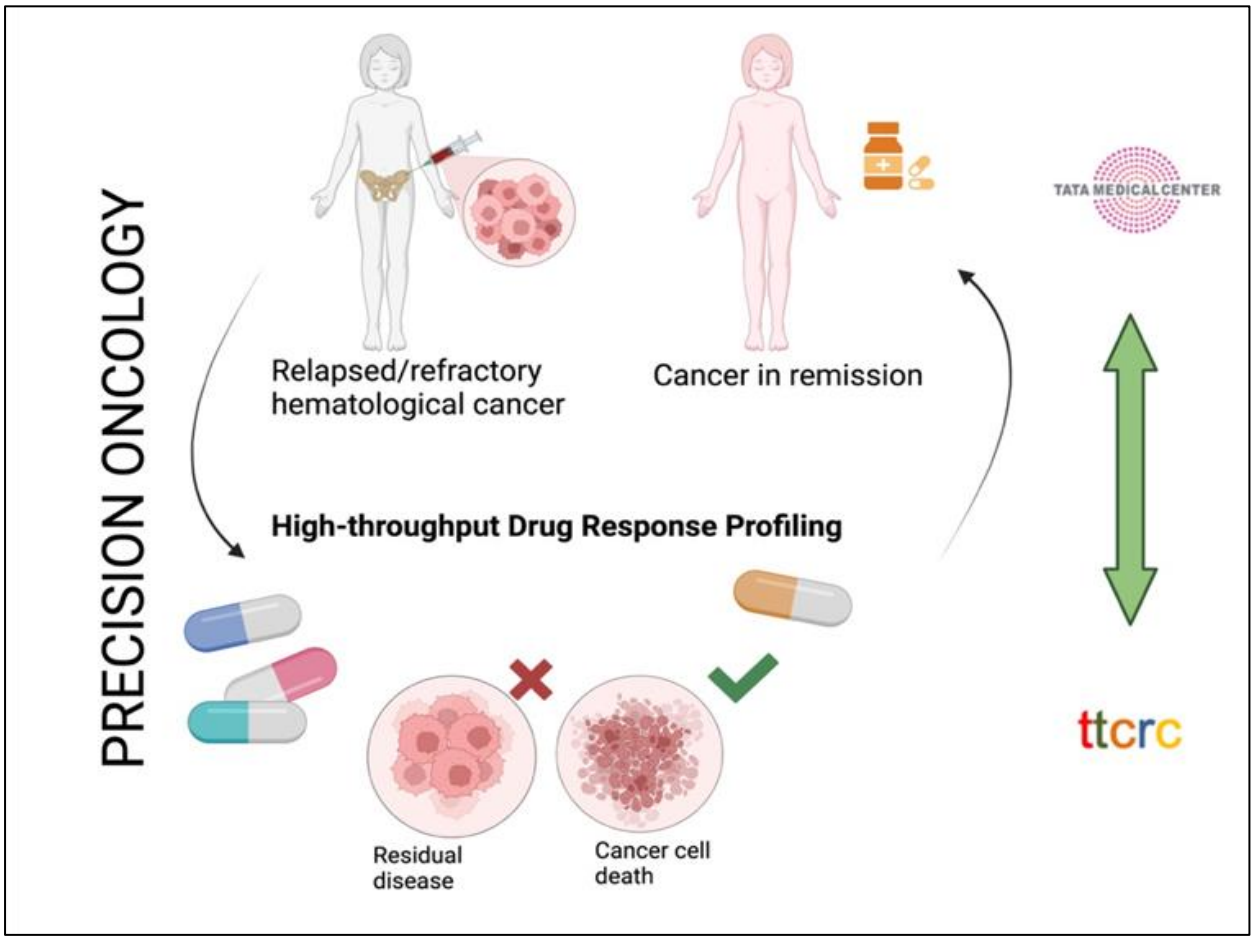


Figure 3. Schematic representation of DRP workflow at TTCRC for relapsed/refractory ALL patients at Tata Medical Center

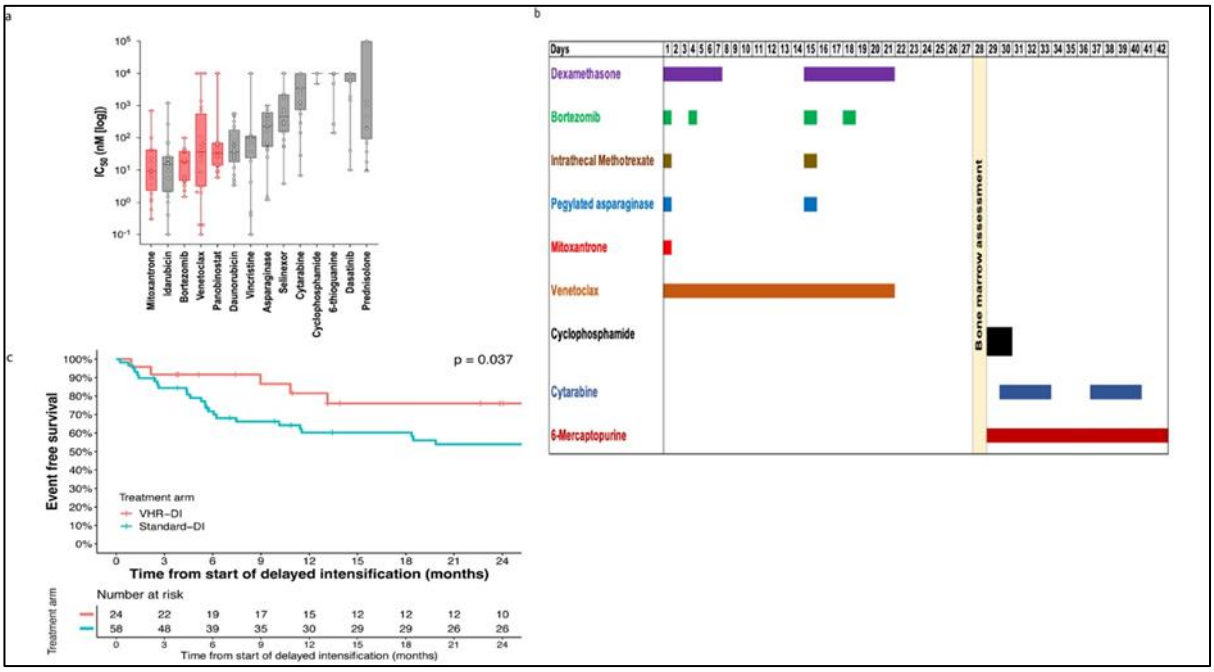


Figure 4. DRP informs therapy on very high risk ALL and significantly improves outcomes

a. Very-high risk ALL primary samples from 25 patients were treated *ex-vivo* with a 14-drug panel. Venetoclax, bortezomib and panobinostat were identified as active agents (IC_{50} <100nM for venetoclax and panobinostat, <10nM for bortezomib) in 71%, 42% and 75% of samples, respectively.
b. Schema of Very High Risk-Delayed Intensification (VHR-DI). Changes were made in delayed intensification phase based on DRP findings- vincristine was replaced with bortezomib and venetoclax was added.
c. Landmark analysis comparing survival of patients(from start of DI) treated on standard DI versus VHR-DI showed significant improvement in 2-year event free survival from 54% (95% CI 40-66) to 76% (95% CI 51-89)(*p*=0.037, log-rank test)

Blasts from 74 patients with ALL (25 VHR, 37 high-risk, 7 intermediate-risk, and 5 standard-risk,) were analysed **(Figure 3)**. Venetoclax (VEN), bortezomib (BZM) and panobinostat (PNB) were identified as active agents (IC50 <100nM for VEN and PNB, <10nM for BZM) in 71%, 42% and 75% of VHR samples, respectively **(Figure 4a)**. Based on drug-sensitivity patterns of VHR ALL, a pilot study was initiated to prospectively evaluate the addition of VEN and BZM in the frontline delayed intensification (DI) phase of VHR ALL patients (N=24) after informed consent (**Figure 4b**). Response was assessed before and after VHR-DI in 46% (11/24) patients. Good response, i.e. decrease in MRD ≥ 1 log after VHR-DI, was noted in 82% (9/11) patients. Landmark analysis comparing survival of patients (from start of DI) treated on standard DI versus VHR-DI showed significant improvement in 2-year EFS from 54% (95% CI 40-66) to 76% (95% CI 51-89) (p=0.037, log-rank test) **(Figure 4c)**. Overall, VHR-DI was tolerated well. No significant difference was noted in grade 4 and 5 toxicities (Common Terminology Criteria for Adverse Events v4.0) between standard DI and VHR-DI (p= 0.053 & p=0.988 respectively, chi-square test). Fever-neutropenia and sepsis were the most frequent toxicities with VHR-DI.

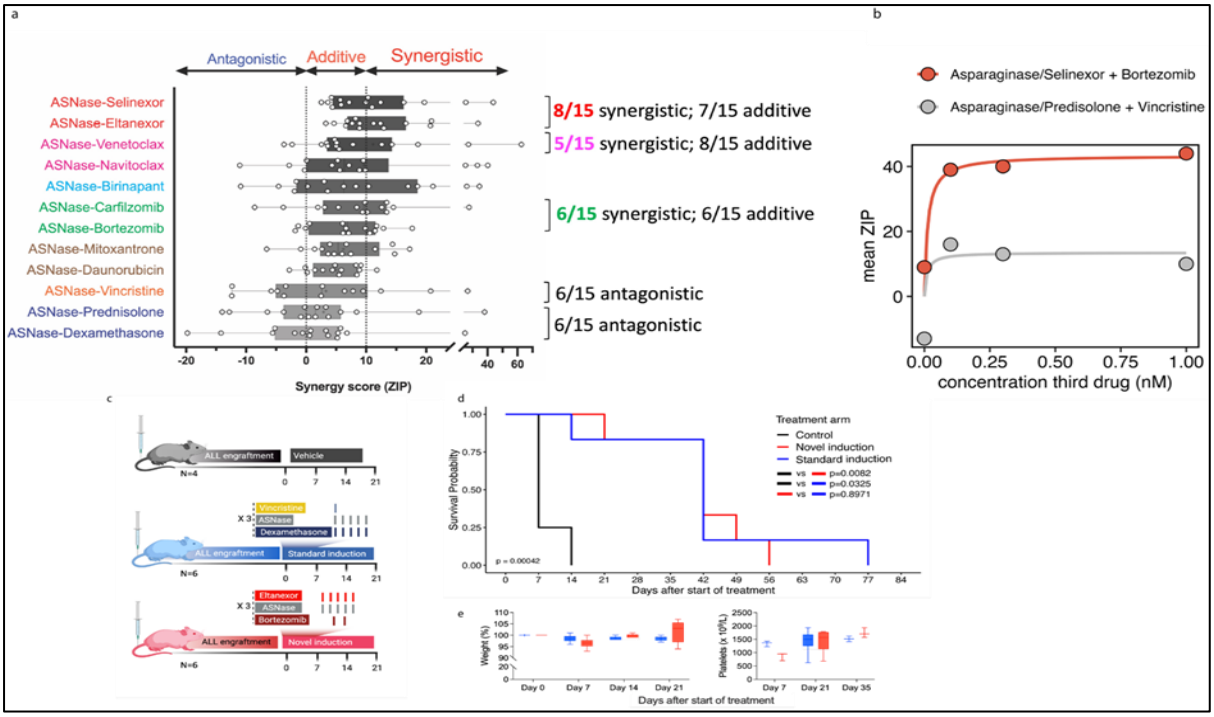


Figure 5. Drug combination profiling identifies synergistic combinations that are effective at low doses and are tolerable

a. Box-plot representation of synergy scores of 12 tested drug combinations. Synergy scores (ZIP) ≥10 represents synergy, 1-10 additivity and ≤0 as antagonism
b. Represention of three drug combinations. Addition of 0.1nM bortezomib doubles the synergy of asparaginase-selinexor combination.
c. Schematic of in-vivo experiment and results. Mice were randomised to 3 treatment groups - vehicle, ASNase-bortezomib-eltanexor (ABE) and ASNase-dexamethasone-vincristine (ADV). Both treatment groups had significant improvement in event free survival (EFS) as compared to the control group (ABE p-value 0.0082; ADV p-value 0.0325) though no significant differences were noted between the 2 treatment arms (p-value 0.8971). Both treatment arms were well tolerated.

We tested combinations of ASNase with the 12 chemotherapeutic drugs using a 4x4 drug matrix in 15 high-risk ALL PDX samples (**Figure 5a**). Synergy scores were calculated using the zero-interaction potency (ZIP) model. Synergy (ZIP score ≥10) or additivity (ZIP score >0 and <10) with ASNase was identified for exportin-1 inhibitors (XPO1i; selinexor, eltanexor), venetoclax and proteosomal inhibitors (bortezomib, carfilzomib) in 100% (15/15), 86.6% (13/15) and 80-86.6% (12/15 and 13/15) samples respectively. Antagonism (ZIP score <0) was noted in more than one-third (6/15) of samples with ASNase in combination with steroids or vincristine. Three-drug combinations were evaluated *ex-vivo* in these 15 PDX samples. Synergy was identified with addition of low doses of BZM or VEN to the ASNase-SEL combination (ASNase-SEL-BZM and ASNase-SEL-VEN) (**Figure 5b**).

Next, 4-6 mice per treatment arm were transplanted intravenously with 1 million ALL cells from 4 high-risk B-ALL patients **(Figure 5c)**. Randomized cohorts were treated with vehicle, ASNase-BZM-ELTA (ABE) and ASNase-dexamethasone-vincristine (ADV). Leukemic burden was assessed weekly using flowcytometry. The

mice tolerated both treatment arms well. Platelet counts recovered within one week after an initial drop in the ABE arm. Both treatment groups had significant improvement in event free survival (EFS) as compared to the control group (ABE p-value 0.0082; ADV p-value 0.0325) though no significant differences were noted between the 2 treatment arms (p-value 0.8971). Seven relapsed/refractory ALL patients were treated with the identified synergistic combination of asparaginase-bortezomib-venetoclax-selinexor (ABVS). Three patients had complete MRD remission while 2 had progressive disease. One patient died due to severe acute pancreatitis.

Understanding drug resistance mechanisms in refractory patients

To investigate the mechanisms of sensitivity to Selinexor, we treated a selinexor-sensitive (P0) and a selinexor-resistant (P1) high-risk ALL patient-derived xenograft (PDX) sample with IC50dose of selinexor for different time-periods, in triplicates at each time point (**Figure 6a**). The protein expression completely changed after 24hours in treated cells as compared to those untreated (**Figure 6b, c**). Upregulation of mitochondrial respiration, lipid and glucose metabolism was noted in sensitive sample. The resistant sample had upregulation of NFkB and inflammatory pathways.

Indication	Age (y)	Cytogenetics	Disease burden before start of ABVS	Response after completion of block
Relapsed refractory BCP ALL	8	High hyperdiploidy	35% blasts at end of consolidation	CR2, MRD <0.01%
Relapsed refractory BCP ALL	12	B-other	90% blasts at end of induction	Progressive disease
Relapsed refractory BCP ALL	18	B-other		Progressive disease
Refractory BCP ALL	5	B-other	Induction failure, 0.3% blasts at end of consolidation	CR, MRD <0.01%
Refractory BCP ALL	11	B-other	MRD ≥0.01% at mid of interim maintenance	n/a, death
Very early relapsed ETP ALL	22	-	88% blasts	CR2, MRD <0.01%
Very early relapsed BCP ALL	8	*IKZF1* del, *DUX4* r	95% blasts	Ongoing treatment

Table 1. Patient characteristics and response outcome after DRP-informed selinexor based combination treatment.

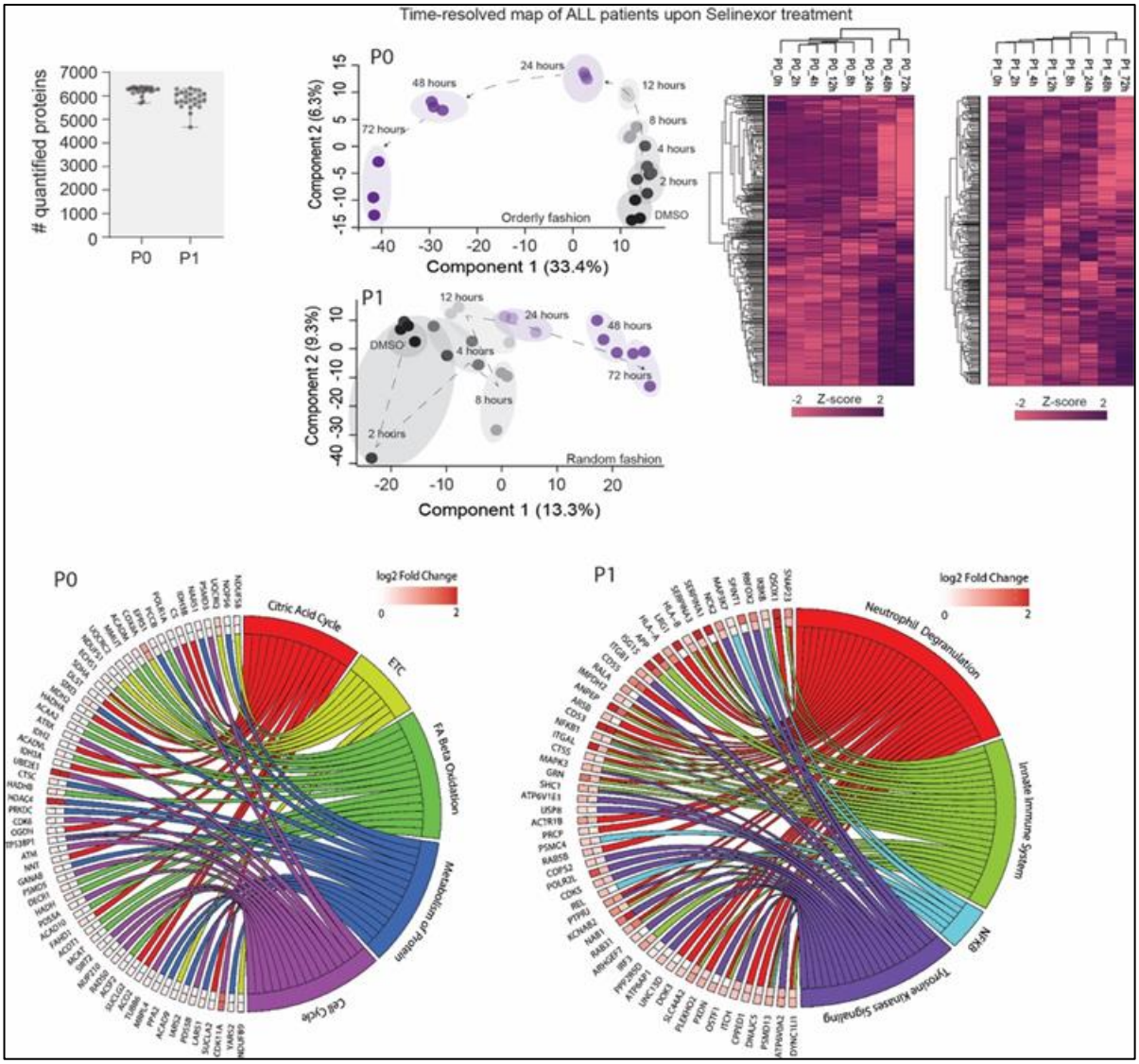


Figure 6. Proteomic analysis of Selinexor sensitive and resistant ALL PDX samples

a. Around 6000 unique proteins were identified in both selinexor sensitive (P0) and resistant (P1) ALL PDX samples
b. Time-resolved map of ALL samples show shift in protein expression at 24hours in both P0 and P1
c. Heatmap showing change in protein expression at serial time points in both P0 and P1
d. Circus plot representing pathway analysis in both P0 and P1 at 24hr time point. There is upregulation of metabolism in sensitive sample while resistant sample has upregulated NFkB pathway.

Future direction:

Short-term goals:

- Continue work in testing and providing best quality asparaginase to the patient at TMC (Recombinant asparaginase – Gennova)
- Widening of drug panel to 100 drugs, combination drug screening and automation of drug response profiling pipeline at TTCRC

- Using CRISPR-Cas9 based approach to identify synthetic lethality with selinexor treatment
- To identify and characterise drug resistant subclones in MRD population (Sree PVF grant-1st phase of grant review cleared).

