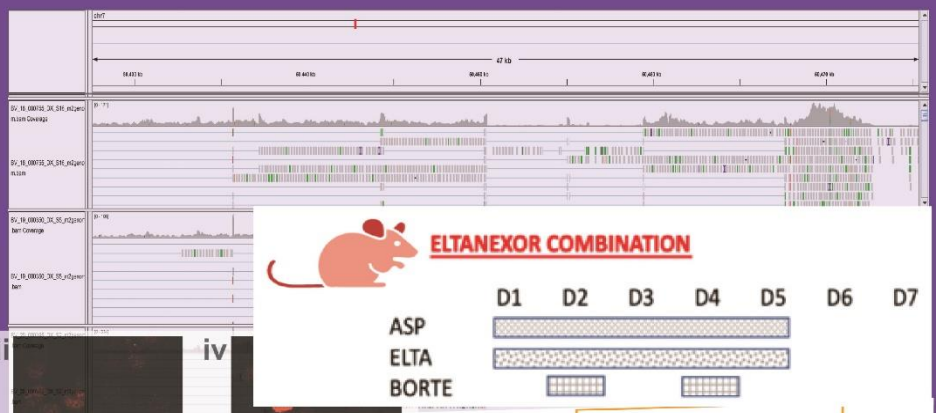
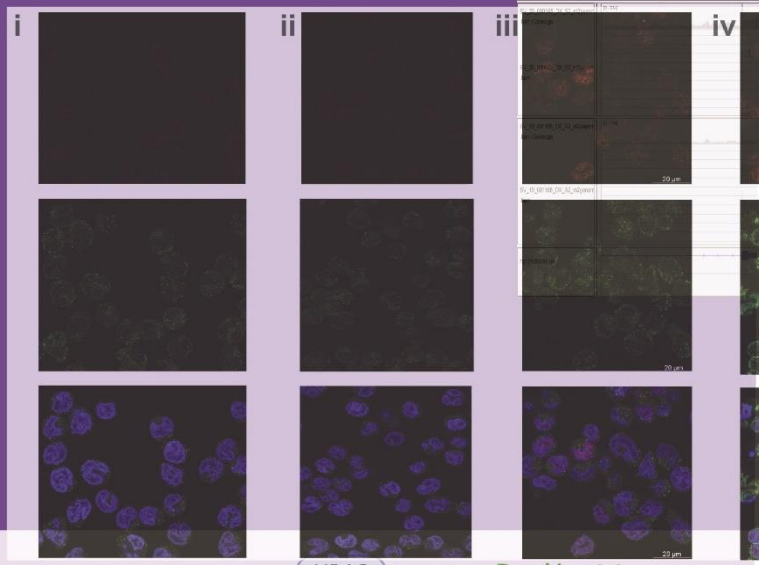
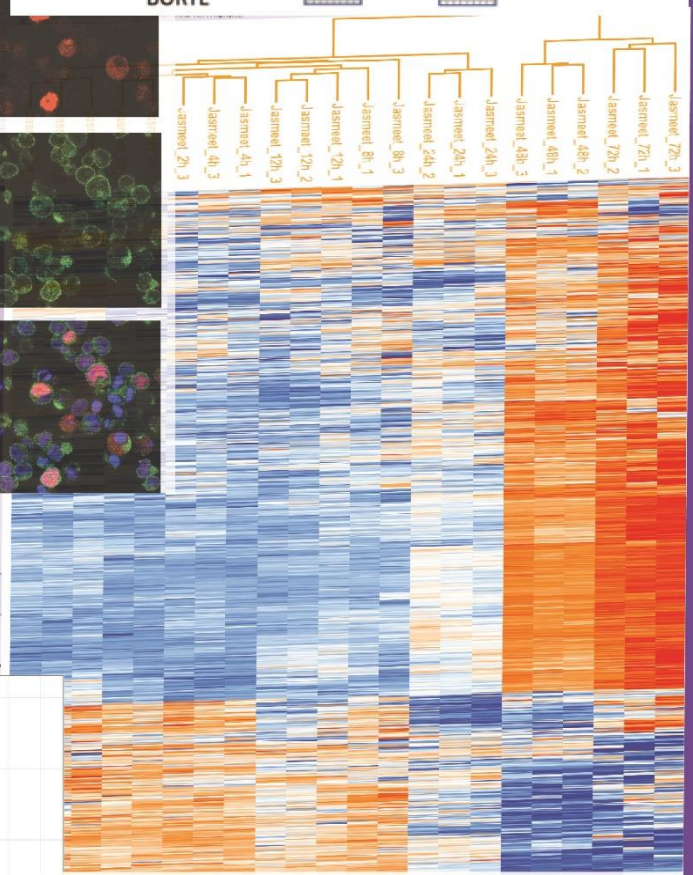
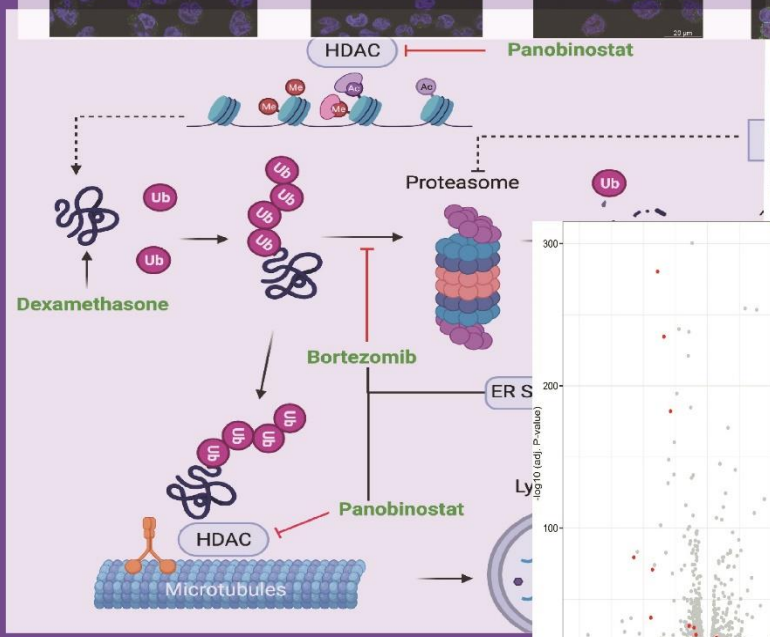
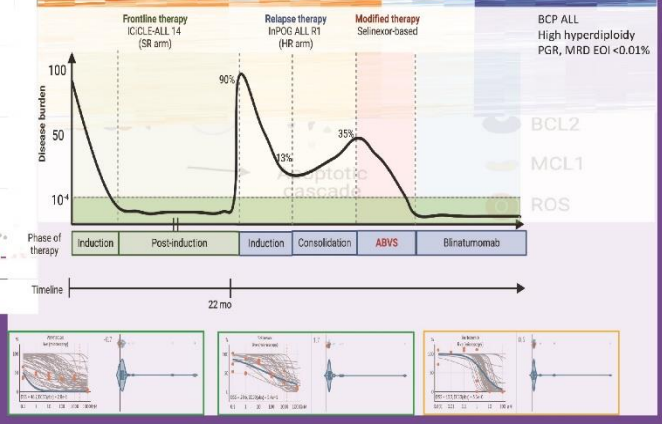
The Annual Scientific Report 2022