Long-term goals

- Systematic evaluation of DRP as potential strategy for very high-risk patients through national ALL study (ICiCLe-2).
- Establishing TTCRC-TMC as a hub for precision oncology in India

Publications and awards (last 3 years)

Research articles

- Sidhu J, Chakraborty A, Das P, Gogoi MP, Dey T, Steffen FD, et al. Delayed Intensification Including Venetoclax and Bortezomib Prolongs Survival in Very High Risk Acute Lymphoblastic Leukaemia. Blood 2023 Vol. 142 Pages 4244
- Sidhu J, Steffen FD, Jimenez IA, Huang Y, Rauwolf. KK, Chakraborty A, et al. Phenotypic Drug Response Profiling Identifies Asparaginase-Based Synergistic Combinations for Very High Risk Acute Lymphoblastic Leukaemia. Blood 2023 Vol. 142 Pages 417.
- Huang Y, Drakul A, Sidhu J, Rauwolf KK, Kim J, Bornhauser B, Bourquin JP. MSC. sensor: Capturing cancer cell interactions with stroma for functional profiling. SLAS Discovery. 2023 Aug 11.
- Sidhu J, Gogoi MP, Krishnan S, Saha V. Relapsed Acute Lymphoblastic Leukemia [published online ahead of print, 2023 Jun 21]. *Indian J Pediatr*. 2023;10.1007/s12098-023-04635-4.
- Sidhu J, Masurekar AN, Gogoi MP, et al. Activity and toxicity of intramuscular 1000 iu/m(2) polyethylene glycol-E. coli L-asparaginase in the UKALL 2003 and UKALL 2011 clinical trials. *Br J Haematol*. Mar 29 2022
- Sidhu J, Saha V, Krishnan S. Reply to: Comment on: Unsatisfactory quality of E. coli asparaginase biogenerics in India-Implications for clinical outcomes in acute lymphoblastic leukaemia. *Pediatr Blood Cancer*. Feb 2022;69(2):e29334.
- Sidhu J, Gogoi MP, Agarwal P, et al. Unsatisfactory quality of E. coli asparaginase biogenerics in India: Implications for clinical outcomes in acute lymphoblastic leukaemia. *Pediatr Blood Cancer*. Nov 2021;68(11):e29046.

Conference papers

- Sidhu J, Steffen FD, Jimenez IA, Huang Y, Rauwolf KK, et al. Phenotypic Drug Response Profiling Identifies Asparaginase-Based Synergistic Combinations for Very High Risk Acute Lymphoblastic Leukaemia. 65th ASH Annual Meeting & Exposition, San Diego, December 2023
- Sidhu J, Chakraborty A, Das P, Gogoi MP, Dey T, et al. Delayed Intensification Including Venetoclax and Bortezomib Prolongs Survival in Very High Risk Acute Lymphoblastic Leukaemia. 65th ASH Annual Meeting & Exposition, San Diego, December 2023
- Sidhu J, Banerjee S, Bhowal A, Das P, Gogoi MP, Siddhanta R, et al. Drug response profiling identifies sensitive synergistic drug combinations for patients with refractory/relapsed acute lymphoblastic leukemia. Pediatric Hematology Oncology Journal. 2022;7(4)

- Sidhu J, Banerjee S, Krishnan S, Saha V. Functional approach for high-risk ALL in India. 3rd Annual Meeting of the European Society for Paediatric Oncology (SIOP Europe), 2022
- Sidhu J, Bodmer N, Manioti D, Baltensperger O, Saha V, Bornhauser B, Bourquin JP. Drug response profiling identifies potent chemotherapy combination therapies for patients with high-risk acute lymphoblastic leukaemia. SPOG Scientific Meeting, 2022.
- Sidhu J, Agarwal P, Mukherjee T, Saha D, Bose P, Roy P, Phadke Y, Sonawane B, Paul P, Saha V, Krishnan S. The variable quality of commercial E. Coli L-asparaginases (EcASNases) in India and the implications for clinical practice. XIIIth SIOP Asia Conference, 2021.

Awards

- ASH Abstract Achievement Award, ASH 2023, San Diego
- Principal investigator, Precision Approach for Treatment of Haematological Malignancies (PATH-01 2023), Tata Medical Center
- First position, Award paper presentation, PHOCON 2022, New Delhi
- Second position, Award paper presentation, SIOP Asia Conference, 2021

Collaborations

Academic

1. University of Zurich – establishment of DRP pipeline
2. KiTZ, Hopp Children's Cancer Center, Heidelberg – proteomic analysis

Pharma

1. VHB Lifesciences - Leucoginase study
2. Karyopharm – Eltanexor donation for in-vivo experiments, Selinexor access for PATH-01 2023 study

References

1. Das N, Banavali S, Bakhshi S, et al: Protocol for ICiCLe-ALL-14 (InPOG-ALL-15-01): a prospective, risk stratified, randomised, multicentre, open label, controlled therapeutic trial for newly diagnosed childhood acute lymphoblastic leukaemia in India. Trials 23:102, 2022
2. Sidhu J, Gogoi MP, Agarwal P, et al: Unsatisfactory quality of E. coli asparaginase biogenerics in India: Implications for clinical outcomes in acute lymphoblastic leukaemia. Pediatr Blood Cancer 68:e29046, 2021
3. Rosa Furneaux LM: The drug was meant to save children's lives. Instead, they're dying., The Bureau of Investigative Journalism. London, 2023
4. Sidhu J, Saha D, Roy P, et al: Therapeutic drug monitoring informs asparaginase dose scheduling in the InPOG-ALL-15-01-ICiCLe-ALL-14 trial. Pediatric Hematology Oncology Journal 2:S2-S3, 2017
5. Liu J, Masurekar A, Johnson S, et al: Stromal cell-mediated mitochondrial redox adaptation regulates drug resistance in childhood acute lymphoblastic leukemia. Oncotarget 43048-64, 2015
6. Ebinger S, Ozdemir EZ, Ziegenhain C, et al: Characterization of Rare, Dormant, and Therapy-Resistant Cells in Acute Lymphoblastic Leukemia. Cancer Cell 30:849-862, 2016
7. Sidhu J, Banerjee S, Bhowal A, et al: Drug response profiling identifies sensitive synergistic drug combinations for patients with refractory/relapsed acute lymphoblastic leukemia. Pediatric Hematology Oncology Journal 7, 2022
8. Frismantas V, Dobay MP, Rinaldi A, et al: Ex vivo drug response profiling detects recurrent sensitivity patterns in drug-resistant acute lymphoblastic leukemia. Blood 129:e26-e37, 2017

SOLI3D Group

Dwijit GuhaSarkar

Lead Scientist –Organoid Laboratory

Payel Guha

Post-Doctoral Fellow

Ankita Dutta

PhD Student

Shinjini Chandra

Research Assistant

Nandita Chowdhury

Research Assistant

Pritha Banerjee

Clinical Research Coordinator

Smrithi JS

Research Assistant

Solid tumour organoid model development and research programme

SOLI3D team:

Dr Dwijit GuhaSarkar (DGS), Lead
Dr Payel Guha (PG), Postdoc
Ankita Dutta (AkD), PhD student
Shinjini Chandra (SC), Research Assistant
Nandita Chowdhury (NC), Research Assistant
Pritha Banerjee (PB), Clinical Research Coordinator

Past members

Smrithi JS (SJS), Research Assistant
Anindita Dutta (AD), Lead

Other teams at TTCRC:

Abhirupa Kar and TiMBR team
Dr Debdutta Ganguly and Genomics team
Dr Ashok Kr. Jayavelu and CPU team

Clinical team at TMC:

Dr Sudeep Banerjee (Consultant, GI-HPB)
Dr Manas Kr Roy (Consultant, GI-HPB)
Dr Paromita Roy (Consultant, Pathology)
Dr Saugata Sen (Consultant, Radiology)
Dr. Sanjit Kr Agrawal (Consultant, Breast Onco-surgery)
Dr. Rosina Ahmed (Consultant, Breast Onco-surgery)
Dr. Indu Arun (Consultant, Pathology)

TCS Research team:

Dr. Rajgopal Srinivasan (Chief scientist, TCS)
Uma Sunderam (Scientist, TCS)
Akshaya VS (Researcher, TCS)

Other collaborators:

Prof. Alberto Saiani and Prof. Aline Miller (Scientist, University of Manchester)

Background:

Solid cancer programme at TTCRC started in April, 2019 with Gallbladder cancer (GBC) program led by Dr. Anindita Dutta. The goal was to establish ex vivo models of gallbladder diseases to understand GBC pathogenesis and identify alternative therapeutic targets. Since then, our group has come a long way. While our group continued to make progress in the gallbladder study, last year we also initiated a new study with breast cancer. In this study, we intend to test the feasibility of predicting neoadjuvant chemotherapy response in patients using ex vivo drug response profiling with patient derived breast cancer organoid (PDBCO) models.

Reflecting the group's wider interest in studying multiple **solid** cancers using patient tissue derived three-dimensional (**3D**) models (organoids) for different research applications, our group (previously known as GBC group) has been rechristened as **SOLI3D** group. The acronym SOLI3D stands for **S**olid tumour **O**rganoid **L**aboratory for **I**nvestigation into (i) **D**isease biology, (ii) **D**rug response profiling and (iii) **D**rug discovery.

In the previous years, we reported how we had overcome the initial challenges in a multidisciplinary project and set up one of the first patient derived tumour organoid laboratories in the country. Last year, we showed how we optimised the protocols for developing and successfully expanding patient derived gallbladder cholangiocyte organoid (PDGCO) models from multiple non-malignant and malignant tissue pathologies. We also demonstrated the developed organoids were functional, they recapitulated the histopathological features of their parent tissues, expressed marker gene expressions and could be banked in living condition for further expansion in culture upon retrieval at a later timepoint when required. We showed high quality biomolecules could be extracted from both the primary tissues and the developed organoids for high throughput molecular

characterisation studies using our optimised protocols.

Progress in the Gallbladder cancer study using PDGCO models to investigate pathogenesis and disease biology:

To delve deeper into molecular characterisation for understanding the disease biology, previously we performed the transcriptomic (Illumina TruSeq Stranded mRNA-seq) analysis in collaboration with the Genomics team at TTCRC and Bioinformatics team at TCS Research. We showed the preliminary results last year, where we found the malignant tissues clustered separately from the non-malignant ones based on their transcriptomic profiles. Notable exceptions were the tissues with xanthogranulomatous cholecystitis (XGC) pathology. XGC is a rare benign inflammatory disease of gallbladder characterised by aggregation of lipid-laden foamy macrophages. XGC tissues clustered closely with the malignant tissues suggesting similarity in their transcriptomic profiles, thus supporting the postulation of long-standing chronic inflammation driven GBC pathogenesis. However, the number of samples were few at that time and the PDGCOs developed from malignant tissues were not analysed.
The study took a complete shape this year as we sequenced a total of 42 primary gallbladder tissues and 38 PDGCO lines so far spread across different pathology groups. Our preliminary observation from last year was further bolstered with more tissues analysed this year and interestingly, the data from the PDGCO lines also supported the same. Hierarchical clustering analysis of the transcriptomic data from the PDGCO models mimicked the similar clustering pattern (primary clustering) as observed for the tissues, further assuring the developed PDGCOs representing the gene expression profiles of the source tissues at the transcriptomic level. Moreover, we observed that irrespective of the culture conditions (canonical Wnt activated or inhibited) used for growing the organoid lines, the primary clustering was always based on pathological states [**Figure 1**]. This observation suggests that the models developed in both the culture conditions represent the disease specific gene expression profiles.
As cellular phenotype is more closely correlated with its proteomic profile, we wanted to perform proteomic analysis of the gallbladder tissues of different pathological states and the corresponding derived organoids. For this project we worked in close collaboration with the Clinical Proteomic Unit (CPU) at TTCRC. We first optimised the protein extraction protocols both from the gallbladder tissues and the organoids followed by sample preparation to be analysed by liquid chromatography with tandem mass spectrometry (Ekspert nanoLC 425 with Sciex TripleTOF 6600+ system). So far, mass spectrometric analyses have been performed for gallbladder tissues collected from 8 patients along with the derived organoids from these tissues. The total ion chromatograms showed expected profiles and we were able to detect peptides from >4000 unique proteins for each sample [**Figure 2**]. More samples need to be analysed to assess whether the PDGCO models represent the proteomic profiles of their parent tissues and to have insight into the protein expressions driving the gallbladder disease conditions leading to GBC.

Breast cancer study for organoid based drug response profiling

In collaboration with Dr. Sanjit Kr Agrawal and Breast Oncosurgery team at Tata Medical Center Kolkata (TMC-K) we initiated this study to test the feasibility whether we can predict chemotherapeutic drug response in triple negative (TNBC) and HER2-enriched breast cancer (HER2-BC) patients using personalised tumour organoid models. To achieve this goal, we are currently working towards two objectives – (i) developing PDBCO models from breast cancer core needle biopsy tissues and (ii) optimisation of PDO based drug response assay. The second objective further has two components – (a) optimisation of biocompatible matrix for reproducible organoid drug assay and (b) optimisation of cellular viability assay for organoids.

i. Developing PDBCO models from breast cancer core needle biopsy tissues:

We have developed PDBCO lines from needle core biopsy tissues collected from TNBC and HER2-BC patients who were planned to undergo treatment at TMC-K in the neoadjuvant chemotherapy setting broadly following the protocols published by others[1,2]. Histological analysis has been performed to confirm that these PDBCO lines recapitulated the cytological and architectural malignant features of the source tissues [**Figure 3**].

ii. (a) Optimisation of biocompatible matrix for reproducible organoid drug response assay:

Extracellular matrix (ECM) plays a crucial role in regulating the tumour cell property. It is also an essential component for supporting organoid growth. Currently, the most commonly used matrix for this purpose is sourced from murine sarcoma extract (commercially available as Matrigel™ or Geltrex™ etc.). Although these murine ECM products are efficient in supporting organoid growth, they suffer from batch to batch variation potentially leading to assay reproducibility issue. Moreover, presence of hundreds of murine proteins in the drug assay would risk influencing the PDO's response to the drugs. Hence, we are testing peptide based biocompatible synthetic hydrogels as a substitute for the murine ECM. For this project we have collaborated with Prof. Alberto Saiani and Prof. Aline Miller's group at Univ. of Manchester, who have developed a number of potential peptide hydrogels that are biocompatible.

We have tested four such peptide hydrogels – Alpha 2 PeptiGel™, Alpha 4 PeptiGel™, Alpha 4 Plus PeptiGel™, Gamma 4 Plus PeptiGel™, having different biophysical and rheological properties with or without functional motifs [**Table 1**]. Among the tested hydrogels, Alpha 4 PeptiGel™ showed best promise, which could support the PDO growth from patient tumour cells and culture maintenance for 3 weeks [**Figure 4**]. However, the growth rate was slower compared to the murine ECM (Geltrex). Histological analysis showed evidence of preserving histological features in the Alpha 4 PeptiGel grown PDOs [**Figure 5**]. Drug assay compatibility is yet to be tested.

(b) Optimisation of cellular viability assay for PDOs:

We have initiated the process of drug assay optimisation for the PDO cultures. The most common approach taken globally for the PDO cell viability assay is a luminescence readout based assay (CellTiter Glo 3D kit, Promega)[3]. This assay relies on the principle of only viable (metabolically active) cells producing ATP, which can then be indirectly quantified in the assay. We first tested the sensitivity and precision of this assay for untreated organoids in the multimode microplate reader (Spectramax M2e). To find the linear range and the lower limit of reliable detection, we tested the luminescence reading for a wide range of organoid cell numbers (100 to 15,000). We found that in the tested system, the variability was too high reflected by the standard deviations ranging between 23% to 107% of the respective mean values for most part of the cell number range tested, particularly below 6000 cells/well [**Figure 6**]. To confirm that this is not specifically due to organoid handling error which is often challenging, we tested this with an

easier to manipulate mammalian cell line (L-WRN), which showed similar variability issue below 8000 cells [data not shown]. From this, we concluded that the sensitivity and reproducibility of the assay was not sufficient in the tested set up and equipment for PDO based drug response assay where the number of organoid cells are limited.

An alternative approach[4,5] is microscopic imaging based viability assay following live/dead cell staining of the organoids that we are currently testing. We found that CyQUANT™ (Thermo Fisher Scientific) live cell staining dye was not compatible with other fluorescent dyes due to quenching issues by one of its component solutions. Hence, we chose Calcein-AM (Thermo Fisher Scientific) as the preferred dye for live cell staining when used in combination with other fluorescent dyes, such as propidium iodide for staining dead cells and Hoechst 33342™ (Thermo Fisher Scientific) for staining all cell nuclei. To test whether Calcein-AM/Propidium Iodide/Hoechst 33342 combination staining can be used for organoid cell viability post drug treatment, we treated the organoid derived (TrypLE digested) cells with 10^5 nM gemcitabine. Treated group showed a large number of cell death compared to dimethyl sulfoxide treated control group as expected [**Figure 7**].

Future direction

In the coming year, we would like to complete the proteomic analysis from the gallbladder tissues and corresponding organoids. We would conduct differential gene and protein expression analyses to understand the disease pathogenesis, which can then be validated using the PDGCO models. This would help to identify actionable targetable pathways in future opening the possibilities of testing alternative therapeutic options for GBC.
For the breast cancer study, we need to optimise the PDBCO culture conditions to expand the cultures optimally for molecular validation of the models and performing drug assays. Fine tuning of the culture conditions would require us to test different concentrations and relative ratios of the Wnt inducers and signalling proteins (Wnt3A, R-Spondin 1, Noggin). As the commercially available purified recombinant proteins are prohibitively expensive, indigenous production of these recombinant proteins are necessary. Gennova Pharmaceuticals has partnered with us to support this project by producing the purified proteins. We plan to test these indigenously produced proteins in the coming year to refine the culture conditions to improve the yield of PDBCO expansion.
In the next few months, our priority will be working towards optimising the organoid based drug assay. We would take three alternative approaches for PDO cell viability assay to see which method is most suitable for our purpose. In the first approach, we would continue to optimise the high throughput confocal imaging based viability assay following live/dead staining post treatment. For this approach, high throughput image analysis of organoids is essential. We have collaborated with TCS research, who will develop ML-based image analysis program for 3D structures. In the second approach, we would test flow cytometry method for the viability assay. Our third approach will be to standardise the CellTiter-Glo 3D assay for drug response testing using organoids, albeit subject to accessibility to a microplate reader having luminometer sensitive enough to detect luminescence from small number (500 or below) of cells. Alpha 4 and other selected synthetic hydrogels, that supported organoid growth for 3 weeks, will be assessed for their compatibility with the organoid based drug assays. Finally, we plan to test and establish automation for conducting the PDO drug assays in high throughput. Once the assay is optimised, we would compare the PDO drug response with the response of the patients in clinic to develop a drug response prediction algorithm.

Publication: Dutta A, Mungle T, Chowdhury N, et al. Characteristics and outcomes of gallbladder cancer patients at the Tata Medical Center, Kolkata 2017-2019. Cancer Med. 2023;12(8):9293-9302. doi:10.1002/cam4.5677

New Research Grant approved by ICMR:

IIRPIG-2023- 0000586: Drug response profiling of patient derived organoids to design personalised neoadjuvant chemotherapy for triple negative and HER2-enriched breast cancer patients.

Conference poster presentations:

1. EMBO conference on 'Organoids: modelling organ development and disease in 3D culture'.

   Venue: EMBL, Heidelberg, Germany
   Date: 18-21 October, 2023

   Presenter: AkD

   Title: An annotated living organoid biorepository of gallbladder diseases

2. The 43rd Annual conference of Indian Association for Cancer Research.

   Venue: IISER-Pune, Pune, India
   Presenters: SC and NC
   Title: Patient derived gallbladder and breast cancer organoid library for drug response testing

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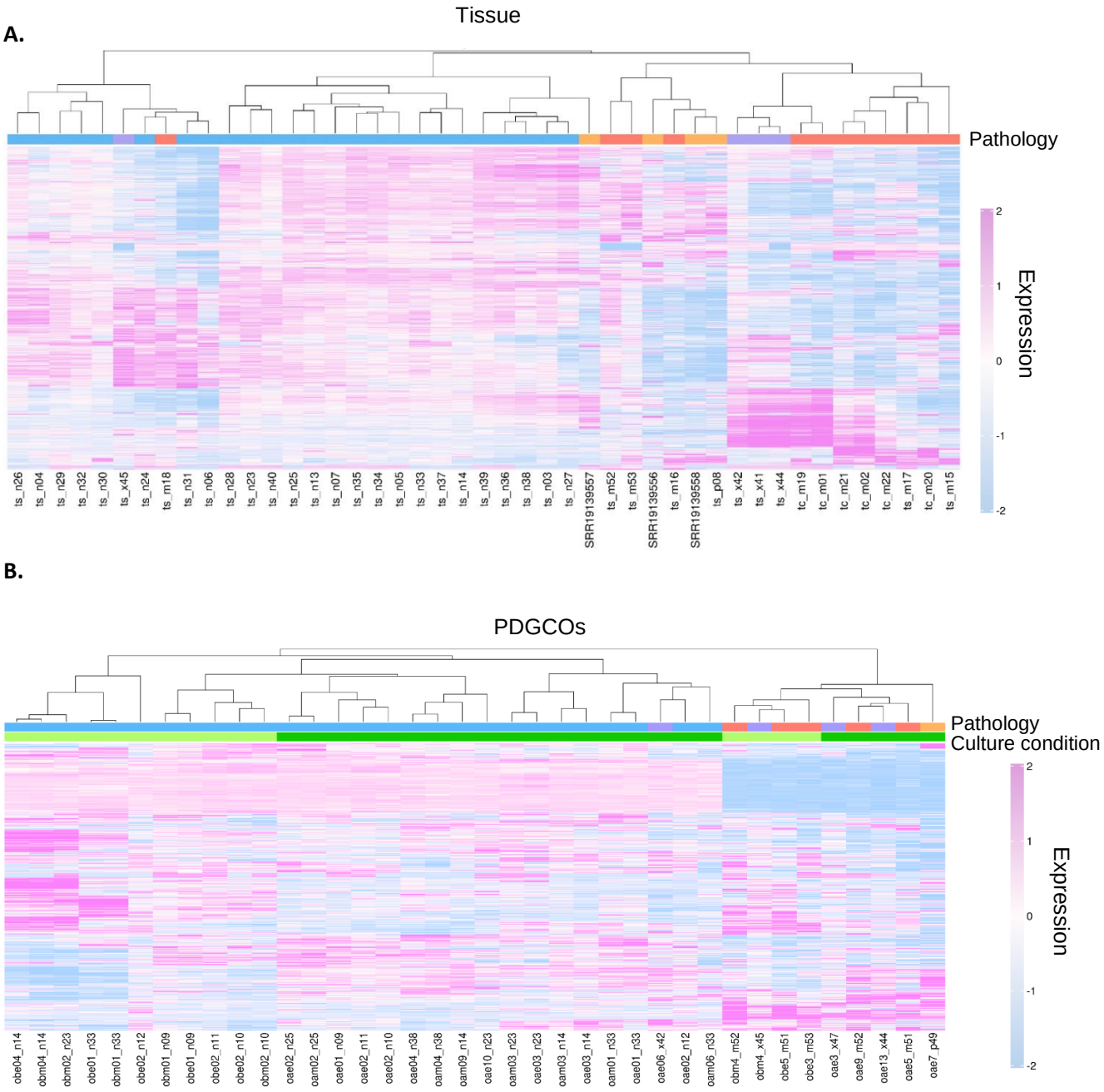
2. Sachs N, de Ligt J, Kopper O, et al. A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity. *Cell* 2018; **172**(1-2): 373-86 e10.
3. Calandrini C, Drost J. Normal and tumor-derived organoids as a drug screening platform for tumor-specific drug vulnerabilities. *STAR Protoc* 2022; **3**(1): 101079.
4. Li X, Fu G, Zhang L, et al. Assay establishment and validation of a high-throughput organoid-based drug screening platform. *Stem Cell Res Ther* 2022; **13**(1): 219.
5. Larsen BM, Cancino A, Shaxted JM, Salahudeen AA. Protocol for drug screening of patient-derived tumor organoids using high-content fluorescent imaging. *STAR Protoc* 2022; **3**(2): 101407.



Pathology Other non-malignant (n) XGC (x) Pre-invasive malignant (p) Invasive malignant (m)

Culture condition Type A Type B

Figure 1. Both primary tissues and GCOs clustered similarly according to the pathological conditions based on their gene expression profile. Hierarchical clustering based on the scaled vst counts of the gene expression profile of **(A)** tissues and **(B)** PDGCOs. Library for mRNA-Seq was prepared using TruSeq Stranded Library Prep kit (Illumina) and 150bp paired end sequencing was performed using NextSeq550 (Illumina). The reads were aligned to the human reference genome (v. GRCh38) with STAR. Based on the gene counts from samples that passed quality checks, protein coding genes with at least 10 reads in 80% of the samples were selected. Counts were transformed with variance stabilization transform (vst) from the DESeq2 package. Hierarchical clustering (with Pearson correlation and linkage clustering algorithm) was performed on top 1000 highly variable genes. Primary clustering was according to pathology for both tissues and GCOs, whereas secondary clustering for GCOs were based on culture conditions. n, normal or inflamed; x, xanthogranulomatous cholecystitis; p, pre-invasive/non-invasive malignant; m, invasive malignant; T and ts, tissue; O, organoids; a, Type A; b, Type B culture conditions; ts, surgically resected tissue; tc, needle biopsy tissue; SRR, external dataset (Yang S. et al. Plos One 2023 Mar 30;18(3): e0283770)

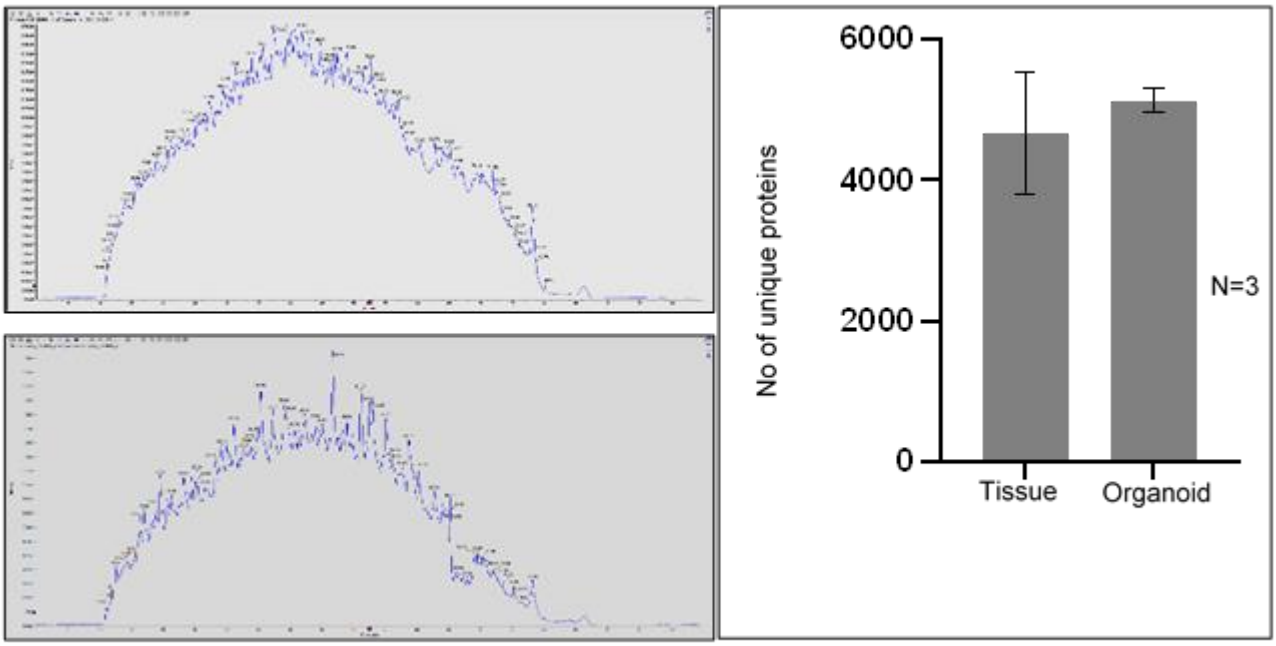


Figure 2. Proteins extracted from the tissues and organoids showed adequality for mass spectrometric analysis. Representative total ion chromatogram showing the mass spectrometric applicability of the tissue (left top) and organoid (left bottom) protein samples in Sciex TripleTOF 6600+ system. Bar graph showing the average number of unique proteins detected from GBC tissue and organoid samples (right panel).

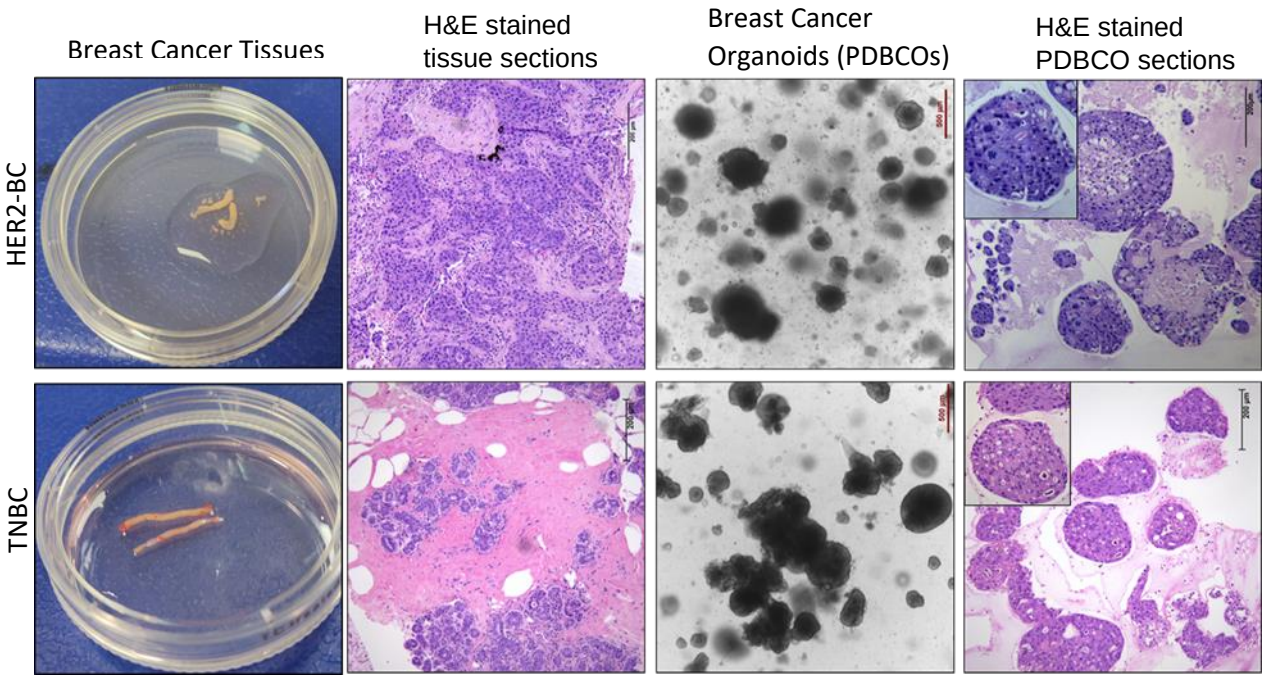


Figure 3. PDBCO lines recapitulated the malignant histopathological features of the source tissues. Representative images of patients tissues collected from TNBC and HER2-BC patients, H&E stained corresponding tissue sections, PDBCO lines developed from the respective tissues and their corresponding H&E stained sections. PDBCO, Patient derived breast cancer organoids; H&E, Hematoxylin and eosin; TNBC, Triple negative breast cancer; HER2-BC, HER2-enriched breast cancer, Microscope – Nikon TS2, Leica DMi8. Scale bar: 200µm. Higher magnification (40x) images of organoid sections shown in the corresponding insets (right most column).

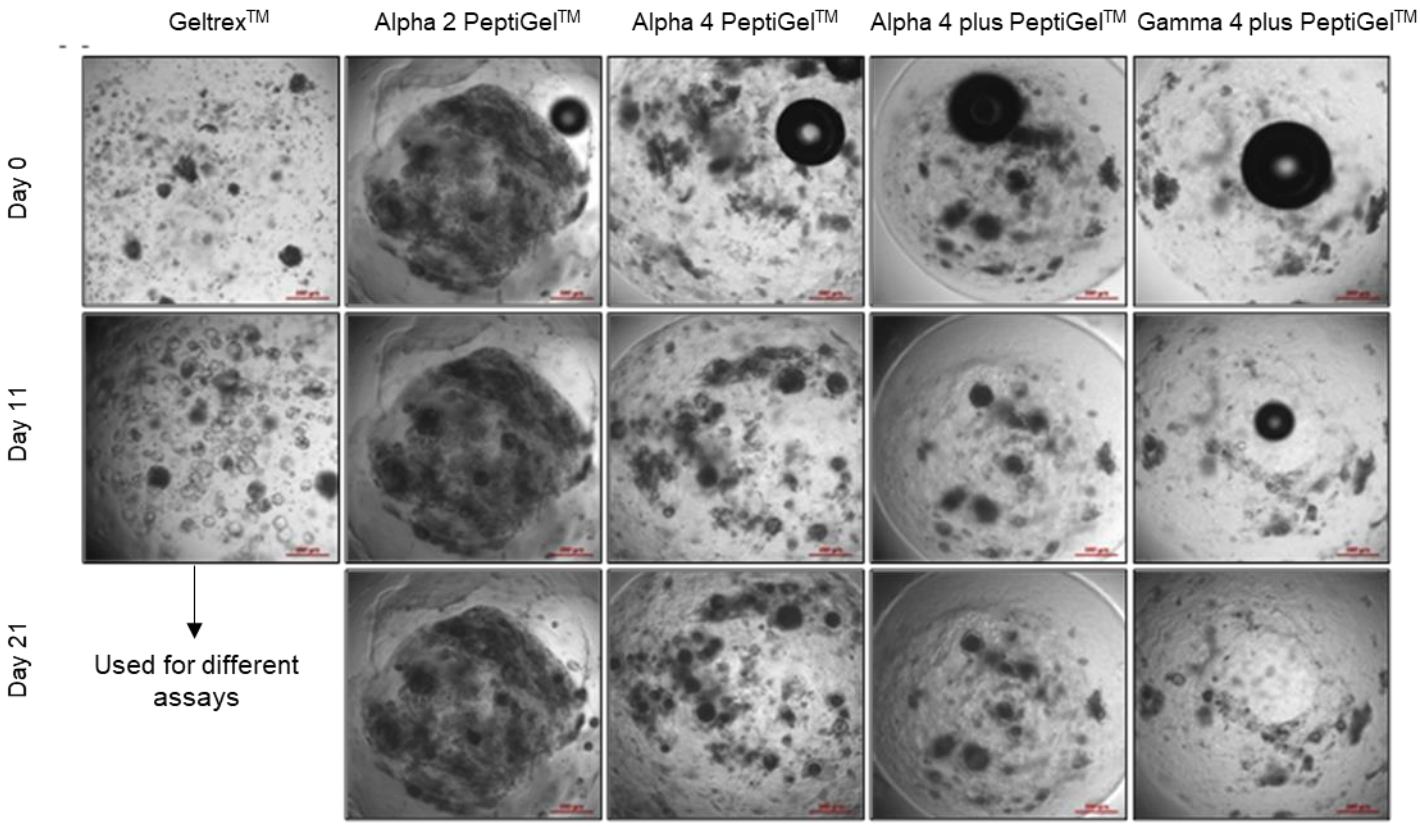


Figure 4. Alpha4 PeptiGel showed best promise among the peptide hydrogels tested for supporting the organoid growth in culture. Bright-field images of PDGCOs cultured for 3 weeks in different peptide hydrogels. Geltrex was used as positive control. Scale bar: 500µm.

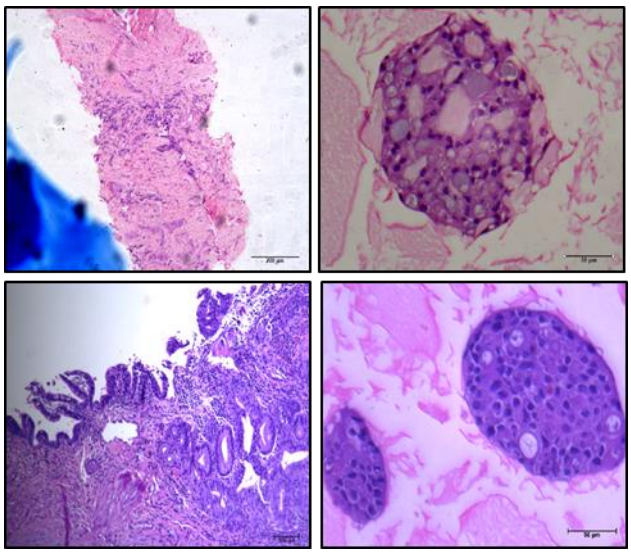


Figure 5. H&E staining analysis shows PDOs grown in Alpha4 PeptiGel maintains the histological features of their corresponding patient tissues. Scale bar: 100µm(left), 50µm

	Geltrex	Alpha 2	Alpha 4	Alpha 4 plus	Gamma 4 plus
Charge	0	+1	+2	+2	+2
Composition	Laminin, collagen IV, entactin, heparin sulfate proteoglycans	Peptides	Peptides	Peptides with RGD, GFOGER motifs	Peptides with RGD, GFOGER motifs
Mechanical Properties (Stiffness)	892 ± 148 Pa	4-5 kPa	1 kPa	0.35-0.7 kPa	0.175-0.35 kPa
Texture	Liquid in 4°C, Gel in RT	Liquid	Gel	Gel	Gel
Gelation	At room temperature (RT)	Upon coming into contact with physiological buffer	Already in Gel form	Already in Gel form	Already in Gel form

Table 1. Composition of different hydrogel matrices

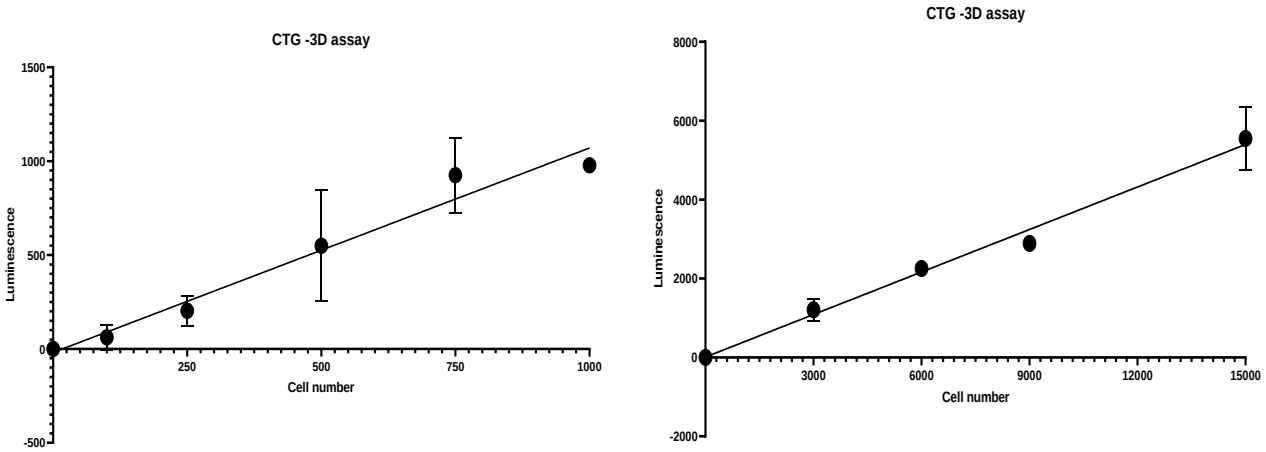


Figure 6. Luminescence based viability (ATP production dependent) assay in the current set up for organoids showed high variability. CellTiter-Glo 3D kit was used for luminescence based cellular viability assay. Cells derived from PDGCO line (BV/23/000249/GIC) by TrypLE digestion was used for the assay. Different number of cells/well were plated in 96-well dish in triplicates to check of reproducibility and linearity range. Left: Cell numbers tested 100, 250, 500, 750 and 1000 per well. Right: Cell numbers tested: 3000, 6000, 9000 and 15000 per well. Luminometer used: SpectraMax M2e multimode plate reader (Molecular Device).