Tata Translational Cancer Research Center

Key to cover page

Picture Courtesy: Research Team

From Top to bottom:

DRP scheme for PDX samples; IGV Snapshot; Pathway Schema; Heat map for Proteomic analysis; p53 dependent *FAS* expression in response to cytotoxic stress; Volcano plot of Gene expression study between p53 wild type and knockout with Mitoxantrone treatment; Patient wise response profile and timeline.

CONTENT

From Director's Desk

Vaskar Saha

Head - Paediatric Hematology

This has been a busy year! With travel opening up in the second half of the year we have a steady stream of visitors. Teams from Gennova, DKMS and Tata Consultancy services visited. The president of the University of Manchester Dame Nancy Rothwell along with other senior members of faculty came to view progress. Gagandeep Kang dropped in for a chat. As I write this, colleagues from the University of Zurich are here for the Annual Review. There has been considerable ebb and flow of staff with many new faces.We were fortunate in Ashok Jayavelu agreeing to take charge of the proteomics laboratory.

Jasmeet returned from a year in Zurich, setting up routine drug profiling for our patients. She was successful in setting up an agreement with Karyopharma to obtain selenexor for patients with refractory ALL. Pritha Dasgupta and Sayantani Mitra returned from a month in Dresden /Berlin and will now move MRD to the next level. Our work with Asparaginase has been recognized internationally and we have active collaborations with pharma to deliver better products to the market. The ICiCLe clinical trial closed to recruitment. Outcomes have considerably improved and costs lowered. The network is due for considerable expansion now as the successor trial taken shape.

Jasmeet received the award for the best oral presentation at PHOCON-2022 and TTCRC was awarded the TATA INNOVISTA Award for *Translational Research for Oncology Patient* in 2022. Well done everyone and keep up the good work.

Administration

Asama Mukherjee

Laboratory Manager

Saheli Biswas

Secretary

Satadru Dey

Administrative Assistant

The Research Administration at TTCRC

The working model

The translational research model (**Figure 1**) at TTCRC is a close-knit assembly of three important teams, the clinical research unit, the biobank and the laboratories that function to provide better, affordable and tailored treatment for cancer patients. Research administration plays a crucial role in supporting a diverse range of functions (**Figure 2**) to help the researchers perform their day to activities. This is achieved through effective co-ordination and support from operational departments at Tata Medical Center. The team focuses on developing and fostering an environment to help the researchers and innovators deliver their best at the workplace.

Figure 1. Function of the Research Administration at TTCRC

Our activities in 2022

In 2022 we have been continuously streamlining operational procedures to enhance, integrate and align resources and infrastructure for meaningful research while adapting to changes primarily in terms of manpower, funding policies and its utilization as well as through expansion of functionalities.

Human Resources Management

Sukanya left in August for adventures abroad and the team welcomed Satadru.

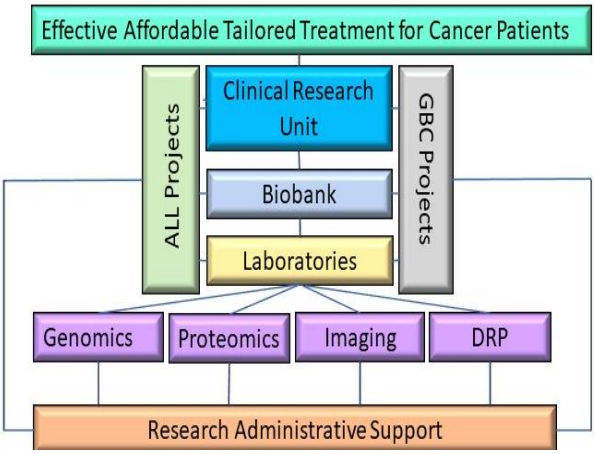


Figure 2. The Translational Research Model at TTCRC

There has been a distribution of responsibilities within the team to strengthen various functions. We are on the process of recruitment for an IT support personnel for efficient co-ordination and management of IT infrastructure right from the desktop to the server level. The research team has been also reorganised with recruitment of another 14 new staff in different groups as replacement for those who have moved ahead. We have also recruited and trained 5 interns during this period. We have initiated a series of induction session including health and safety for the new joiners with participation from CRU, Biobank, Genomics and Cell Biology groups. We will continue to hold these sessions periodically as refresher training for the team.

Finance Management

We have improved considerably in finance management in this year by adopting a co-ordinated procedure in preparing budget, money allocation between groups, tracking and recording expenditures, doing quality checks of the records, preparing a month-wise/quarter wise expenditure reports (allocated vs spent) followed by reconciliation with the finance.

Our greatest challenge in this year was adapting to the new and elaborate protocol for material acquisition as set up by our core funder, the Tata Consultancy Services Foundation. The transition to the new protocol initially slowed down our material acquisition process from this funding. We worked with the team leads to optimise the ordering by identifying procurement of several items from alternate funds and managing the existing supplies effectively. We have also received considerable support from TMC purchase, TMC finance, TCS project team and TCSF to expedite ordering of urgent items which are required for patient reporting. The admin team has played a key role in streamlining the new protocol with effective co-ordination between the various stakeholders involved in the process-the TCS project, TMC finance, TCSF, TMC purchase, users as well as vendors. We have been working hard to maintain the balance between user demand for their essential supplies while adhering to the new protocol which requires a significantly overall higher turnaround time. We are working to reduce this turnaround time by continuously working with the stakeholders at each level through improvising procedures.

Inventory Management

To streamline material management at TTCRC, we have expanded our central stock facility by accommodating items for common use such as plastic consumables and reagents each from the research groups. Processes are in place to avoid duplication in ordered items and encouraging the sharing of materials between teams, thereby attempting optimal utilization of available resources, both material and funding. Currently a common inventory is maintained manually in spreadsheets. We plan to switch to an electronic laboratory inventory management system.