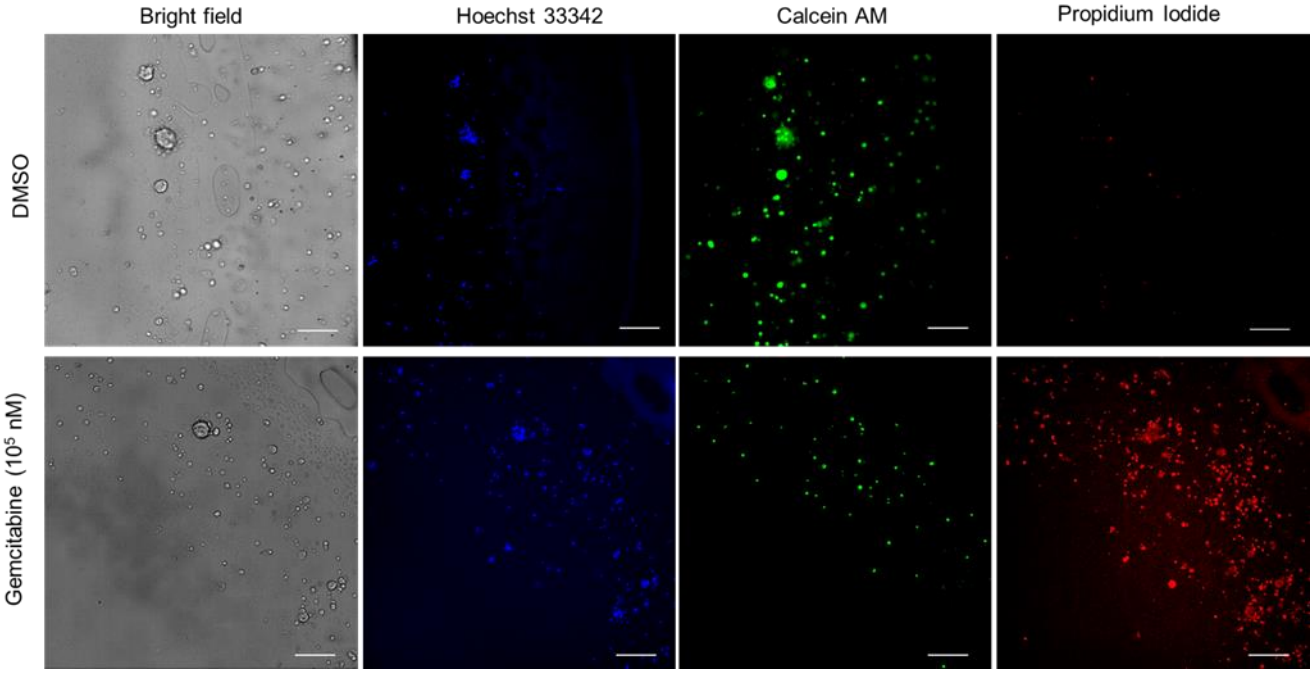


Figure 7. Live/dead cell staining and microscopic fluorescence imaging could be used to detect cell death upon drug treatment of patient derived organoids. Confocal bright field and fluorescence images of Gemcitabine (105 nM) or Dimethyl sulfoxide treated PDGCO derived cells for drug response testing. Organoids were TrypLE-digested and seeded into Geltrex one day prior to treatment. Treated cells were stained with viability dyes (Calcein AM as live cell dye, Propidium Iodide as dead cell staining dye, Hoechst 33342 for all nuclei staining dye) and imaged 72 hrs post-treatment. 2D projection images were captured at 10X magnification using ImageXpress Microconfocal High Content spinning disk confocal microscope (Molecular Devices). Scale bar: 200 µM.

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The Cancer Genomics Laboratory in TTCRC has two arms; it is operational as a core facility infrastructure within TMC as well as providing support to translational research projects.

1. Illumina-based Approach

As a part of the core facility lab since 2018, it has established Illumina-based platforms for RNA-Seq, targeted panel sequencing and single nucleotide polymorphism screening (for copy number detection) to improve 'Disease Classification' along with

Genetic Subtype	1	2	3	5
Hyperdiploid*	Flow Ploidy	Karyotype	FISH	SNP^
ETV6-RUNX1	FISH			
DUX4	RNAseq			
NUTM1	FISH	RNAseq		
TCF3-PBX1	Karyotype	FISH		
MEF2D	FISH			
ZNF384	FISH			
ETV6-RUNX1-like	RNAseq			
Others	Karyotype	FISH	RNAseq	
PAX5 alt/P80R	RNAseq			
BCR-ABL1	FISH			
KMT2A	FISH			
iAMP21	FISH			
Hypodiploid**	Flow Ploidy	SNP		
Ph-like	FISH	RNAseq		
TCF3-HLF	Karyotype	FISH		
IKZF1 N159Y	RNAseq	Sequencing		
TP53	RNAseq	Sequencing		
KMT2A-like	RNAseq			
ZNF384-like	RNAseq			
BCL2/MYC	Karyotype	FISH		
*IKZF1**	FISH	SNP		
*CRLF2**	Flow	FISH		

* includes low hyperdiploid, ** includes low and haploid,
^ to exclude hidden hypodiploid

Figure 1. ALL Disease Classification using RNA-Seq and SNP Array as Companion Diagnostic Tool

standard diagnostic tests for e.g. cytogenetics and karyotyping (Figure1). The combined efforts helped us to develop and optimise new tools and pipelines for companion diagnostics (CDx) which can be delivered in parallel with the standard investigation tools for improved patient diagnosis and personalised therapy. Genomics unit has played important role to plan precise treatment strategies for patients particularly belongs from high-risk groups (*IKZF1*-plus, *DUX4*r, *MEF2D*r, *TP53*-mutated etc). Till date we have sequenced almost 137 total RNA and mRNA libraries prepared from patient samples across different ALL subtypes like High-hyperdiploid (HeH), B-Others, *ETV6-RUNX1*, *ZNF384*r, *PAX5*alt, *IKZF1*-del etc. We have also genotyped almost 74 patients from different time-points using the Illumina 850K array and CytoScan HD platform from Affymetrix. Single-gene deletion (*IKZF1*, *PAX5*, *CDKN2A/2B*, *ERG*, and *PAR1* locus) along with gene-expression profiles will add to risk stratification of ALL and also of immense prognostic relevance. One single approach to identify both copy number variation (CNVs) and gene-expression signatures is always not found suitable and requires more comprehensive approach for analysis.

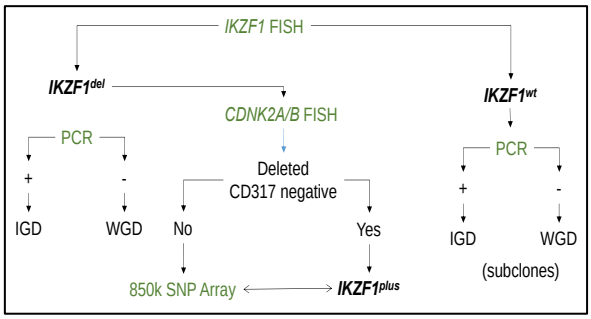

Figure 2. Schematic of Current Workflow to Identify and Characterise *IKZF1DEL* and *IKZF1*^{plus} patients

1.a. High-Risk CNV Detection

Using bulk RNA-Seq approach alone, we are not capable of identifying *IKZF1* deletions accurately as clonal frequency varies to sub-clonal range (<0.25). FISH approach works in most of the cases using

probes for *IKZF1*, *PAX5*, *CDKN2A/B* and *PAR1* deletions but not able to pick up *ERG* deletion (which is important to identify the *IKZF1*-plus) (Figure 2). As a companion diagnostic approach within a clinical setting, we tested the suitability of exploring techniques like CytoScan HD and Illumina 850K although both of these approaches are high-throughput and expensive. Alternatively, we are exploring rapid and cost-effective solutions for screening *IKZF1* plus which is associated with higher relapse rates in ALL. MLPA (multiplex ligation-dependent probe amplification) and in-house PCR assays were developed with some success but the sensitivity of both these approaches vary. Therefore, we are investigating a new digital MLPA workflow which runs on the Illumina platform and offers rapid as well as low-cost high throughput screening of CNVs for ALL which later can be developed as a common approach to serve other network hospitals included in ICiCle-2 trial.

1.b. Gene-Expression Classifier

We are also working on developing an RNA-Seq based gene-expression classifier for predicting distinct genomic subtypes for B-ALL in collaboration with St Jude Children's Research Hospital, Memphis using the platform called '**DIVIA**' (**D**iagnostic **I**nnovations using **V**alue-based implementation models to **I**ncrease **A**ccess). **DIVIA** is a secured cloud-based platform to analyse transcriptomic data generated through Illumina platforms. We are working now to conduct a feasibility study on establishing whole transcriptome sequencing as an adjunct method for the diagnosis and classification of B-ALL at Tata Medical Center (TMC) in Kolkata. A total 43 ALL retrospective samples will be sequenced in six independent Illumina sequencing runs designed to generate ≥100 million reads per sample. The objective of the study will be to test the diagnostic utility of Illumina-based transcriptomic sequencing as an additional method in parallel or in replacement to the orthogonal diagnostic workflow (flow-cytometry/ FISH/ Karyotyping) in future. The study is funded by SJCRH. The common RNA-Seq classifier if developed can be used for expanded ICiCle network.

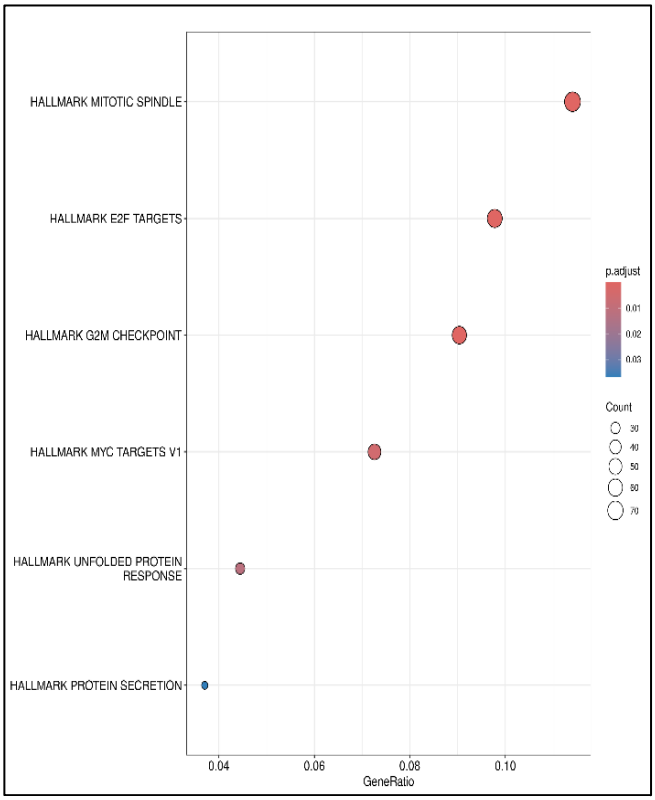

Figure 3. Pathways identified from Global Splice Variant Analysis between IKZF1-deleted vs wild-type patients

1. c. Splice Variant Detection

In order to investigate the splice variants as a surrogate molecular marker for predicting disease genetics in ALL, we were interested in looking for kinase alterations in IKZF1 deleted patients. Interestingly, the transcript variant profile of IKZF1 showed expression of full-length isoform (IKZF1-001) as well as dominant-negative (IKZF1-202) and non-protein coding isoforms (IKZF1-009) in both IKZF1 wild-type and deleted patients, leaving us with a conjecture of some regulatory events happening at the level of global transcriptome. Upon further probing, our global splice variant analysis showed about 150 genes are differentially regulated in IKZF1-deleted patients, with the top ones being TP53, BRCA2, TSC1, FANCG, ATR and PTPN. The pathways identified were suggestive of mechanisms of chemoresistance; apoptosis and DNA

damage repair genes- thereby leading to cell-survival and relapse. This data also supports the findings from our previous study on TP53. (Pritha Paul *et al.* P53 regulated metabolic adaptation and DNA damage response repair mediates drug tolerance in BCP-ALL, manuscript under preparation).

2. Nanopore Sequencing

In order to improve the accessibility and affordability of sequencing-based diagnostics, we have optimised and established the pipelines for DNA and RNA based sequencing workflows using the third generation sequencing from Oxford Nanopore Technology. As a rapid, more cost-effective alternative and flexible in terms of the number of samples required for pooling, we wish to adapt Nanopore sequencing for real-time reporting of samples in a clinical setting.

2.a. Nanopore-based Gene-Expression Classifier

As first step, we have done a pilot study with few ALL patients as a proof-of-principle experiment, which was sequenced using the Nanopore. Our collaborators, St. Jude's Children's Research Hospital and University of North Carolina, Chapel Hill have developed an AI/ML algorithm based gene-expression classifier (called **DARGAN**), which can predict lineage and subtype classification from transcriptome sequencing data based on gene expression profiling. In order to evaluate the specificity and sensitivity of the classifier (specifically rarer subtypes), we will perform Nanopore sequencing for 100 patients (75 ALL and 25 AML) and compare against the gold standard-cytogenetics. This work is being supported by an extramural funding from St. Jude Global. Furthermore, using in-house pipelines being developed, we would be calling for fusion transcripts from the transcriptome data generated from this project as a validation to the orthogonal sequencing platform (Illumina) (Figure.4).

2.b. Targeted Gene Sequencing using Adaptive Sampling Approach

In addition, we had previously optimised candidate single-gene amplicon sequencing using Nanopore for rapid mutation analysis.TP53 mutations are enriched in ALL relapses and are

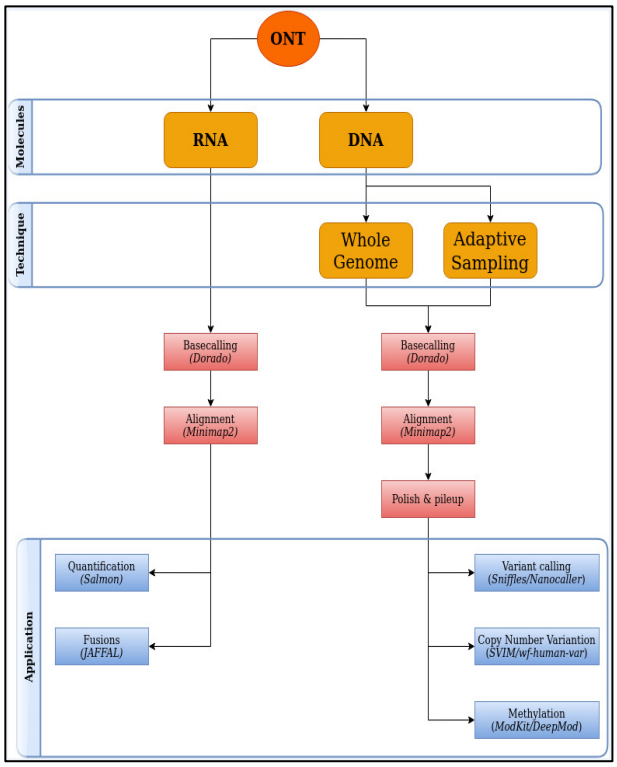

Figure 4. In-house Pipeline for ONT data

independently predictive of poor response to therapy. However, targeted gene panel amplicon sequencing comes with disadvantages of customised panel designs, PCR amplification bias, sequencing and informatic challenges. At present in our institute, we have separate workflows running for detecting copy number alterations, mutations and polymorphisms, which add up to the costs for separate library preparation and sequencing. Therefore, we thought of addressing this issue by developing a comprehensive approach, which unifies all our objectives, 'Integrative 'One for ALL' profiling using Nanopore sequencing (ION)'. Using the 'adaptive sampling' technology by Nanopore, we have developed a panel of 124 genes of interest with respect to leukemia biology and ALL

drug-response, whereby we could sequence our genes selectively, using computational method rather than any complex library preparation. This potentially save cost, time and eliminates bias associated with PCR duplicates or bisulphite conversion for detecting epigenetic changes in genome. Identification of the recurrent copy number alterations, structural variations, methylation, mutations and polymorphisms in patients with ALL from low-pass sequencing (Fig.4), would potentially add on to the existing risk-stratification for therapeutic management strategies of patients.

As a proof-of-concept, we proposed to develop a workflow for adaptive sampling, to identify high-risk subgroup of patients with IKZF1 deletions or IKZF1 plus phenotypes, who are at an early risk of relapse and would benefit from an additional year of maintenance according to DCOG-ALL studies. In our first pilot experiment, we have sequenced three ALL cell-lines; Jurkat, NALM6 and REH along with two patients of interest who are well-annotated by molecular genomic studies. We are building informatics pipelines for achieving these objectives, testing them against the data generated.

Future Directions

To support the translational research projects in TTCRC, we are also working in collaboration with other groups like SOLI3D and FORE teams. We are working on developing the pipelines for mini-bulk RNA-Seq as well as Single-cell sequencing in collaboration with Prof Anindita Roy, University of Oxford. We are also trying to optimise the workflow for ATAC-Seq to explore the state of chromatin remodelling in ALL.

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The PREDICT team, formerly known as MRD Team, is focussed on Personalised Residual Disease Tracking in Cancer.

The Team experienced a dynamic 2023 filled with significant milestones. The year started with elevated enthusiasm as two of the team members returned from a four-week training program at DKMS LSL and the University of Charité. In addition to conducting routine RQ-PCR MRD for prospective patients, the team found inspiration to integrate a novel technology—NGS MRD based on unique molecular identifiers (UMI). The excitement reached new heights with the approval of a substantial 3.4 Crore DBT grant for a three-year period.

One half of the team started working hard on prospective reporting to support the clinical decision of paediatric ALL patients. Bringing down the non-informative patients according to the EuroMRD guidelines (of <5%) had been a challenge since 2015. Finally, we had only one non-informative patient (3.4% of the cohort) this year and around 75% patients were reported with two or more than two targets (Figure 1). We also participated in EuroMRD quality assessment (QA) for maintaining EuroMRD accreditation. Despite many challenges, the team successfully qualified in both the rounds (QA42 and QA 43).

The other half of the team focussed on implementing UMI-NGS MRD, named as HemaTrack-ALL. The purpose is to check if the new technique can be a better alternative for MRD monitoring. This required strategic plans to design the study as well as hands-on training to other team members. At TTCRC, execution of the strategic plan started with sample curation, documentation, database generation and extramural grant application (submitted in 2022). Ulf-Peter Guenther from DKMS-LSL visited TTCRC in the beginning of the year (February) to initialise the implementation of the HemaTrack-ALL protocol. Pilot runs were targeted with cell lines and positive controls (combination of cell lines). It took a lot to overcome all initial challenges ranging from bead based cleaning (AMPure XP *vs* SPRI select) of the PCR products to well size of different gel apparatus playing a contributory role. Finally, the data from the pilot runs showed the desired reproducibility and sensitivity of the assay (Figure 2). We then started with a retrospective study to compare RQ-PCR MRD and HemaTrack-ALL. Initial HemaTrack-ALL data from a cohort of 29 patients and 67 follow-up time points showed a 91% of concordance and a sensitivity of 94% suggesting its comparability to RQ-PCR MRD (Figure 3). The initial study was presented at the national haematology conference (Haematocon 2023). We are further aiming to extend the assay to a bigger cohort of 70 patients having 140 follow up time points.

The team bid good bye to Atreyi (during mid 2023). She got an excellent PhD offer from University of Otago, New Zealand. We welcomed Anusree, Preyashi and Sunanda by the mid of the year. They were trained sufficiently by the end of the year to continue the prospective RQ-PCR MRD when Aishwarya and Sreyasree left to attend ESLHO 2023 followed by training at DKMS LSL and University of Charité. We said auf wiedersehen to Sayantani, whose contribution for implementing HemaTrack-ALL at TTCRC has been outstanding. She has secured a PhD position in the University of Cologne, Germany. We will miss Atreyi and Sayantaniand wish them all the very best in their adventures.

Future direction: Along with the prospective RQ-PCR MRD, validation of HemaTrack-ALL is required in a bigger cohort (around 70 patients having 140 follow up time points). We plan to move ahead with a prospective HemaTrack-ALL tracking for selected patients at Tata Medical Center, Kolkata by mid-2024. This would give us the opportunity to have a cost comparison and turnaround time (TAT) evaluation. In addition to cost and TAT, HemaTrack-ALL offers higher throughput making MRD assays available to more patients.

Year	Patient reported	1 Target (%)	2 or >2 Target (%)	Non-informative (%)
2015 - 2020	114	60 (52.6%)	34 (29.8%)	20 (17.5%)
2021	35	13 (37.1%)	19 (54.3%)	3 (8.6%)
2022	36	11 (29%)	22 (64.5%)	3 (6.45%)
2023	30	7 (23.3%)	22 (73.3%)	1 (3.3%)

Figure 1. Patient reporting (from 2015 till 2023). 29 patients are reported in 2023. As compared to previous years, two targets reporting has improved with a decrease of one target reporting and non-informative patients.

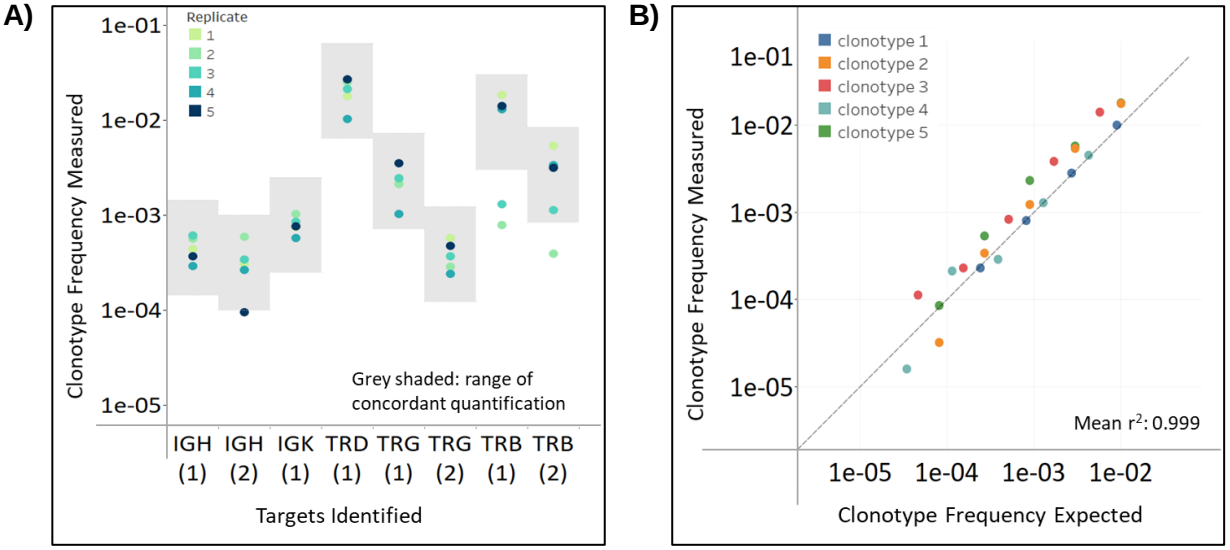

Figure 2. Pilot study of HemaTrack-ALL. (A) Positive controls showing repetitive identification and quantification of clonotypes within range of concordance. (B) Control clonotypes accurately quantified in dilution experiments.

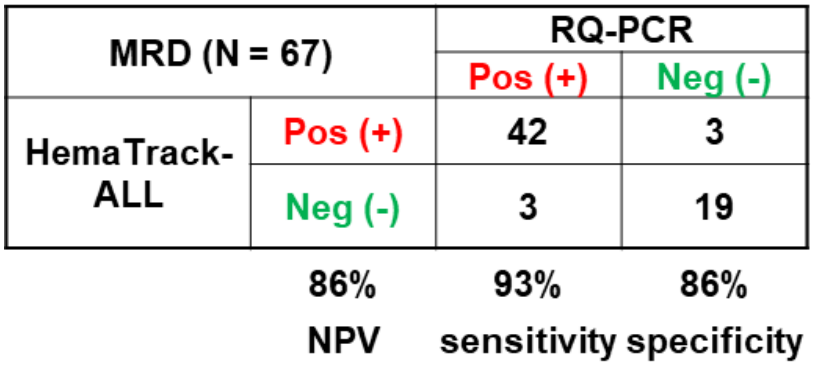

Figure 3. Comparative study of HemaTrack-ALL *vs* RQ-PCR MRD. HemaTrack-ALL was performed for 67 follow up time points. 2X2 contingency table shows HemaTrack-ALL has a specificity and sensitivity of 86% and 93% respectively with a negative predictive value (NPV) of 86% as compared to RQ-PCR MRD.

Clinical Proteomics Group

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Review Report

In the expansive realm of life sciences, the advent of Mass Spectrometry-Based Proteomics has emerged as a cornerstone, enabling scientists to decipher the intricate language encoded within the proteome. This innovative approach, characterized by its capacity to scrutinize proteins at the molecular level, has propelled research into new frontiers, offering unprecedented insights into the structure, function, and interactions of these fundamental biological entities. Currently our facility is equipped with TripleTOF 6600 mass spectrometer (AB Sciex), connected to a nano LC system (Eksigent) coupled with a nano electrospray ion source for bottom-up MS-based proteomics. We have implemented an advanced sample preparation workflow, as illustrated in Figure 1, which boasts superior high throughput and remarkable cost-effectiveness. This cutting-edge methodology, adopted from Dr. Ashok Kumar Jayavelu, Proteomics and Cancer Cell Signalling lab at DKFZ, Heidelberg, Germany, enables the processing of 384 samples in a day. Dr. Jayavelu, is currently serving as the clinical proteomics team lead at TTCRC and enabled the development and optimization of this innovative workflow.

Clincial Proteomics Unit Published Work

Determination of amyloids in plant seeds (collaboration with IIT, Kanpur)

The detection and roles of functional amyloids are well-studied in various organisms, but less in plants. Seed storage protein bodies (SSPBs) play a vital role in seed germination and seedling growth, aided by an intricate relationship of plant hormones and proteases. Mass spectrometry based distinct amyloid protein bodies identification was performed using AB Sciex TripleTOF 6600. The trypsinization and liquid chromatography with tandem mass spectrometry (LC–MS/MS) of the dissected areas and SSPBs, revealed multitude of proteins, over 300 amyloidogenic proteins using both Data Dependent Acquisition (DDA) and Data Independent Acquisition (DIA) mass spectrometry acquisition mode. The above work resulted in a publication entitled “Protein reservoirs of seeds are amyloid composites employed differentially for germination and seedling emergence” in The Plant Journal (2023) doi: 10.1111/tpj.16429.

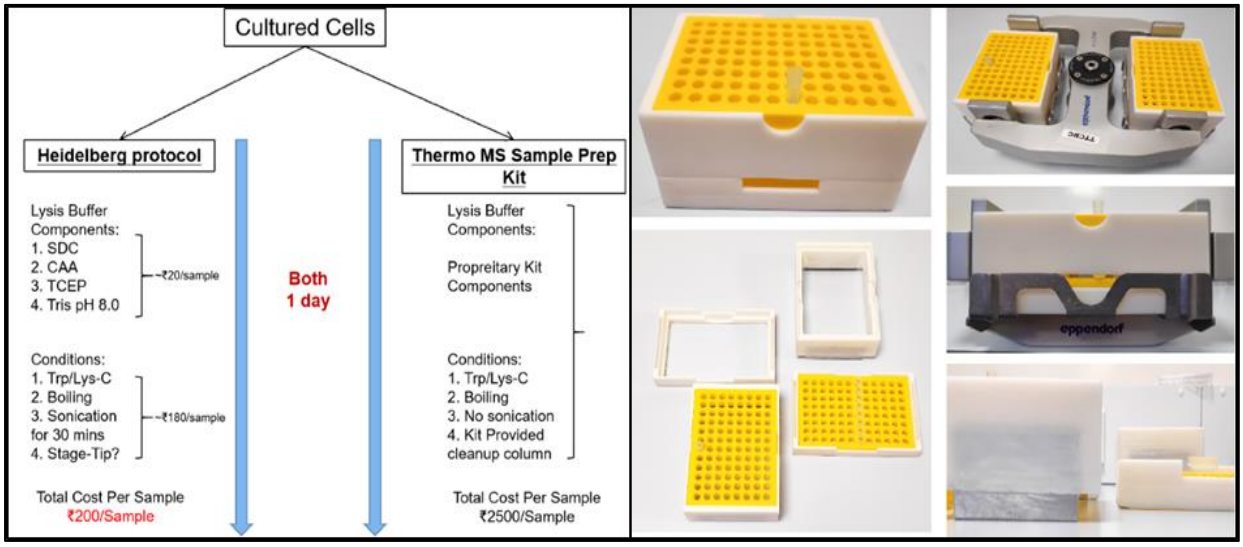

Figure 1. HighThroughput Sample preparation workflow

Ongoing Projects

Mechanism of chemo therapeutic drug Selinexor in ALL cell lines

Over 80% of children with Acute Lymphoblastic Leukaemia (ALL) treated successfully with an intensive combination therapy. However, 20% of patient disease recurrence emerges due to generation of drug tolerant clones. Selinexor is a small molecule, first-in-class, selective inhibitor of nuclear export (SINE) which acts through blockade of exportin-1 (XPO1) which is widely used in the treatment of Multiple Myeloma (MM) and other cancer entities. To understand the synergy between Selinexor and treatment of ALL, we conducted a comprehensive analysis of the time-dependent proteome profiles in Selinexor-sensitive and resistant patient-derived xenografts (PDX) as well as cell lines subjected to the drug over various time intervals. The samples processed as per our HT protocol, subjected to both DDA and DIA acquisition, raw data processed through advanced tools such as MaxQuant & DIANN and data visualized using Perseus & R (Figure-2). In total we identified 4500 proteins (TripleTOF 6600, TTCRC) and the analysis is continuing.

Protein expression analysis in Gall Bladder tissue and organoids from different pathologies

The Gall Bladder Cancer Research team at TTCRC has pioneered the development of advanced 3D culture models, specifically organoids, designed to replicate the intricate extracellular matrix microenvironment of gallbladders. These models cater to various pathologies, offering a sophisticated platform for research and clinical applications. Integral to the validation of these models' clinical relevance and the comprehensive comprehension of disease biology is the utilization of proteomics analysis. This technique plays a pivotal role in not only confirming the models' fidelity but also in uncovering novel targetable pathways critical for gallbladder cancer research.

In the initial phase, an analysis employing a modest sample set revealed the presence of over 5000 proteins, meticulously extracted from both the native tissues and the organoids. This analysis was conducted using the Data-Independent Acquisition (DIA) mode, and the data were processed through tools such as DIANN, Skyline, and Perseus. As the research progresses, the team aims to elevate the scale of their study by working with a more extensive sample set.

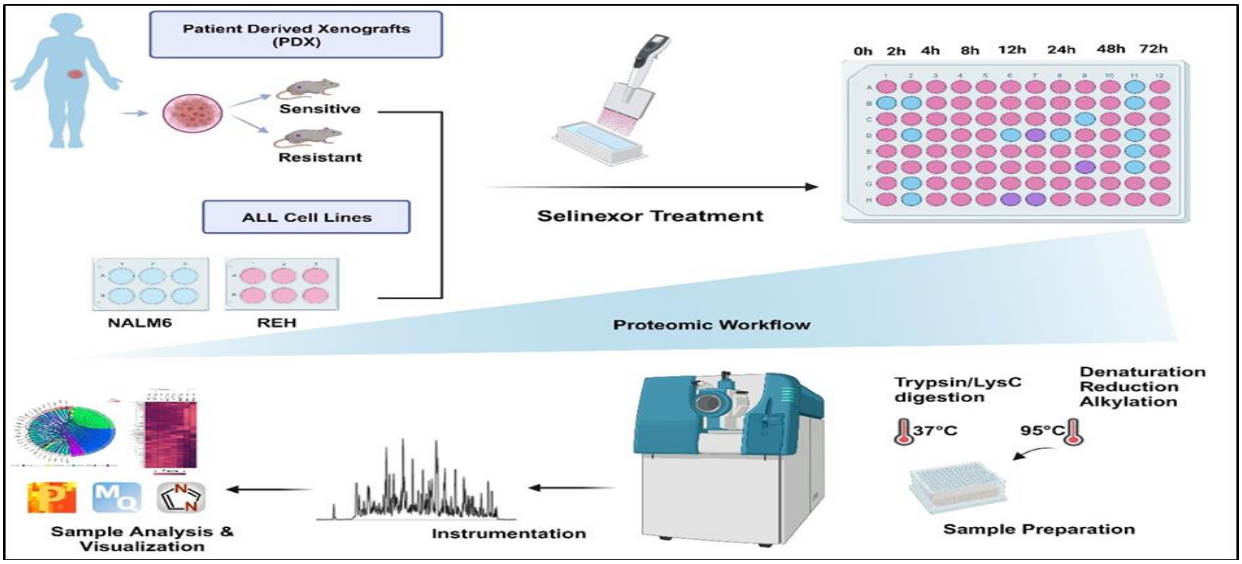

Figure 2. Data Independent Acquisition (DIA) of Selinexor sensitive and resistant cell lines treated with the drug at different time points

Plasma biomarker discovery in ALL

A biomarker can be defined as a biological characteristic reflecting an individual's normal or altered state of biological processes. At the TTCRC, we are actively developing a cost-effective plasma depletion strategy to improve the detection limit of these low-level biomarkers. Our modified method addresses both the depletion of Human Serum Albumin (HSA) and the most abundant immunoglobulins (see Figure 3). To validate this approach, banked plasma samples were obtained from both Pediatric BCP-ALL patients at various time points before and after chemotherapeutic treatments, as well as Normal Healthy Plasma (NHP). Through the utilization of DDA (Data-Dependent Acquisition) and DIA (Data-Independent Acquisition) methods, we successfully detected over 200 proteins. Analysis was performed using advanced tools such as ProteinPilot and DIANN, contributing to a more comprehensive understanding of the biomolecular landscape in our study cohorts. This concerted effort underscores our commitment to advancing biomarker discovery and refining diagnostic methodologies for improved patient care. Our current progress and plasma proteome depth is largely limited due to lack of robustness, reproducibility and sensitivity of TripleTOF 6600.

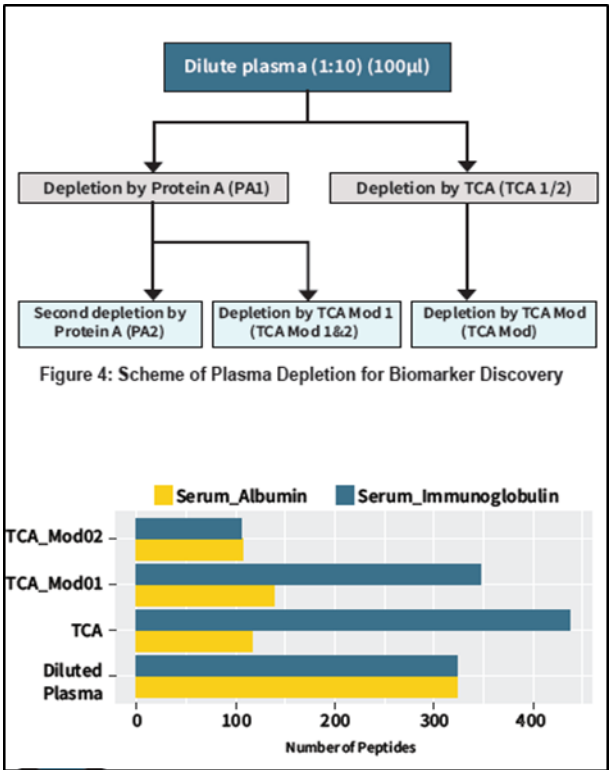

Figure 3. Depletion of human plasma. The plasma depletion workflow and the total number of proteins detected before and after plasma depletion.

Future Outlook

Development of a cost-effective plasma enrichment strategy using spions

In our pursuit to advance the development of highly reproducible, cost-effective, and high-throughput methodologies for capturing the dynamic plasma proteome in patient samples—a critical stride toward its imminent clinical application for diagnostic purposes—we are poised to employ an innovative nanoparticle-based enrichment strategy targeting low-abundant proteins.
Our chosen approach hinges on the utilization of Spions (super-paramagnetic iron oxide nanoparticles), which have recently demonstrated efficacy in enriching plasma proteins. By harnessing the unique properties of Spions, we aim to not only enhance the reproducibility and throughput of our methodology but also to ensure the capture of the intricate dynamics within the plasma proteome. This cutting-edge approach holds immense promise for its potential application in clinical settings, paving the way for a more robust, highly cost-effective and efficient diagnostic platform that can revolutionize patient care. (Figure 4).

Characterization of Phyllodes Tumours by MS based spatial proteomics

Phyllodes tumors (PTs) represent an exceptionally rare category of fibroepithelial lesions within the breast, constituting only 0.3-1% of all primary breast tumors. This distinctive pathology manifests through the concurrent proliferation of stromal and epithelial cells. Typically observed in individuals around the median age of 40-43 years, PTs are

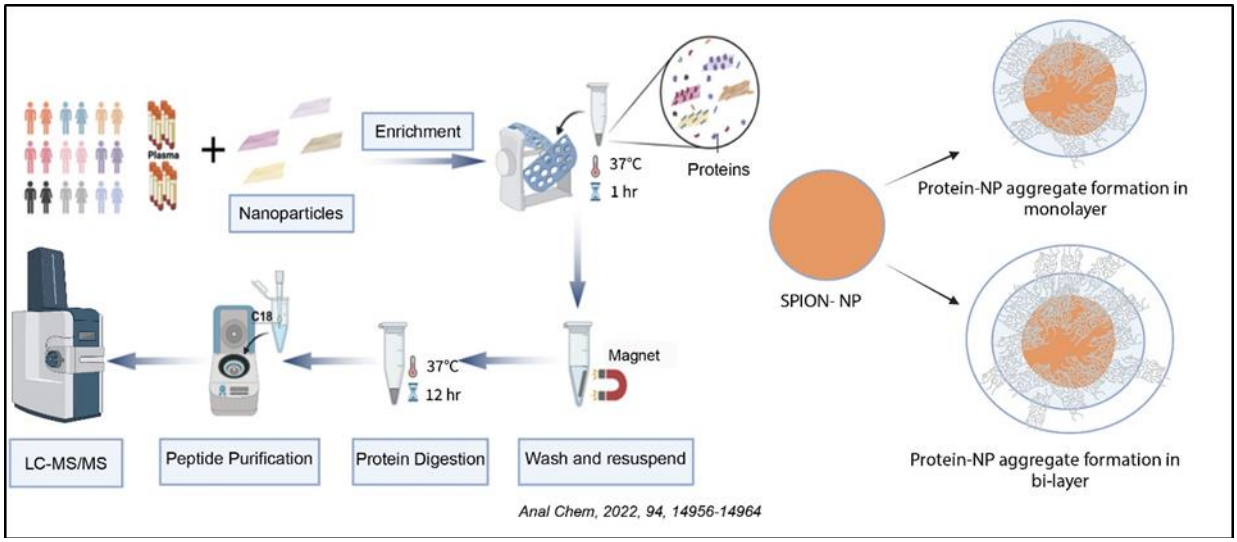

Figure 4. Plasma protein enrichment workflow using SPIONs

currently classified into benign, borderline, and malignant subtypes by the World Health Organization (WHO). This categorization hinges on crucial histological features, including tumor borders, stromal cellularity, stromal atypia, mitotic activity, stromal overgrowth, and the presence of malignant heterologous elements. The diverse grades of PTs exhibit varying risks of local recurrence and metastasis, with malignant PTs standing out as the most aggressive, showcasing a substantial 25% rate of distant metastasis. The optimal approach to managing malignant Phyllodes, particularly concerning adjuvant radiotherapy and chemotherapy, remains elusive to date. Notably, standard chemotherapy often proves ineffective for most patients with metastasis, leading to a stark reality where many succumb within three years of their initial treatment. The primary objective of our research endeavours is to identify distinctive protein biomarkers exclusive to malignant Phyllodes, unravelling the intricate proteome of other subtypes (benign and borderline). This comprehensive exploration aims to deepen our understanding of Phyllodes tumors, paving the way for enhanced diagnostic precision and targeted therapeutic interventions.