Data Management

We have prepared a Data Management Policy last year which has been enforced and implemented in this year. We have encouraged each staff to share a data management plan through e-forms for each of the projects along with their appraisals which will be reviewed in each year. As we look forward to more collaborative projects involving high-throughput data sharing and analysis, the admin will continue to play a critical role in providing the infrastructure for data storage, back-up, accessibility, visualization while ensuring safety and security of shared data.

Feedback 360^0

In order to have an understanding on the performance of the admin team and expectations of the researchers on the kind of mentorship, skill development opportunities, training needs, difficulties faced by them at their different standpoints, and hear their voice on how to improve our organisation we have developed a questionnaire, and taken anonymous feedback from the team through a Google Form. There were 65% responders from among the staff. The feedback analysis provided information which helped us to improve processes. This helped us to understand the importance of mental health wellbeing of staff and how it can impact their performance at workplace. With this motivation we went ahead in organising a workshop on mental health wellbeing for our staff to support them deal with workplace.

Abhirupa Kar

Bio repository Manager

Sayak Manna

Senior Bio repository Officer

Paromita Biswas

Bio repository Officer

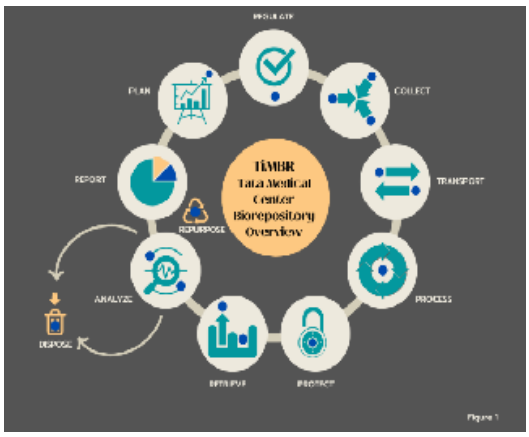
Subhajit Kundu

Project Research Officer

How TiMBR is making a difference in 2022?

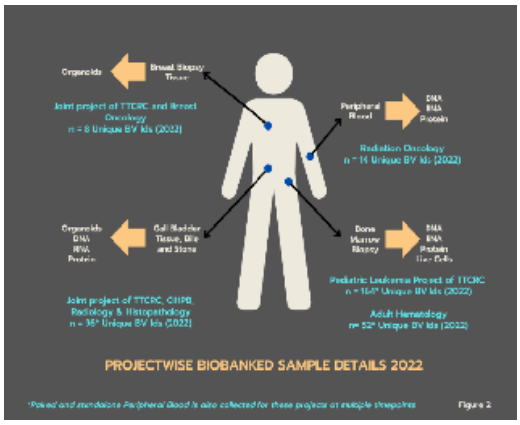
The Tissue Biorepository (TiMBR) at Tata Medical Center assists researcher and clinicians in processing, cryopreserving and storing of blood, tissues and other specimens for archival and research-oriented projects. From 2020 onwards, TiMBR has evolved from supporting hypothesis-driven research activities to making real-time impacts on clinical decisions. Our biobank holds a wealth of well annotated clinical biospecimens that ensure and promotes maximum sample integrity and enhances research collaborations in patient health.

Established in 2014 and re-furbished in 2021, the biorepository is the first cancer biorepository in eastern India with more than 50,000 high quality samples. Figure 1 demonstrates the journey of a sample, which a patient altruistically contributes, to the development of precision oncology.



Biorepository Capacity

The current overall vial storage capacity is up to 1.5 lakh sample vials. TiMBR is equipped to process and store whole blood, buffy coats, sera, plasma, cerebrospinal fluid, DNA, RNA, tissues and other biospecimens. All biospecimens are tracked throughout their life cycle—from collection to sample disposition—using a customized laboratory information system - LabVantage. The laboratory information management system integrates manual and automated workflow processes, captures biospecimen annotated data, controls permissions, assigns unique identifiers, provides reports and maintains an audit trail. Following Figure 2 showcases the project-wise distribution of samples at TiMBR in the year 2022.



Governance

The biorepository is governed by an Oversight Committee (OC) and a Scientific Advisory Committee (SAC). The OC provides accurate guidance for biobank functioning, SOP compliances and operational issue resolution of the biorepository whereas the Scientific Advisory Committee, comprising project investigators, provides strategic scientific guidance on resource development and quality management.

Commitment to the Research Participant:

TiMBR safeguards and provides research opportunities along with accession to specimens of the highest quality and integrity through conscientious and scientific custodianship, while also minimizing risk to patient privacy and

confidentiality. This important function is a cornerstone to the academic integration with Tata Translational Cancer Research Centre and the continual evolution and improvement of the research mission here at Tata Medical Center to provide better care to our patients.

The decision to store or use biospecimens is an important act of responsibility. The value of any request for advancing medical knowledge must be weighed against the irreplaceability of the specimen, as well as any ethical, legal or scientific challenges arising from the proposed research purpose. The Oversight Committee provides this crucial governance function, and its authority is supported by the inclusion of a diverse, volunteer membership consisting of physicians, surgeons, scientists, Institutional Review Board representatives, and biorepository management.

Achievements

TiMBR is currently supporting the setting up of companion diagnostics for the pediatric leukemia group. TiMBR provides fuel to India's only Ig/TCR based PCR MRD and Drug Response Profiling laboratories, which tailor a successful personalized treatment plan for pediatric leukemia patients to achieve clinical remission.

Challenges and Strategies

Ensuring the highest sample quality is the overarching goal of TiMBR. TiMBR core members continuously engage with the oversight and advisory committee to maximize sample integrity and utility. In December 2022, TiMBR underwent an internal operational audit by Dr. Rizwan Javed, Consultant, Clinical Hematology and Cellular Therapies at Tata Medical Center. The following table highlights the challenges, recommendations and action plans post-audit discussion.