The Clinical Proteomics Unit at TTCRC works as a core support group aiding in the research of other groups functioning within and outside TTCRC.

- The main objective is to provide a cost-effective diagnostic solution to the diseased. Establishment of high-throughput protocol which that is capable of determining protein targets from a single cell is our long-term goal.
- We envision the expansion of our facility with the procurement of a highly sensitive Bruker timsTOF HT Mass Spectrometer due to its unmatched performance for conducting large scale proteome study and diagnostic purposes.
- Implementing new analysis packages, automated pipelines, AI/ML for large datasets is currently our focus as well.
- We also plan to hold a national level MS training workshop in the next financial year.
- Recently we have received approval from IRB to conduct our study with the Phyllodes Tumours. As a next preparation phase, we aim to acquire a grant from ICMR for this study.

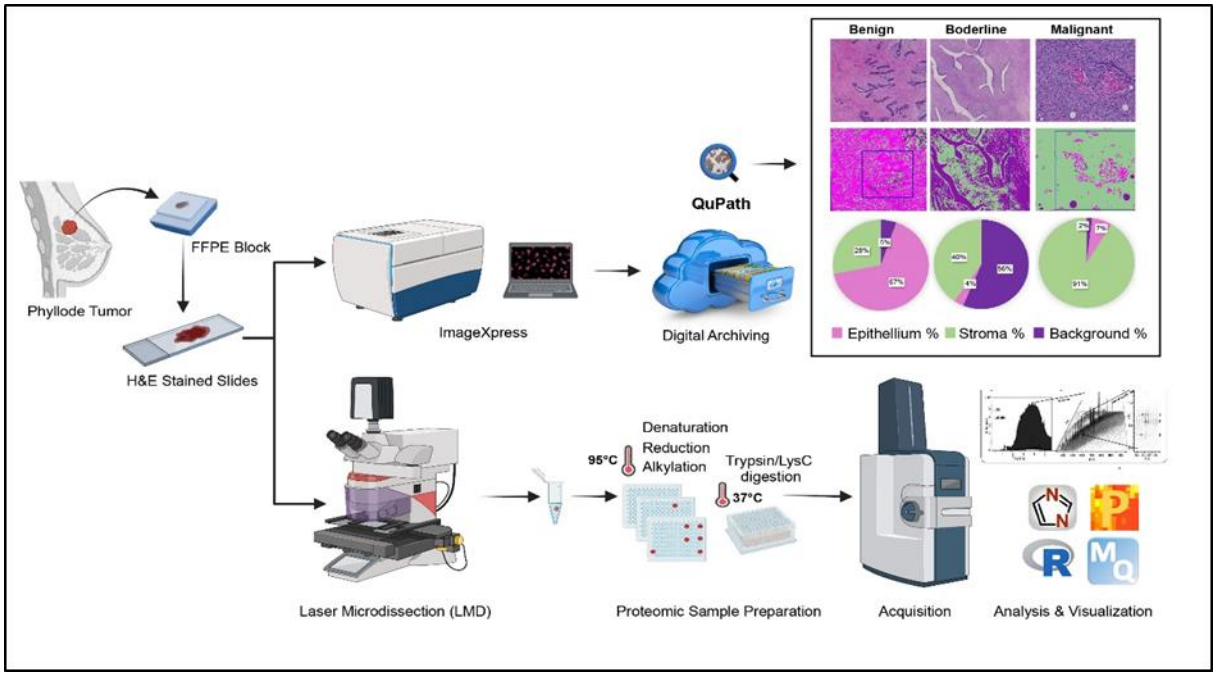

Figure 5. AI-integrated mass spectrometry-based proteome workflow for Phyllodes Tumours

Planned collaborations outside TTCRC

I. Study of amyloid protein detection from human cataract eye lens to elucidate the target proteins responsible for the disease. Further work on amyloid protein detection from chick-pea seeds. This will be a collaborative work between Dr. Ashwani Kumar Thakur and his group, Biological Sciences and Bio-Engineering, IIT Kanpur and TTCRC.

II. Study of p53 amyloid pathology in collaboration with Dr. Shinjinee Dasgupta and her team, at Amity Institute of Molecular Medicine and Stem Cell Research, Amity University, Noida.

.

TCS @ TTCRC

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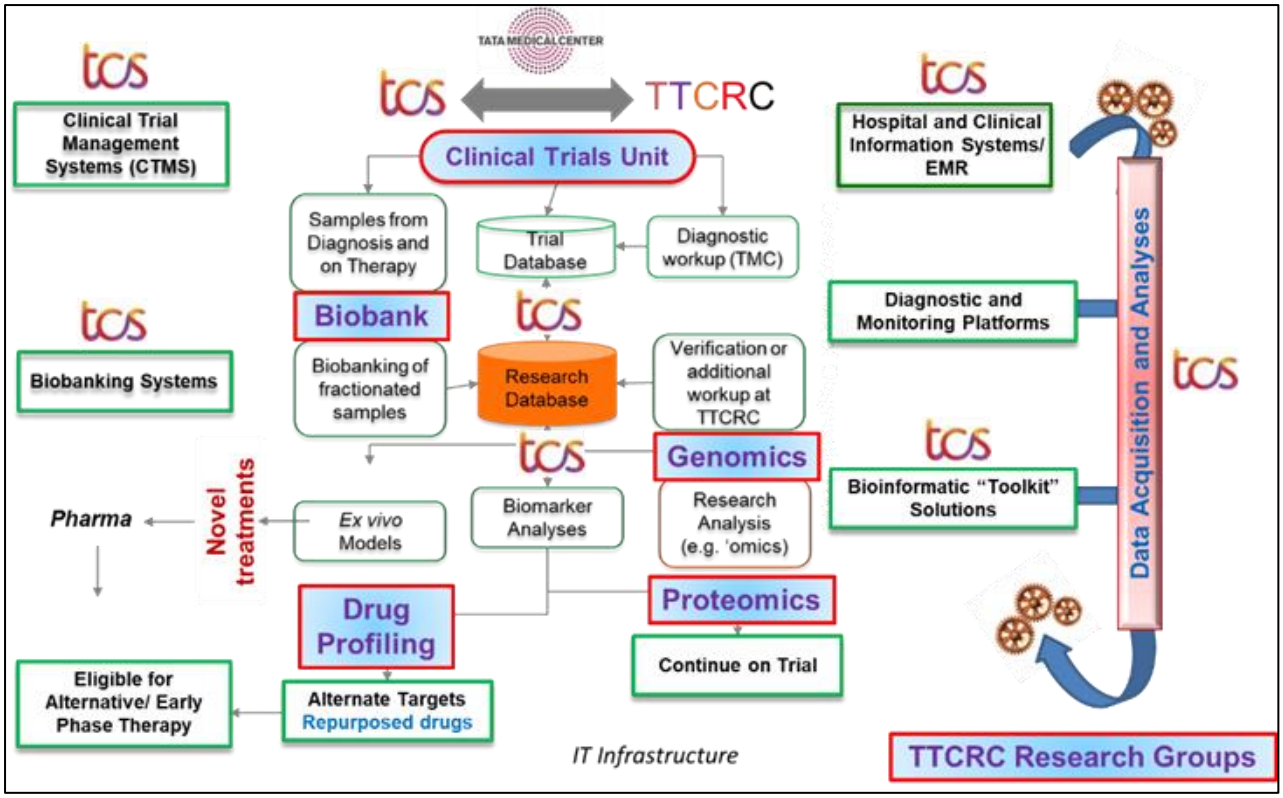


TCS collaboration with TTCRC continues to yield transformative benefits. Focus has been around accelerating data analysis, thereby expediting the identification of potential therapies, and enhancing precision medicine, development of Advanced algorithms facilitating personalized therapy, automation to optimize operational efficiency and real time collaboration solutions to enable seamless information exchange with global researchers. Additionally, we bring in technologies like advanced data analytics and AI/ML models to several use cases in translational research. Overall, we believe our synergy amplifies the pace of discovery to advance the fight against cancer and improve patient outcomes.

Enablement of clinical studies

TCS ADD® IDM : Clinical Trial Management Solution

TCS ADD® Integrated Data Management is a one-stop solution that caters to all needs of clinical trials including core electronic data capture (EDC) solution, terminology management, medical coding, non- case report form (non-CRF) data management and automated digital document generation. It offers an integrated, cloud deployed, self-service solution that is modular, intuitive, and ready-to-onboard with minimal training.

The Icicle trial, a prospective, risk stratified, randomized, multicenter, open label, controlled therapeutic trial, runs on this platform. The entire study has been running on our platform for the past 5 years. Recently all legacy data has been migrated to the latest version of the platform which is a cloud-based solution with premium analytical features and optimal architecture.

The Relapsed ALL clinical study also continues to be implemented on the platform.

TCS teams also provide the associated services to develop the clinical trial database, including conducting testing, maintaining a record of database features and functionality, and developing an SOP for database-related queries. Our data analysts have been developing and

generating reports to extract insights for research and regulatory purposes.

Personalized Diagnostics

TCS brings in its computational biology research capabilities to enhance and augment biomarker discovery programs in TTCRC. These discoveries were developed with and alongside TTCRC clinical research team and the analyses were performed on data from TTCRC studies. We have used data from public domain to augment and validate the analysis pipelines and models.

ALL – IKZF1 deleted subgroup characterization.

Deletions in IKZF1 have been associated with poor prognosis in BCP-ALL, and patients with additional deletions in genes such as CDKN2A, CDKN2B or PAX5 in the absence of ERG deletions (IKZF1 plus) have been shown to have an even more severe prognosis. Both intra gene and whole gene deletions are observed in the patients. The primary objective of this study was to explore if mRNAseq data could a) Detect the deletions reliably and b) Characterize the differences between the 3 subgroups viz., IKZF1 intragene deleted, IKZF1 whole gene deleted and IKZF1 WT (wild type).

We developed multiple approaches to analyse the data from the cohort with the 3 phenotypes. Fusions in genes of interest and of high confidence were identified in the IKZF1 deleted group and selected for PCR validation. The transcript abundance of IKZF1 was characterized for these samples, specifically comparing the relative abundance of these transcripts between the 3 groups. Identification of CNVs were done with multiple tools and sensitivity and specificity evaluated. In addition, global splice variant analysis was also performed to compare the splice differences between the groups.

Our findings identified pathways that showed significant variations between the groups and could provide mechanistic understanding of the prognosis difference.

RNAseq as a single assay for diagnosis

The variance in gene expression between different phenotypic groups being compared could be driven by different factors. Bulk RNAseq data generated for such studies is being explored as a single assay for better mechanistic understanding of these patterns observed. In addition to transcript isoform abundance variation, methods to identify CNVs, variants, fusions in the RNAseq data are being evaluated for sensitivity and specificity. Best practises and constraints for optimal outputs are being evaluated with the ALL cohort as well as published data. These have potential for diagnostics for better risk stratification and clinical decision making.

Personalized Therapies & Clinical decisions

Differential gene expression analysis of TP53 knockout in ALL

TP53 gene is one of the master regulators of cellular stress adaptation and has been reported to be associated with relapse in several cancer types. The objective of the study was to understand the effect of knocking out TP53 in Acute Lymphoblastic Leukaemia (ALL) cell lines (NALM6 and RS411) and to analyse the differential response of TP53 knocked out cells as compared to wild type cells under stress conditions that provide them survival advantage.

High throughput RNA sequencing data was obtained for the following conditions – 1) Wild type ALL cells, 2) TP53 knocked out ALL cells, 3) Wild type ALL cells treated with Mitoxantrone, 4) TP53 knocked out ALL cells treated with Mitoxantrone, 5) Wild type ALL cells treated with Nutlin3a and, 4) TP53 knocked out ALL cells treated with Nutlin3a. The RNAseq analysis for all the conditions were performed on two ALL cell lines, NALM6 and RS411. The knockout and wild type samples formed distinct clusters for the protein coding genes, indicating significant differential expression for the two groups. The gene set

enrichment analysis identified the significantly upregulated pathways in knocked out samples such as Oxidative phosphorylation, DNA repair and G2M checkpoint, and the significantly downregulated pathways such as P53 pathway and apoptosis. Gene ontology (GO) enrichment analysis was carried out to identify the biological processes that were significantly enriched by the upregulated and downregulated genes. The analysis pipeline was followed for the differential gene expression analysis of stress induced knockout and wild type samples. The analysis helped in identifying the genes and interconnected pathways that provide survival advantage to the TP53 knockout cells under the stress condition.
RNAseq analysis was also performed on two patient cohorts from TMC Kolkata and Berlin with 3 and 25 patients respectively. For each patient, samples were collected during initial diagnosis and relapse. Differential gene expression analysis was performed for relapse verses initial diagnosis and compared with the cell line analysis. We found concordance of the patient data analysis with cell line analysis which provides validation to the cell line analysis.

Gall Bladder Cancer Organoids

Organoids are one of the best 3D representations of tissues in vitro, and our objective was to use transcriptomics to study how well the organoids established from gallbladder tissues from various pathologies could represent the source patient tissue.
We interrogated the data in several ways. Clustering techniques were used to analyse the tissue and the organoid data. Copy number variation segments were also compared between the source and derived organoids in addition to SNVs and small Indels. Interesting fusions found in the malignant tissue and the corresponding organoids were also characterized. Gene expression patterns between the parent tissue and the derived organoids were also compared and significantly enriched pathways based on the pathologies examined.
Our studies concluded that the derived organoids replicated the pathology and other source tissue characteristics.

Predictive models based on Histopathology and clinical data for aiding prognostication and interventions in Oral Cancer

We collaborate with the Department of Histopathology ((PI: Dr. Geetashree) to develop AI/ML models that can be used to classify, estimate and predict disease progression and survival based on images of histopathology slides stained with hematoxylin and eosin (H&E) and clinical data from patients with OSCC. A retrospective study is being planned which includes tongue and gingivobuccal patients. Currently, the team works on public data repositories and has participated in several prestigious challenges such as MIDOG 2022, Kaggle, etc.

Translational Research Solutions

TCS brings its expertise in life sciences and healthcare focused solution building and advanced data analytics towards translational research and clinical decision support areas.

Adaptive Maintenance Therapy

ALL treatment requires dose regulation during the entire course (approx. 2 years) of Maintenance therapy (MT). Delivering optimal therapy by monitoring blood counts and prescribing the maximum tolerated dose of 6-MP and MTX are the mainstay of MT.

ADAM Solution

ADAM is a clinical decision support solution to aid physicians in determining dose titrations for children undergoing maintenance treatment in Acute Lymphoblastic Leukemia. The solution predicts the dosage values of two medicines namely Mercaptopurine(6MP)

and Methotrexate (MTX) based on individual response to drugs, patient history and dosage rules & algorithms. The solution aids doctors/clinicians save effort and time, standardizes treatment among clinicians of various experience levels, reduces medication errors, visualize patient's past chemotherapy schedule and dose distribution for the predicted drug values.

The solution is deployed in a clinical setting with promising outcomes. The system is able to maintain an average agreement rate of over 75%. Disagreements and the reasons thereof are being captured and will provide valuable insight and data store for future AI/ML models. The system is also being enhanced for cloud enablement and multi center implementation.

Solution Features & Enhancements:

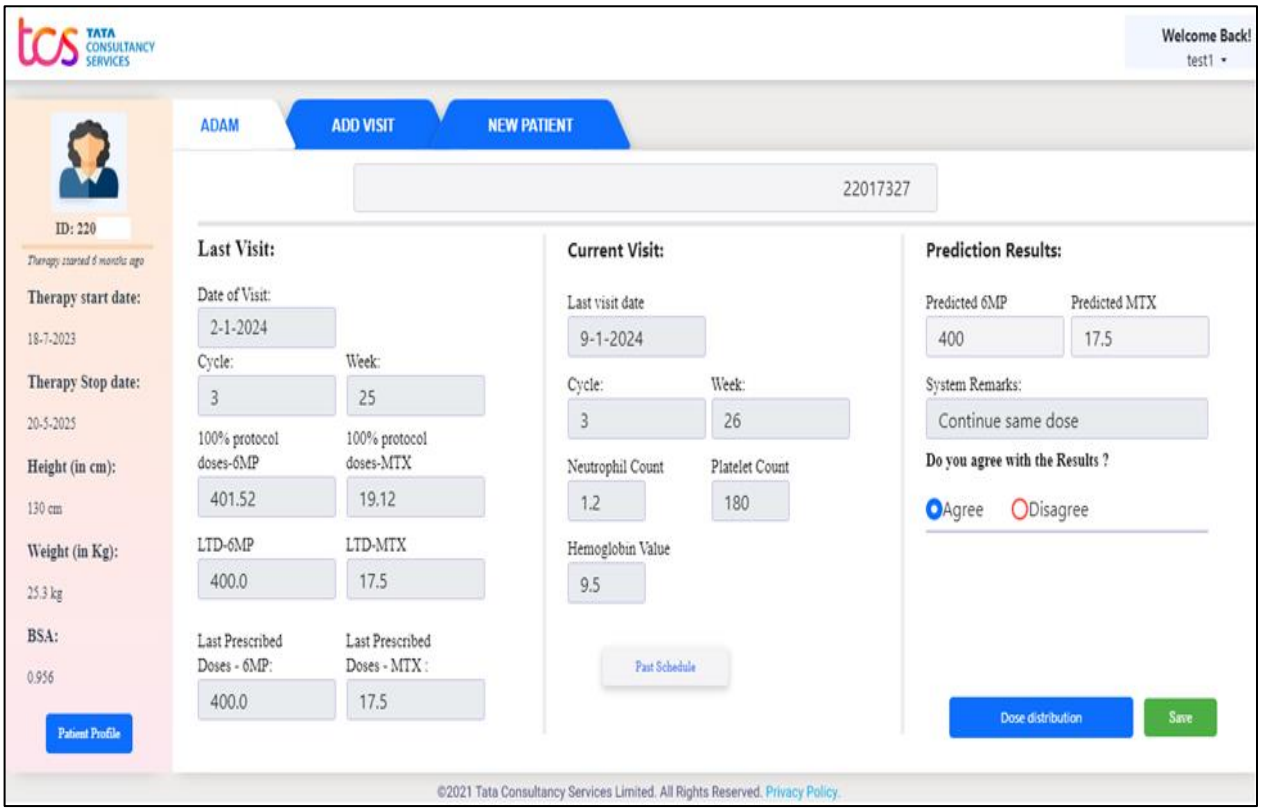
The solution gives the facility to predict the next doses using the prediction button. Prediction is based on the set of well-defined rules in the back. The leftmost column in the app projects the basic information about the patient.

Patient Profile:

The patient profile button on the leftmost column renders the modal which shows the patient's previous records for each visit. **For better user experience the highlighting of the escalated dose and stop dose has been added.** The feature to download the patient profile is also being provided if in case clinician requires the detailed information.

Interactive Patient Plot:

To visualize the past chemotherapy schedule in an interactive manner, a patient plot button is provided.

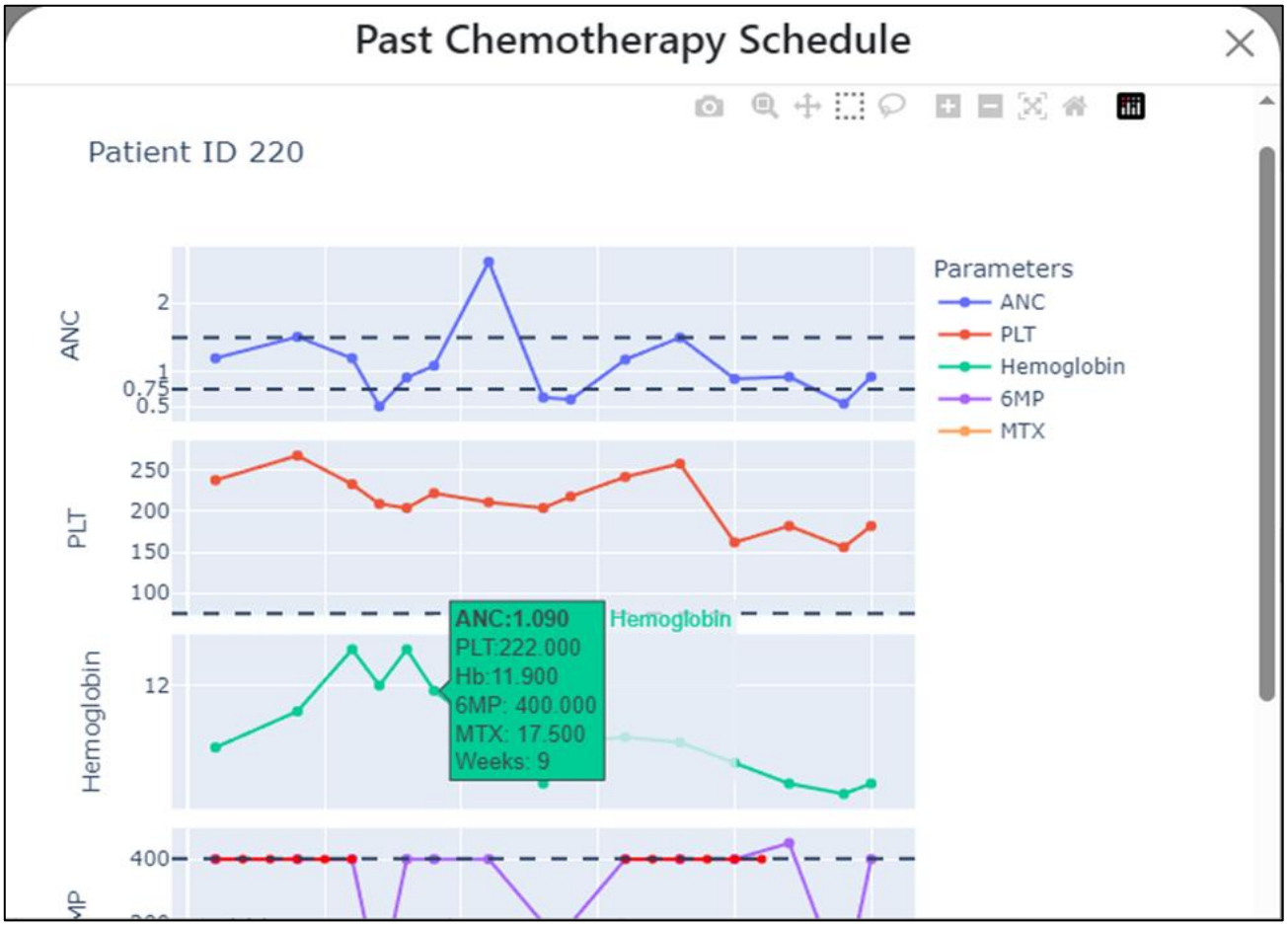


Patient Information for Patient ID: 220

Date of visit	Body surface area(BSA)	Cycle	Week	ANC(10^9/L)	PLT(10^9/L)	HB(g/dl)	Prescribed 6MP	% Prescribed 6MP dose (current dose/starting dose *100)	Prescribed MTX	% Prescribed MTX dose (current dose/starting dose *100)	System remarks	Physician Remarks	Physician Comments
Jan. 2, 2024	0.956	3	25	0.93	182	10.1	400.0	99.62	17.5	91.53	Resume last tolerated dose	Agreed with predicted remarks	
Dec. 26, 2023	0.956	2	24	0.54	156	9.9	0.0	0.0	0.0	0.0	Stop dose	Stop dose	Borderline counts
Dec. 12, 2023	0.956	2	22	0.93	182	10.1	450.0	124.53	17.5	91.53	6MP dose escalation	Agreed with predicted remarks	
Nov. 28, 2023	0.956	2	20	0.9	162	10.5	400	99.62	17.5	91.53	Continue same dose	NA	Automated
Nov. 14, 2023	0.956	2	18	1.5	258	10.9	400	99.62	17.5	91.53	Continue same dose	NA	Automated
Oct. 31, 2023	0.956	2	16	1.18	242	11.0	400	99.62	17.5	91.53	Resume last tolerated dose	NA	Automated
Oct. 17, 2023	0.956	2	14	0.6	218	10.9	200	49.81	10.0	52.3	Reduce dose	NA	Automated
Oct. 10, 2023	0.956	2	13	0.63	204	10.1	200	49.81	10.0	52.3	Reduce dose	NA	Automated
Sept. 26, 2023	0.956	1	11	2.6	211	11.1	400	99.62	17.5	91.53	Continue same dose	NA	Automated
Sept. 12, 2023	0.956	1	9	1.09	222	11.9	400	99.62	17.5	91.53	Continue same dose	NA	Automated
Sept. 5, 2023	0.956	1	8	0.92	204	12.7	400	99.62	17.5	91.53	Resume last tolerated dose	NA	Automated
Aug. 29, 2023	0.956	1	7	0.5	209	12.0	0	0.0	0.0	0.0	Stop dose	NA	Automated

Download Table

Close



Add Visit:

This section of the app provides the facility to add new record for the exiting patient, Update Patient Details feature has been provided to correct any record before dose prediction and Delete Last Record feature has also been provided to rectify the data post dose prediction.

Upload File:

Users can upload a new patient's complete maintenance therapy data at one time using this application. User can also delete the uploaded data if incorrect data is uploaded.

Translational Research Platform

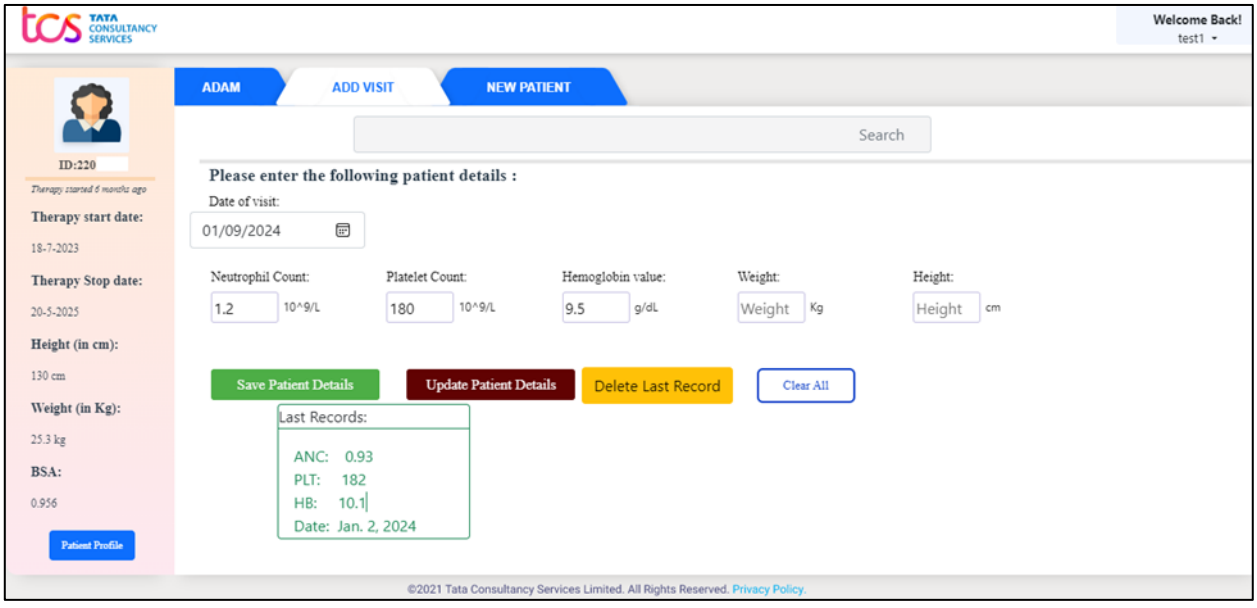
One of the key benefits of platform/solutions to manage and analyze real-time translational research data includes improved patient outcomes due to timely and informed clinical decision support. This is facilitated by improved access, collaboration, and communication among different stakeholders in ALL cancer treatment by integrating and analyzing longitudinal patient data from various sources like biobanks, imaging platforms, MRD etc.

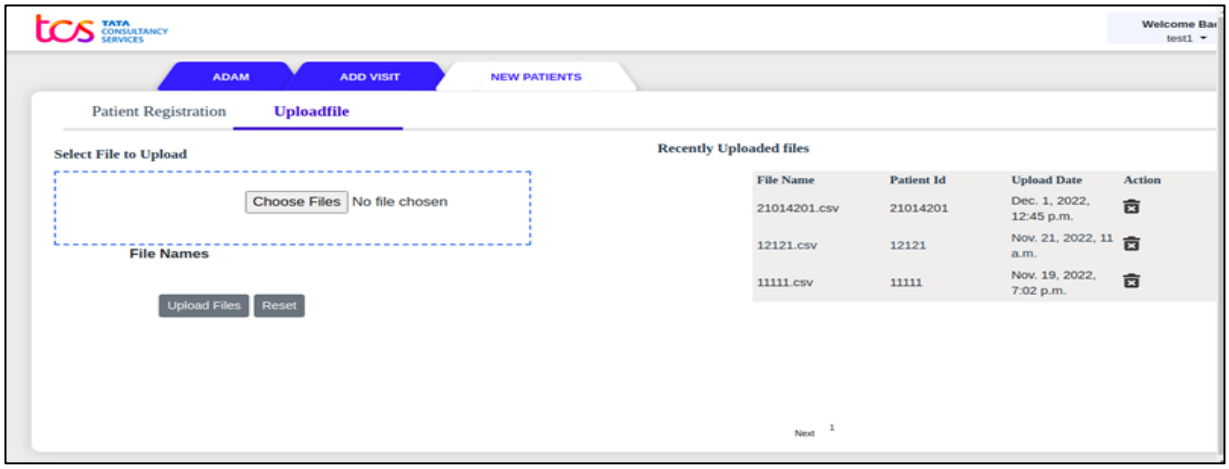
Such platforms and ecosystems also help clinicians and researchers manage and explore real world data to support decision making, demonstrate value and improve outcomes for the patients.

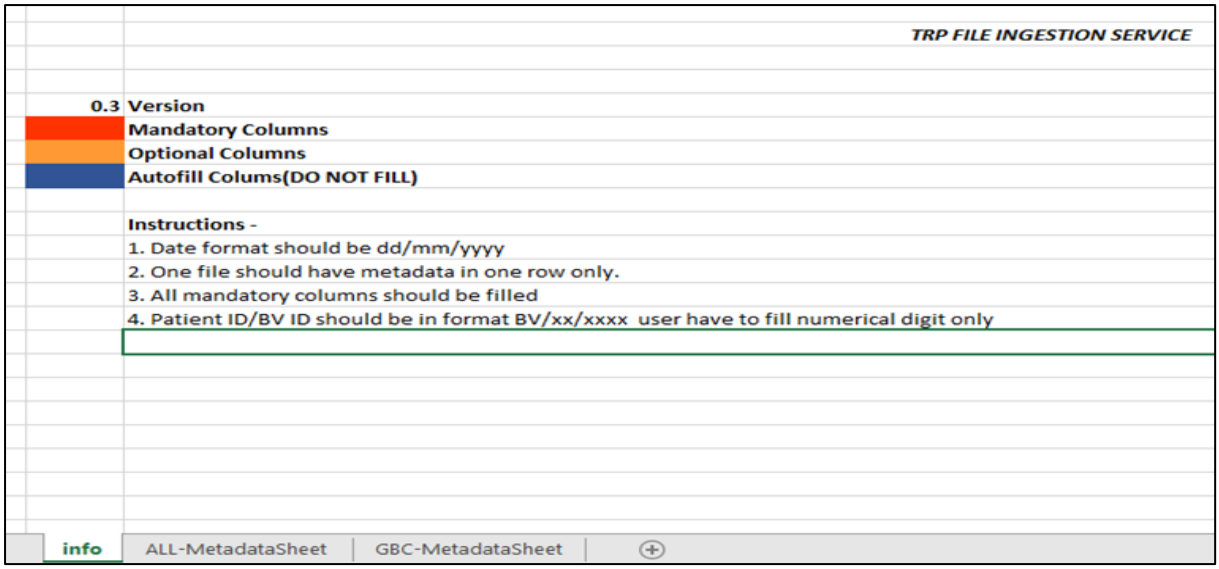
Moreover, advanced analytics using AI/ML and deep learning techniques, done on data acquired via such platforms have many other potential applications like helping identify new sub-groups, optimizing personalized treatment regimens, monitoring the quality of chemotherapeutic drugs and personalizing therapies based on patient characteristics and behaviors.

Scientific Data Ingestion and Management

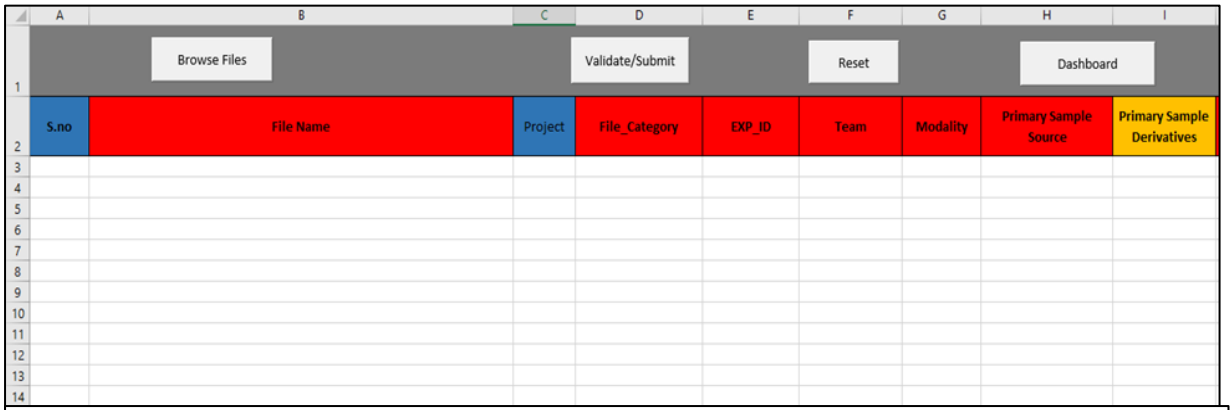
Research datafile ingestion is accomplished from TTCRC shared storage server to TRP Data repository without any manual intervention. The configurable, auto suggested & semi-auto populated GUI metadata template for ALL & GBC projects has been provided to users. This aids in metadata Extraction, Validation & tagging, as well as track file ingestion status and update latest versions.



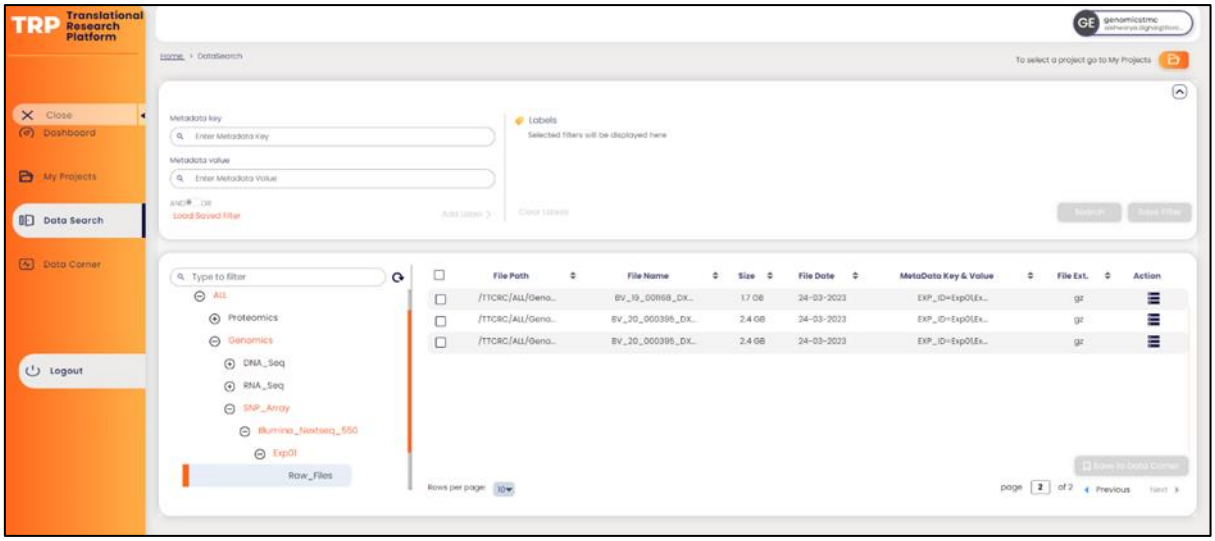




Input Metadata GUI Template:



Serial No.	Log Time	User ID	File Upload Status	File hierarchy	File Name	Project	File_Ca
1	2023-05-05 12:32:55	1950883	file with "test789.txt" filename already exist on TRP. Please try uploading with unique file name	GBC/CellBiology/RNA_Seq/Illumina_Nextseq_550/Exp01/Raw_Files	test789.txt	GBC	Raw_
2	2023-05-10 12:12:08	2934533	Upload successful	GBC/CellBiology/WES/Illumina_Nextseq_550/Exp01/Raw_File	flow_master.drawio	GBC	Raw_
3	2023-05-17 12:08:19	1950884	Failed! Connect with TCS-TRP support team	GBC/Cell_Biology/WES/Illumina_Nextseq_550/Exp01/Raw_File	flow_master.drawio	GBC	Raw_
4	2023-05-17 12:17:25	1950883	Failed! Connect with TCS-TRP support team	GBC/Cell_Biology/RNA_Seq/Illumina_Nextseq_550/Exp01/Raw_File	flow_master.drawio	GBC	Raw_
5	2023-05-17 12:20:27	2340883	Upload successful	GBC/CellBiology/RNA_Seq/Illumina_Nextseq_550/Exp01/Raw_File	flow_master.drawio	GBC	Raw_
6	2023-05-23 14:31:04	1950883	Upload successful	GBC/CellBiology/RNA_Seq/Illumina_Nextseq_550/Exp01/Raw_File	asd.txt	GBC	Raw_
7	2023-05-24 12:12:06	1950883	me already exist on TRP. Please try uploadir	GBC/CellBiology/RNA_Seq/Illumina_Nextseq_550/Exp01/Raw_Files	asd.txt	GBC	Raw_
8	2023-05-24 12:29:46	1950883	me already exist on TRP. Please try uploadir	GBC/CellBiology/RNA_Seq/Illumina_Nextseq_550/Exp01/Raw_Files	asd.txt	GBC	Raw_



Files Ingested to TRP

Users will be able search these files using metadata-based search. Using data corner, users will be able to upload and download data files as required.

Data Extractors:

MRD

This utility automates the extraction of required patient timepoints data with lower PCR-MRD levels from MRD pdf reports for all four phases of treatment and provides desired master patient tracker. Users will be able to upload multiple patient reports (MRD) in one go from TTCRC common storage server and will be able to view master patient tracker inclusive of CRU + Biobank required data (in excel format). The processed reports will be archived. This will serve the analysis purpose of prospective & retrospective patient timepoints in consolidated manner for researchers.