Challenges	Strategies	Action Points	Timeline
SOP development, optimisation and issue resolution	Formation of Scientific Advisory Committee with project investigators, quarterly feedback meetings	Incorporation of recommendations of the Advisory Committee in the SOP (annual version update) Monthly review meetings	December 2022
Storage facility maintenance	Improved vigilance and enhanced engagement of the Estate team	Daily (including Sundays and Holidays) vigilance strategy to be deduced Breakdown and Back Up Policy to be circulated so that all stakeholders are informed	January 2023
Adhoc sample collection with limited information to the banking team about collection point, time	Improved coordination with the clinical team	Timely updates from CRC/CRU about sample collection through daily emails	Ongoing
Sample retrieval: Delays, missing samples and incorrect information	Advance notification from users, appropriate authorisation, immediate checking out, regular audits	At least 3 working days' notification for sample identification and retrieval Use of Dry Ice for locating samples from boxes Launching Stability Program for TiMBR samples uniformity in storage and retrieval SOP	February 2023
Storage space shortage	Sustainable management of banked samples	Stability program to be launched which will define the shelf life of a sample type at TiMBR Discard Policy (in progress) to be activated in consensus with the advisory and oversight committee accordingly	February 2023

Meet the Team

The TiMBR team is led by Ms. Abhirupa Kar, Biorepository Manager, who has a research experience of 9 years in the field of microbiology and biotechnology. She oversees the functioning of TiMBR and is assisted by Mr. Subhajit Kundu, who joined TiBMR as a Project Research Officer after completing his yearlong internship at Biobank. In 2022, two dynamic members joined TiMBR and became an integral part of the Paediatric Leukaemia Biobank core services. Sayak Manna joined Biobank as a Senior Biorepository Officer. Sayak has 5 years of experience in cancer and stem cell biology and is preparing to submit their Ph.D. thesis focussing on the therapeutic efficacy of cord blood plasma factors on leukaemia. Ms. Paromita Biswas joined Biobank as Biorepository Officer with 2 years of experience in clinical and diagnostic areas. We are also grateful to our previous members- Mr. Ritam Siddhanta and Dr. Kankana Das who paved way for our new members and retired from the team to explore other opportunities. We wish them all the best.

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

Biswaranjan Jana

Data Manager

Bony Dasgupta

Data Manager

Tushar Mungle

Post Doc Fellow

Amit K Mehta

Data Manager

Srijani Goswami

Research Assistant

Subhajit Kundu

Project Research Officer

Domains of CRU work include 1. Late phase clinical trials, 2. Early phase clinical trials and studies, and 3. Data driven studies

1. Late phase clinical trials

a. ICiCLe Clinical Trial

CRU continues to coordinate the Indian Paediatric Oncology Group's multicentre Indian Collaborative Childhood Leukaemia randomised clinical trial in children 1-18 years old with newly diagnosed ALL (InPOG-ALL-15-01-ICiCLe-ALL-14, Clinical Trials Registry of India CTRI/2015/12/006434), following the pre-trial phase that started in 2013. The trial concluded enrolment in August 2022. ICiCLe is the largest multi-centre paediatric ALL study conducted in India in terms of cohort size, having treated ~6000 children. Trial observations and analyses demonstrate a sustained lower rate of treatment-related mortality and improved outcome compared with previous reports in India (**Figure 1**). These observations have generated interest across paediatric oncology centres in the country to introduce ICiCLe-based risk-stratified management as standard of care for newly-diagnosed ALL at their respective institutions. After establishing the ICiCLe protocol as the standard of care in paediatric ALL in India, we move forward to implement it in public hospitals with an aim of building capacity and expertise in regional cancer hospitals for managing paediatric ALL.

b. InPOG-ALL-R1 Clinical Trial

CRU also coordinates The Indian Paediatric Oncology Group collaborative multicentre treatment protocol for children and adolescents with relapsed acute lymphoblastic leukaemia (InPOG-ALL-19-02; CTRI/2019/10/021758). Study enrolment is underway and increasingly, families opt for intensive treatments aimed at cure or good quality remission.

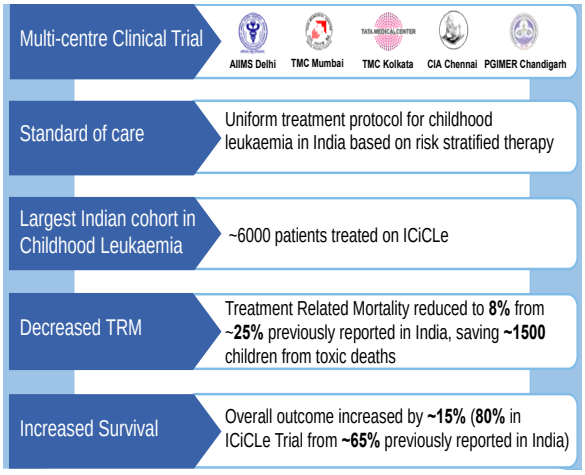


Figure 1. Diagrammatic representation of the observations from ICiCLe clinical study

2. Early phase clinical trials and studies

a. Asparaginase in ALL

In 2022, the team prioritised strengthening patient and sample coordination, along with streamlining the process of sample collection, so as to maximise collection while minimising patient inconvenience. Bony along with Bishwaranjan focussed on patient and sample coordination. Bishwaranjan takes the credit for developing a comprehensive BioBank-Laboratory tracker for Asparaginase samples. This allows tracking progress of sample collection real-time, and provides longitudinal data of the samples. Sample collection was disrupted in 2021, largely due to the 'Omicron' effect. This year we could bring our house in order through systematic coordination with patient families, our nursing and clinical team, and TiMBR. More than 90% of all targeted samples were collected, processed and stored.

We further focussed to re-build our laboratory team. Subhajit transitioned from an intern to a Project Research Officer and Srijani joined as a Research Assistant. Between both of them, they focussed to re-evaluate the SOPs and performed analytical validation and quality check for the determination of Asparaginase activity. Going ahead, we aim to partner with Gennova Biopharmaceuticals in conducting the anti-drug antibody assays for select samples from the Hamsyl study.

As part of a monitored study in collaboration with Gennova Biopharmaceuticals, we continued to provide our patients with a PEG-conjugated E. coli Asparaginase biogeneric (Hamsyl). With the help of a donation program sponsored by Gennova and a vial sharing program developed and managed by CRU, we were able to provide our patients with a PEGylated formulation of Asparaginase at a substantially reduced cost. More than 90% of samples collected during induction and post-induction stages, demonstrated therapeutic asparaginase activity of more than 100 IU/L, across all time points and risk groups. Rate of incidence of clinical hypersensitivity and pancreatitis were within expected range.

Looking forward in 2023, we propose to shift our choice of biogeneric PEG-Asparaginase from Hamsyl to Asviia (Zydus Cadilla), as the primary PEG-Asparaginase provided to our patients (paediatric + adults) with ALL (first presentation and relapse). Vial sharing program, a strong force behind our Hamsyl program, will be discontinued for logistic reasons. With Asviia, we envision pharmacokinetic monitoring of few select patients with first presentation of ALL (paediatric patients), to establish the dose that provides satisfactory therapeutic activity, in tandem with continuous monitoring of all patients treated with Asviia both at first presentation and relapse, for both therapeutic activity and drug safety.

Towards the latter half of the year, CRU would be coordinating a Phase I/II clinical trial to investigate the safety, therapeutic activity, suitable dose and suitable treatment schedule of an affordable indigenously manufactured recombinant PEG-conjugated asparaginase biotherapeutic, administered intramuscularly in children 1- 18 years old with newly diagnosed ALL.

b. Treatment of Very High Risk ALL: PATH-01 2023

Although the outcome of childhood ALL with current multiagent regimens has gone up to >80%, there is a group of patients with very poor outcome (EFS of <25%). This group include patients with high risk cytogenetics, very early medullary relapse, and those with poor response to treatment. PATH-01 2023 is a prospective, open label, single arm phase 1b/2 study with a novel treatment protocol developed to improve outcomes of these patients (**Figure 2**). The regime introduces or replaces drugs used in a specific treatment phase of ICiCle (Delayed Intensification) with drug(s) in use for other malignancies identified through drug response profiling.

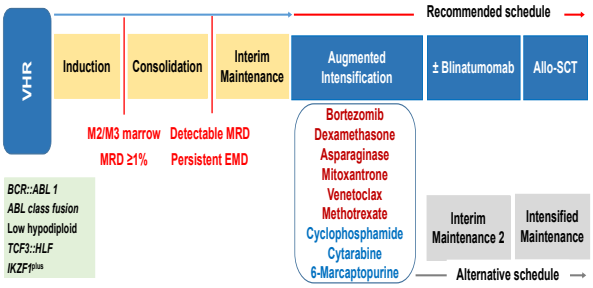


Figure 2. Treatment schema of PATH-01 2023 clinical protocol

3. Data driven studies

a. Maintenance therapy in ALL

CRU supports the clinical monitoring of the maintenance treatment (MT) phase in first-presentation and relapsed ALL. Research studies highlighted the problem of suboptimal antimetabolite drug dosing in the majority of patients and identified potential strategies to address this shortcoming. Following measures were taken to optimise the clinical practice with their outcomes.

I. Implementation of excel-based data recording for MT to aid systematic data capture and access for clinical practice as well as research studies.
II. Re-educate and train the clinical team to optimise the practice. The analysis of the intervention (or exercise) has shown improvised clinical practice as compared to pre-intervention, in terms of treatment intensity (**Figure 3**).

III. Development of an automated dose decision advice system (ADAM: Automated Dose Advice in ALL Maintenance) built on protocol-based dose rules of antimetabolite drug doses based on longitudinal information from blood counts, dose tolerance and time elapsed from last dose changes (in collaboration with TCS).

The focus will be to evaluate the testing, validation and implementation of automated dose advice tools along with visualisation tools in maintenance management. The tool could serve as one of the potential means to solve the problem of physicians' compliance to dosing guidelines, alongside reducing the time for clinical decision making during the clinic by providing patients historical data (**Figure 4**). Next, we aim to develop a data driven

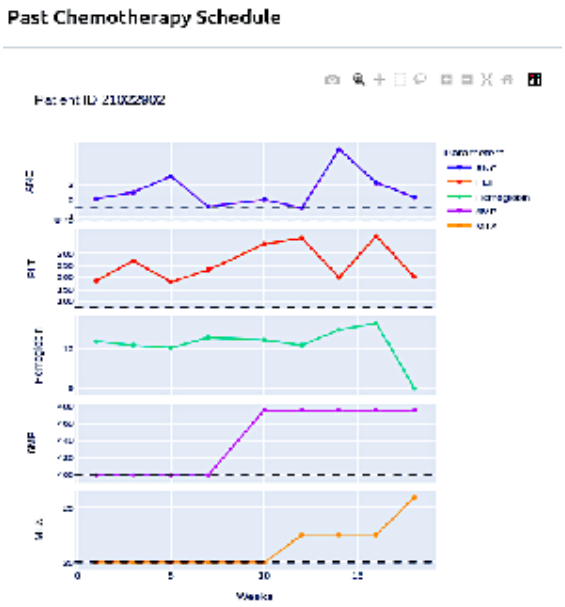


Figure 3. ADAM graphical visualization of the patient's historical showing blood counts and physician's prescribed doses of 6MP and MTX (antimetabolite drugs) since beginning of the treatment.

strategy to identify 6-MP intolerance patients having risk polymorphism for *NUDT15*, *ITPA* and *TPMT* genes. We believe the proposed data driven model would identify the intolerant patients before the start of MT thereby avoiding the need of pre-emptive testing for risk polymorphism and extra cost that may in incurred by every patient.

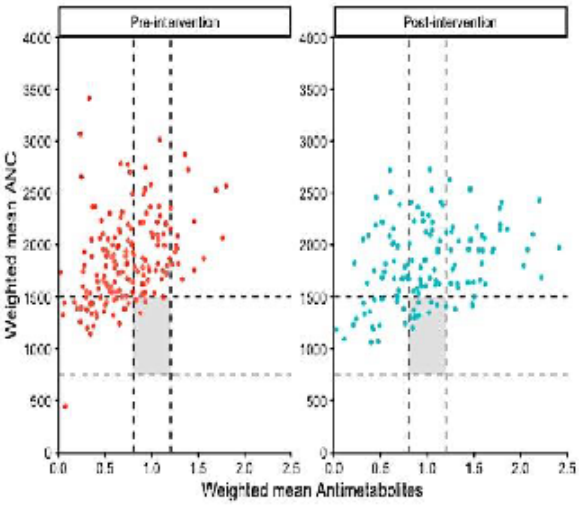


Figure 4. Scatterplot representing the weighted mean antimetabolite doses against weighted mean ANC counts for n = 311. Grey area indicates optimal mean antimetabolite dose (0.8 -1.2) vs target ANC (750 -1500). Red dots represent patients (n=175) who have been advised prior to protocol intervention, blue dots represent patients (n=136) who have been advised post protocol intervention. Date of intervention: 01/06/2017.

Flow Cytometry Facility

Arunima Maiti

Proteomics Technologist

The Flow Cytometry Facility 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 personnel cover the entire demand for cell sorting and user assistance needs in experimental design, advice and training.