Date_of_Extraction	Date_of_Reporting	PCR-MRD status	PCR-MRD levels	Target	SR	QR	Lateral Side	Abundant Marker	Remarks
17/06/2023	09/09/2023	Positive	1.06E-02	Vd2Dd3, Vd2Ja29	5*10^5	5*10-4	n/a	Vd2Dd3	2 Targets
30/05/2023	09/09/2023	P.B.N.Q	1.13E-04	VH1_7JH4, Vd2Dd3	10^5	5*10-4	n/a	VH1_7JH4	2 Targets
21/07/2023	09/09/2023	Positive	4.42E-04	VH1_7JH4, Vd2Dd3	10^5	5*10-4	n/a	VH1_7JH4	2 Targets
15/05/2023	08/09/2023	Positive	2.08E-03	VH3JH5	5*10-5	5*10-4	n/a	VH3JH5	1 Targets
04/08/2023	08/09/2023	Negative	0.0	VH3JH5	5*10-5	5*10-4	n/a	VH3JH5	1 Targets
13/06/2023	04/09/2023	Positive	1.16E-02	DH3JH6, Vg11JP1/2	5*10-5	10-4	n/a	DH3JH6	2 Targets
18/07/2023	04/09/2023	Positive	1.22E-02	DH3JH6, Vg11JP1/2	5*10-5	10-4	n/a	DH3JH6	2 Targets
26/07/2023	01/08/2023	Negative	0.0	VK2Kde, Vg1-8Jg1/2	5*10-5	5*10-4	n/a	VK2Kde	2 Targets
19/07/2023	25/07/2023	Negative	0.0	VH3JH4	5*10-5	5*10-4	n/a	VH3JH4	1 Targets
19/07/2023	25/07/2023	Negative	0.0	VH3JH5, Vg1-8Jg1/2	10-5	5*10-5	n/a	VH3JH5	3 Targets
09/06/2023	05/07/2023	Positive	7.88E-04	VH1-7 JH4, Vg1-8 Jp1-2, Vk2 Kde	5*10-5	10-4	n/a	VH1-7 JH4	3 Targets
23/06/2023	28/06/2023	Negative	0.0	Vd2Dd3, Vg9-Jg1_2	5*10-5	10-3	n/a	Vd2Dd3	2 Targets

MRD Tracker

Asparaginase

This utility also automates the extraction of ALL patient reports (word doc format) and maintains the required master patient tracker in excel. Users will be able to upload multiple Asparaginase patient reports from TTCRC common storage server and will be able to view updated master patient tracker (in excel format) where in few columns have been populated based on some calculations of the existing columns. The processed reports will be archived and backed up. This utility enables prospective & retrospective study of the Asparaginase drug activity in various patients in a consolidated manner.

	Diagnosis	Risk_Group_Induction	Risk_Group_Final	Phase_of_Treatment	Dose_number	Administration_date	Collection_date	Sample_Type	Activity_Data_(IU/L)	Day_after_dose
53	Relapsed BCP-ALL	BHR	BHR	R1 Induction	1	31-March-23	07-April-23	Peak	1167	7
53	Relapsed BCP-ALL	BHR	BHR	R1 Induction	1	31-March-23	14-April-23	Trough	668	14
53	Relapsed BCP-ALL	BHR	BHR	R1 Induction	2	14-April-23	18-April-23	Hepatotoxicity	776.5	4
53	Relapsed BCP-ALL	BHR	BHR	R1 Consolidation	2	14-April-23	18-April-23	Trough	776.5	4
53	Relapsed BCP-ALL	BHR	BHR	R1 Consolidation	2	19-April-23	19-April-23	Hepatotoxicity	776.5	0

Asparaginase Tracker

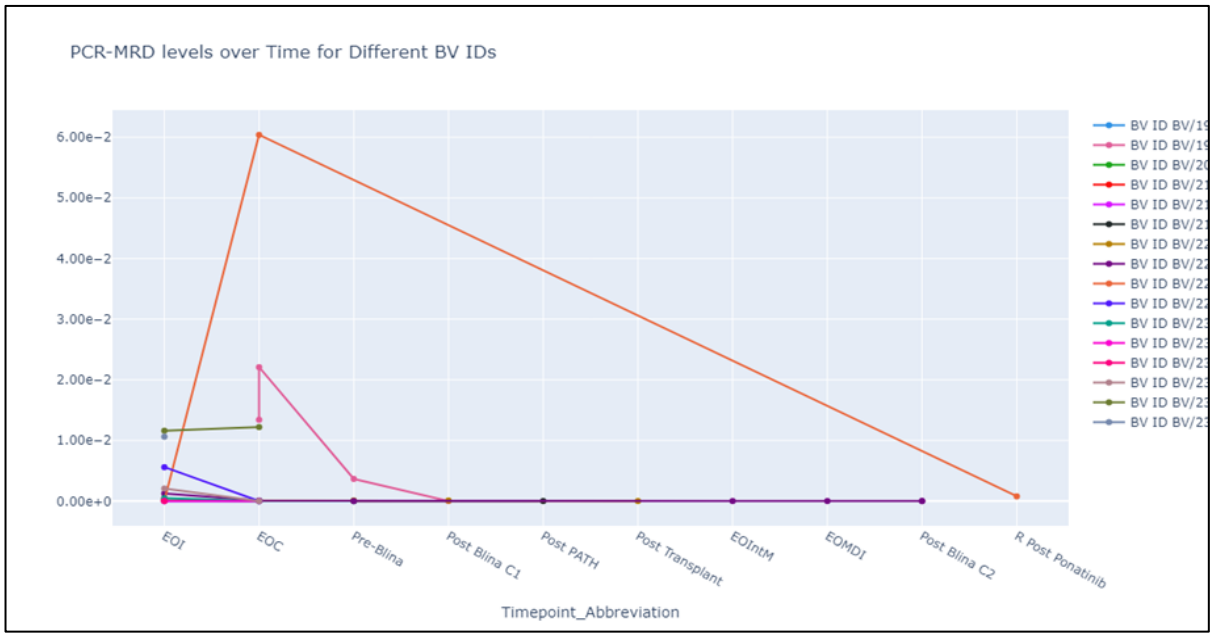
Patient Data Visualization

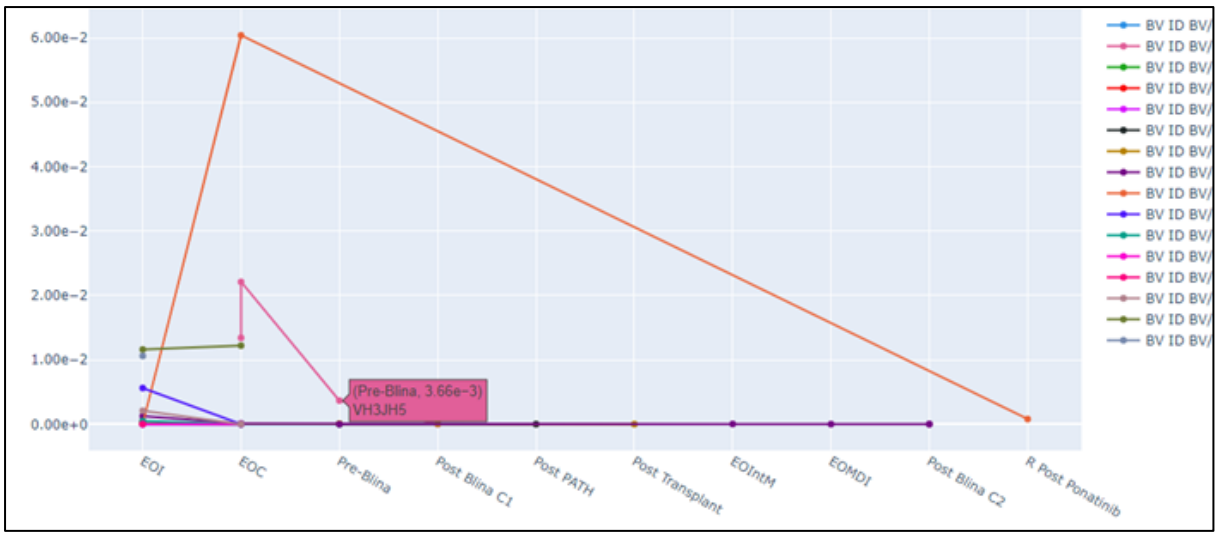
Patient interactive visualization plots showing MRD quantification levels w.r.t various patient different timepoints considering most abundant marker and different phases of treatment as per Icicle protocol for all the patients in consolidated manner. These plots are generated using MRD tracker which would help researchers and scientists for further analysis.

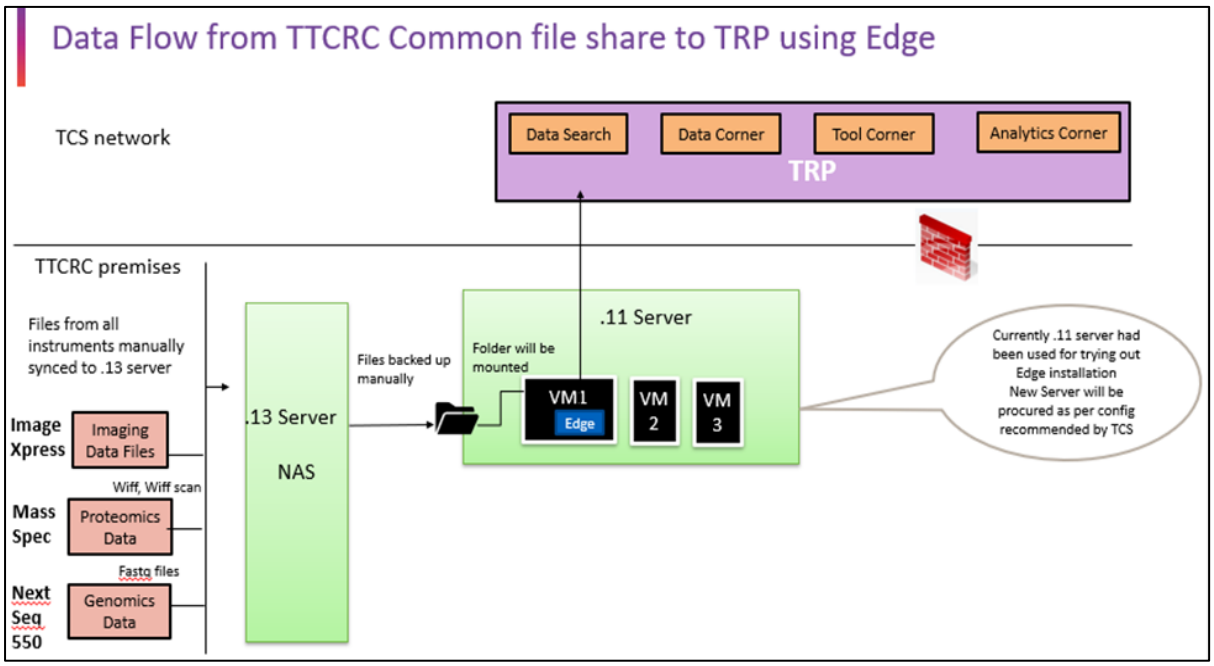
Instrument Connectivity

Instrument connectivity is required to fetch instrument raw and analyzed data files directly from the instruments for all research groups without manual intervention to TRP Data repository for metadata search and scientific data management.

Edge box has been installed on the TTCRC server to fetch raw instrument files and other processed & analyzed files directly for all research groups without manual intervention from the file share system. Currently, this activity is initiated with two high throughput instruments, **Mass spectrophotometer** & **Next Seq 550**.







POCs

Two significant POCs have been carried out for TTCRC.

- Deploy and run Proteomics Pipelines over Azure cloud.
- Secure Azure architecture development for Scientific Data management and analytics for sharing between Collaborators.

Research Publication-2023

1. Dutta A, Mungle T, Chowdhury N, Banerjee P, Gehani A, Sen S, Mallath M, Roy P, Krishnan S, Ganguly S, Banerjee S, Roy M, Saha V. Characteristics and outcomes of gallbladder cancer patients at the Tata Medical Center, Kolkata 2017-2019. Cancer Med. 2023 Apr;12(8):9293-9302. doi: 10.1002/cam4.5677. Epub 2023 Feb 13. PMID: 36779618; PMCID: PMC10166897.
2. Mungle T, Mahadevan A, Das P, Mehta AK, Gogoi MP, Jana B, Ghara N, Ghosh D, Saha V, Krishnan S. Hybrid Email and Outpatient Clinics to Optimize Maintenance Therapy in Acute Lymphoblastic Leukemia. J Pediatr Hematol Oncol. 2024 Jan 1;46(1):39-45. doi: 10.1097/MPH.0000000000002796. Epub 2023 Dec 12. PMID: 38096154; PMCID: PMC10756697.
3. Sidhu J, Chakraborty A, Das P, Gogoi MP, Dey T, Steffen FD, Basu S, Ganguli D, Ghosh D, Ghara N, Kundu S, Kar A, Bornhauser B, Bourquin JP, Saha V, Krishnan S. Delayed Intensification Including Venetoclax and Bortezomib Prolongs Survival in Very High Risk Acute Lymphoblastic Leukaemia. The American Society of Hematology. https://doi.org/10.1182/blood-2023-185989
4. Sidhu J, Steffen FD, Jimenez IA, Huang Y, Rauwolf KK, Chakraborty A, Krishnan S, Saha V, Bornhauser B, Bourquin JP. Phenotypic Drug Response Profiling Identifies Asparaginase-Based Synergistic Combinations for Very High Risk Acute Lymphoblastic Leukaemia. The American Society of Hematology. https://doi.org/10.1182/blood-2023-172885
5. Sidhu J, Gogoi MP, Krishnan S, Saha V. Relapsed Acute Lymphoblastic Leukemia. Indian Journal of Pediatrics. https://doi.org/10.1007/s12098-023-04635-4
6. Bhattacharya S, Saha V. How to write a research grant proposal. Indian Journal of Medical Microbiology.
7. Moreira DC, Metzger ML, Antillón-Klussmann F, González-Ramella O, Gao Y, Bazzeh F, Middlekauff J, Fox Irwin L, Gonzalez ML, Chantada G, Barr RD, Garrington T, Hastings C, Kutluk T, Saab R, Khan MS, Saha V, Rodríguez-Galindo C, Friedrich P. Development of EPAT: An assessment tool for pediatric hematology/oncology training programs. Cancer. 2023 Nov 1;129(21):3448-3456. doi: 10.1002/cncr.34946. Epub 2023 Jul 7. PMID: 37417913.
8. Berger A, Rennie S, Aijaz J, Johnson LM, Antillon F, Roberts MC, Chitsike I, Kambugu J, Saha V, Bhakta N, Davis AM, Alexander TB. The role of relative advantage for development of sequencing-based diagnostics for pediatric cancer in low- and middle-income countries. Cancer. 2023 Oct 16. doi: 10.1002/cncr.35065. Epub ahead of print. PMID: 37843081.
9. Berger A, Rennie S, Aijaz J, Johnson LM, Antillon F, Roberts MC, Chitsike I, Kambugu J, Saha V, Bhakta N, Davis AM, Alexander T B. The role of relative advantage for development of sequencing-based diagnostics for pediatric cancer in low- and middle-income countries. Cancer. 2024;130(2):173-178. doi:10.1002/cncr.35065

Staff Joined & Exited TTCRC in 2023

Staff joined in 2023

Staff Name	Designation	Joining Date
Anusree Martina Bor	Research Assistant-Genomics	18/01/2023
Nikhilesh Chowdhury	System Support Assistant-IT	02/03/2023
Kazim Ali Iqbal	Data Scientist-CRU	16/05/2023
Anusree Goswami	Research Assistant-MRD	15/06/2023
Ayan Halder	Research Assistant- Genomics	19/06/2023
Tanima Dey	Research Assistant-Cell Biology	03/07/2023
Sunanda Bera	Research Assistant-MRD	14/07/2023
Preyashi Karmakar	Research Assistant-MRD	14/07/2023
Bhaswati Tarafdar	Research Assistant	01/08/2023
Annwesha Roy	Research Assistant	16/10/2023
Roopsa Ghosh	Research Assistant	13/11/2023

Staff Left in 2023

Staff Name	Designation	Leaving date
Pritha Paul	Postdoctoral Fellow- Molecular Biology	04/03/2023
Arunima Maiti	Proteomics Technologist	21/01/2023
Tushar Dilip Mungle	Postdoctoral Fellow - Imaging	31/03/2023
Sayantani Mitra	Research Assistant	31/12/2023
Arnav Bhattacharya	Research Assistant	31/12/2023
Ananya Mahadevan	(Intern-15/09/2020), Research Assistant	28/04/2023
Dr. Shruti Banerjee	Research Assistant	13/05/2023
Amit Kumar Mahta	Data Analyst	30/09/2023
Atreyi Dutta	Research Asistant	31/05/2023
Smrithi J S	Research Assistant - Cell Biology	15/10/2023
Rupsha Mukherjee	Data Analyst	14/04/2023
Suparna De	Research Assistant	31/08/2023
Anusree Martina Bor	Research Assistant-Genomics	17/04/2023
Kazim Ali Iqbal	Data Scientist-CRU	30/09/2023

Poster presentation in Annual Review 2023

Poster No.	Title	Presenter
P1	Systematic clinical and pharmacological monitoring of biogeneric native and PEG-conjugated L-Asparaginase in paediatric patients with newly diagnosed acute lymphoblastic leukaemia	Bishwaranjan Jana
P2	Qualitative Detection and Quantitative Estimation of Plasma anti-Asparaginase (IgG) antibody in Patients with Acute Lymphoblastic Leukaemia	Srijani Goswami
P3	Treatment related deaths and non-fatal severe toxicities during intensive treatment phase of ICiCLe-ALL-14 risk-stratified treatment protocol for newly-diagnosed acute lymphoblastic leukaemia	Parag Das
P4	Biobanking For the Next	Abhirupa Kar
P5	Reassessment for refinement and optimization of Pediatric Leukaemia Bone Marrow Aspirate Biobanking protocol	Sayak Manna
P6	Assessing biobank Sample Quality through gold Standard flow cytometric analysis for enhanced downstream application	Paromita Biswas
P7	Facilitating personalized Treatment – Strategies for addressing Low cell Counts & low BLAST percentages	Subhajit Kundu
P8	Tracking down the "Rogue" through its genomic footprint: an advancement in MRD detection	Aishwarya Dighal, Sreyasree Dhar
P9	*IKZF1* Deletions and Biological role of Splice Variants	Sangramjit Basu
P10	Clinical Proteomics Unit	Roopsa Ghosh
P11	Ex-vivo Drug Response Profiling (DRP) identifies SINE (Selective inhibitors of Nuclear Export) as sensitive and synergistic compounds in combination with L-asparaginase in drug-tolerant Acute Lymphoblastic Leukaemia	Arijit Chakraborty; Tanima Dey
P12	An annotated living organoid biorepository of gallbladder diseases	Ankita Dutta
P13	Patient derived gallbladder and breast cancer organoid library for drug response testing	Nandita Chowdhury, Shinjini Chandra
P14	Optimization of cell viability assay for organoid based drug response testing	Payel Guha
P15	Isolation of rare leukaemic population for phenomic study using modernised sorting approach	Bhaswati Tarafdar
P16	The Research Infrastructure Development at TTCRC: Our Journey Through the Decade 2014-24	Asama Mukherjee

Picture Gallery

Legend to photos

1. Visit of Mr. Balaji Ganapathy - Global CSR Head,Mr. Joseph Sunil Nallapalli - CSR India Head Mr. Praphul Pradeep - CTO & Head of Purpose Partnership, Dr. Sanjay Singh CEO of Gennova with team, Mr. Amit Saxena, Ms. Anju Goel, Dr. Rajgopal Srinivasan on 9th December, 2022
2. Visit of TCS-TCUP team at TTCRC on 20th September, 2023
3. Visit of CEO of TATA TRUST Mr. Siddharth Sharma on 26th June, 2024
4. Visit of new Trustees of Tata Trust -Ms. Maya Noel Tata, Ms. Leah Noel Tata and Mr. Neville Noel Tata along with Mr. Vijay Singh and Mr. H D Malesra on 20th April, 2023
5. The German collaborators & Dr. Ester Mejstrikova's visit during MRD Workshop 20th January, 2024
6. Participation in TMC Annual sports 2024. TTCRC champions of Tug of War on 03rd February, 2024

7-8. TMC Annual Programme 2023- TTCRC's participation in cultural programme on 20th May, 2024

9. Diwali celebration at TTCRC in 2023. Rangoli made by staff members
10. Visit of TCS steering committee on 31st January, 2024