Equipment

We have one benchtop cell analyser, BD Accuri™ C6 Plus (2 laser, 4 colour) and one sophisticated and highly sensitive cell analyser cum sorter, *BD FACSAria*™ (5 laser, 18 colour). BD Accuri™ C6 Plus can be used in simultaneously analysing multiple physical characteristics, like, relative size, internal complexity, and fluorescence intensity. While, *BD FACSAria*™ is with cell sorting ability, from heterogenous cell populations based on their relative size, granularity, viability and antigen expression using up to eighteen fluorochromes. Sorting up to 4 separate pure populations simultaneously at BSL-2 level is possible in this machine.

Activity

The activity in the facility can be described in terms of experiments performed in both the instruments as shown in table below.

Equipment	No of experiments (2022)
BD Accuri™ C6 Plus	
• Apoptosis assay	62
• Mitochondrial health assessment	23
• Cell cycle analysis	11
BD FACSAria™	
• Immunophenotyping	39
• Invitro fitness assay	20
• Mitochondrial health assessment	13
• Assessment of cellular stress	7
• Limiting dilution assay	17
• Cell proliferation (Ki67)	3
• Assessment of DNA damage	7
• Apoptosis assay	1
• Sorting	16

Additionally, hands on training, teaching, of the technology is provided.

Development

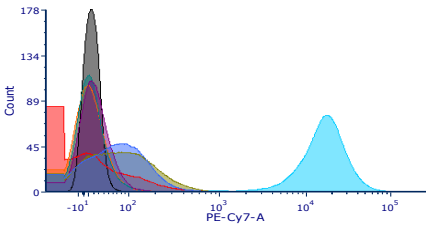
Our facility promotes development of new applications both based on the needs of research community. Few new assays have been standardised this year, main highlights are:

- Immunophenotyping up to 10 parameters (2 scatter+ 8 colour) for cell lines and bio banked plural effusion
- Assessment of cellular stress
- Estimation of cell proliferation
- Limiting dilution assay
- Assessment of DNA damage
- Enrichment of blasts from bio-banked bone marrow sample through immunophenotyping followed by sorting

Additionally, this year we were able to cater external institutes like, Indian Institute of Technology, Kharagpur and Indian Institute of Chemical Biology, Salt Lake, Kolkata for cell sorting.

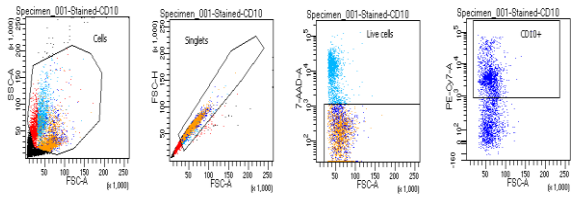
Few representative images are:

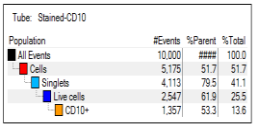
Immunophenotyping: Differential Expression of CD34 in ALL cell lines



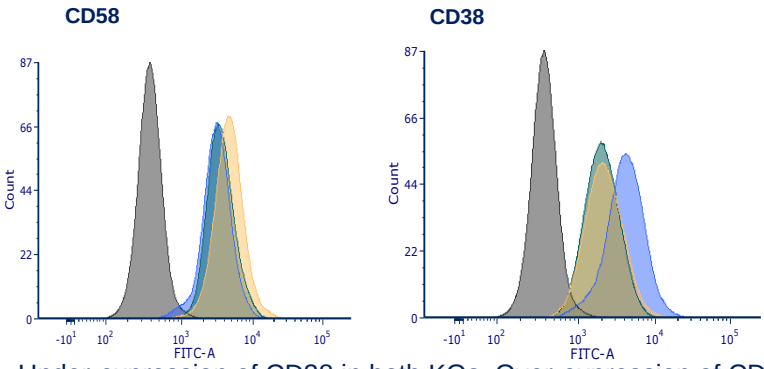
Sample	MFI
Control	-2.3
NALM6	-13.6 (Neg)
RS4;11	-6.72 (Neg)
REH	-5.92 (Neg)
SUP B15	12660.95 (Bright)
697	1.73 (Dim)
SEM	50.34 (Mod)
KOPN8	48.32 (Mod)

Enrichment of Blasts (CD10+) from Cryo-preserved Primary Samples by sorting



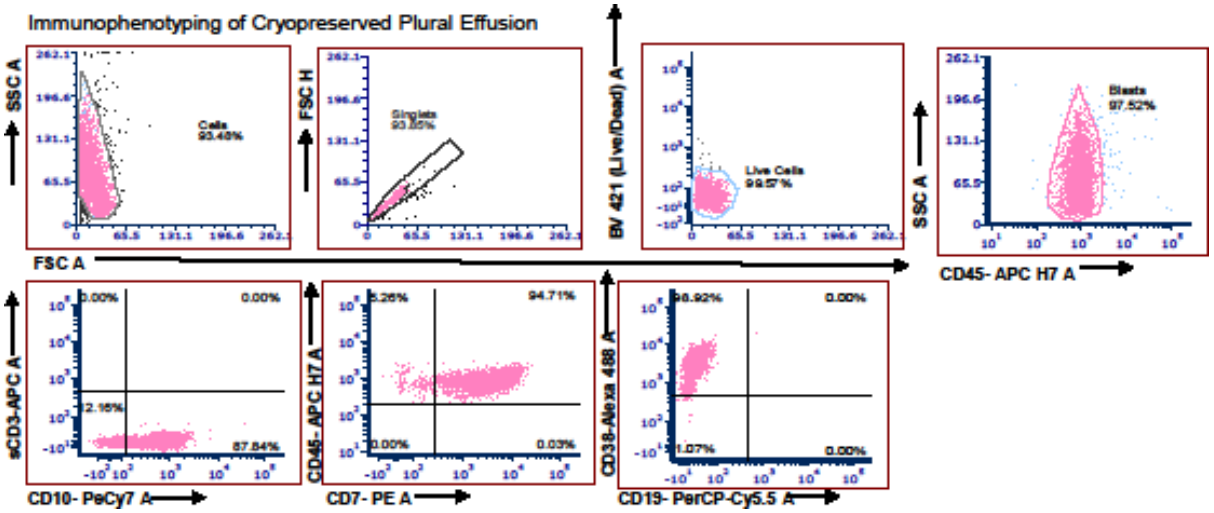


Immunophenotyping: Differential CD marker Expression in genetically modified ALL cells



Sample	MFI: CD38	MFI: CD58
Control	302.96	311.21
NALM6 WT	3184.22	2373.81
IKAROS KO	1562.87	2633.13
P53 KO	1647.01	3424.37

Under-expression of CD38 in both KOs, Over-expression of CD58 in P53 KO; WT: wild type, KO: knock out



Population	Events	%Parent	%Total
All events	30,000	-	100
Cells	27,743	93.48	93.48
Singlets	25,80	93.85	83.6
Live cells	24,739	99.57	82.5
Blasts	22,331	97.52	81.7
CD38+	18,226	94.92	79.22
CD38+/ CD19+	0	0	0
CD38-/ CD19-	205	1.07	0.8
CD19+	0	0	0
CD45+	685	5.26	5
CD45+/ CD7+	18,046	94.71	58.46
CD45-/ CD7-	0	0	0
CD7-	0	0	0
sCD3+	0	0	0
sCD3+/ CD10+	0	0	0
sCD3-/ CD10-	1026	12.16	8.26
CD10-	17,605	87.84	85.33

Drug Response Profiling Group

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Developing therapeutic strategies for patients with very high risk acute lymphoblastic leukaemia

Background

Risk-stratified response-based treatment protocols have led to over 90% survival rates in children with acute lymphoblastic leukaemia (ALL) in the west. In India, early results of the ICiCLe-ALL -14[1] risk stratified randomised clinical trial in childhood ALL suggests outcomes of nearly 85% in the last 5-years compared to ~65% in the previous two decades. 10-15% of ALL patients have either very high levels of MRD at the end of induction therapy or persistent MRD is detected at subsequent time points despite intensified therapy. Outcomes for these patients remain poor even with stem cell transplantation and therapeutic strategies remain to be defined.

Of the drugs used in ALL treatment, asparaginase (ASNase) is the most effective single agent for clearing disease. Except for ASNase, all other drugs are administered at maximum dose tolerance. Intensification of ASNase therapy is not associated with an increase in toxicity [2]. We, and others, have shown that there are considerable individual differences in ASNase pharmacokinetics and have introduced therapeutic drug monitoring along with individualisation of dosing[3-5]. Thus, investigation into drugs that synergise with ASNase, help overcome resistance to the drug without increasing toxicity and may be of benefit as alternative induction therapies in childhood ALL.

Ex-vivo drug response profiling (DRP) with single agents and combinations can help design sensitive chemotherapy protocols for such resistant disease patients, potentially leading to improved outcomes. Key to this strategy is combination chemotherapy and assessing synergy of the drug combinations used.

Aim: To identify strategies to decrease minimal residual disease (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
2. Identify drug resistance mechanisms using an *ex vivo* model of MRD

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 [6,7]. Leukemic cells in the marrow niche adapt to cytotoxic stress through exchange of nutrients and redox adaptation [6]. To mimic this marrow microenvironment *ex vivo*., we established an experimental workflow using a co-culture system. Primary ALL cells are cultured along with bone marrow mesenchymal cells (BMSCs) prior to drug exposure and then analysed by drug profiling (**Figure 1**) [8]. A panel of 4 – 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.

We analysed ficoll extracted blast cells obtained from pre-treatment bone marrow aspirates obtained from 68 patients with ALL treated at Tata Medical Center (between July 2020 to February 2023), identified to either have slow response to therapy, induction failure or relapsed disease (**Table 1)**.

Figure 2 represents the sensitivity and resistance profiles of these samples against the common chemotherapeutic agents. Both bortezomib (BZM) and venetoclax (VNX) had promising sensitivity profiles. We designed a protocol incorporating venetoclax in relapse induction backbone with bortezomib, dexamethasone, mitoxantrone and

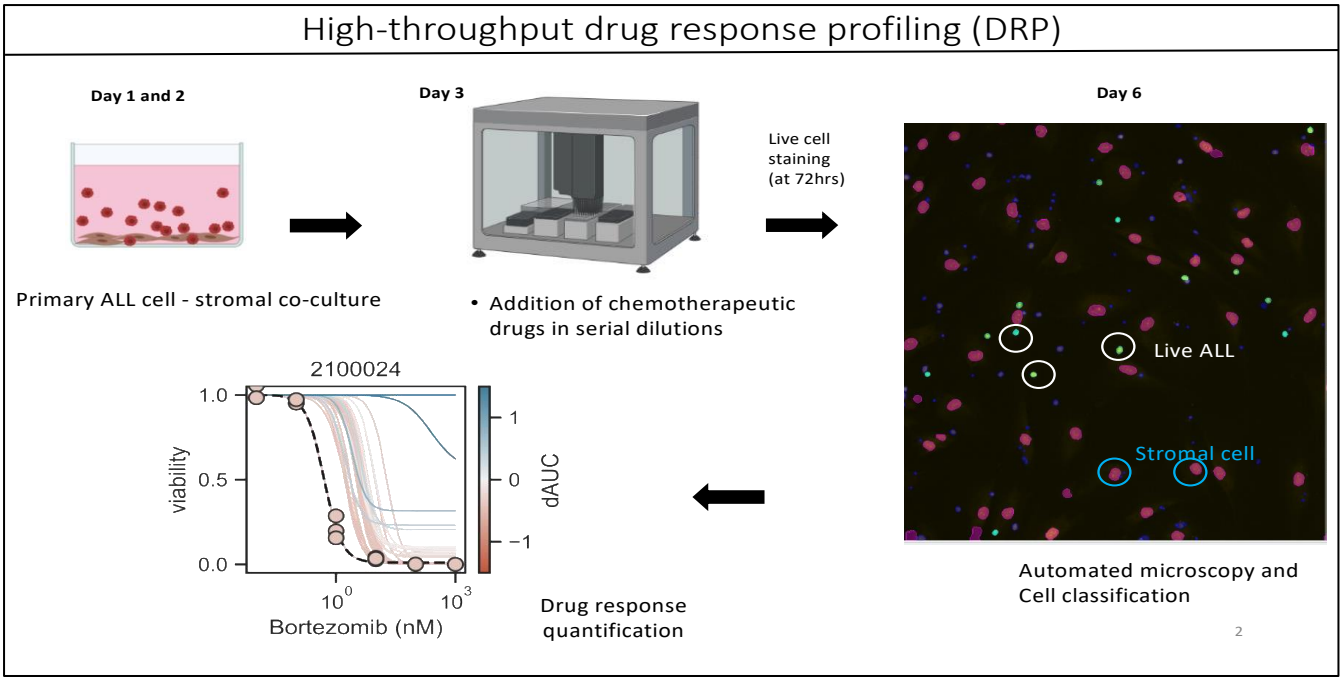

Figure 1. Schematic representation of high-throughput drug screening pipeline
Day 1: 2500 MSCs are seeded per well in 384 well plate in AIMV media.
Day 2: Primary ALL cells are added, 10000/well, in AIMV media on top of MSCs.
Day 3: Chemotherapeutic drugs are added in serial dilutions and in triplicates
Day 6: Live cell dye, CyQUANT, is added to cell suspension as per manufacturer's protocol and allowed to incubate for 45min at 37degC. High-throughput imaging is done at 10X magnification using ImageXpress microscope. Image analysis is done to quantify number of viable ALL cells per well using BIAS software (Peter Horvath, Single Cell Technologies). Drug response quantification is done using non-linear regression and curve fitting (The DB). The response to bortezomib in sample 2100024 is shown as an example in the figure. Dots represent ratio of viable ALL cells at different concentrations. Lighter curves in the background represent response to bortezomib in previously screened samples. This sample is very sensitive to bortezomib as AUC is the least.
ALL, acute lymphoblastic leukaemia; nM, nanomolar; dAUC, delta area under curve for bortezomib; hrs, hours.

asparaginase. The treatment protocol was well tolerated and more effective in very-high risk ALL (selected high risk cytogenetics and poor response to chemotherapy) patients (**Table 2**).

Table 1: Characteristics of patients in whom drug response profiling was performed

		N	%
		68	
Disease stage	Frontline	32	47
	Relapse	36	53
Gender	Male	41	60
	Female	27	40
Age (y)	<1	2	3
	1 to 10	41	60
	>10	25	37
Immunophenotype	BCP ALL	54	79
	Pro B	4	6
	ETP	3	4
	T ALL	5	7
	MPAL	1	1
	AML	1	1
Cytogenetics	*BCR::ABL1*	4	6
	CSF1R r	1	1
	KMT2A r	5	7
	TCF3::HLF1	1	1
	TCF3::PBX1	4	6
	B other	16	24
	ZNF384 r	1	1
	CRLF2 r	1	1
	ETV6::RUNX1	9	13
	High hyperdiploidy	15	22
	IKZFplus	2	3
	iAMP21	1	1

iAMP21, intrachromosomal amplification of chromosome 21

Results of DRP were also used for an early relapsed refractory BCP ALL patient found to be sensitive to selinexor, bortezomib and venetoclax. The 3 drugs were added on the backbone of intensification protocol, without mitoxantrone. The patient went from having 30% blasts in marrow to complete MRD remission.

We have now initiated an IRB-approved clinical study to inform treatment of

Table 2: Outcomes of patients treated with standard versus venetoclax-based ALL therapy

	Standard therapy n=46	Venetoclax-based therapy n=22
Complete remission	26%	73%
Relapse	59%	9%
Progression	9%	5%
Death	7%	0%

refractory patients with DRP results, **PATH-01 2023.**

antioxidant rescue experiments to confirm this hypothesis. Serial proteomic analysis

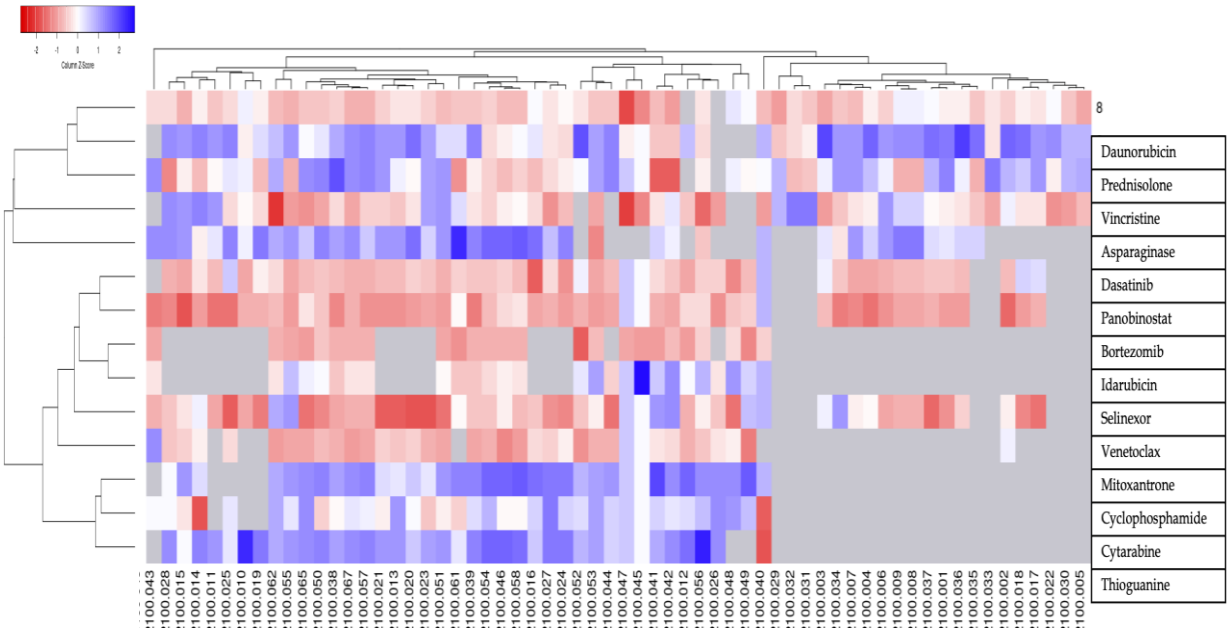

Figure 2. Distinct drug activity and response patterns detected with high-throughput drug screening for the whole cohort and individual patients of interest. Heatmap showing relative *ex-vivo* drug response (z-scored logIC50) to drugs screened across primary ALL cells of varying genomic subtypes and MRD response (N = 68). Grey boxes represent drug(s) not tested for patient. Blue represents resistance and red represents sensitivity.

Dissection of synergistic drug combinations for high risk ALL

As submitted in previous year's annual report, we identified a synergistic combination of asparaginase-bortezomib-venetoclax-selinexor through ex-vivo drug profiling of high-risk ALL PDX samples. Selinexor is an exportin-1 inhibitor that blocks export of over 1000 proteins from the nucleus to cytoplasm in human cells [9]. Its use in ALL has not been explored. We identified Selinexor sensitive and resistant ALL cell lines (NALM6 and REH, respectively) through DRP and apoptosis assays (**Figure 3A, B**). Published literature suggests intranuclear accumulation of p53 as the cause for apoptosis induction [10]. However, we noted p53 accumulation in both sensitive and resistant cell lines on treatment with Selinexor (**Figure 3C**), suggesting alternative mechanism of apoptosis induction. Mitochondrial assays show a distinct difference of oxidative stress induction and mitochondrial activity in both cell lines (**Figure 3D**), suggesting a probable metabolic cause for cytotoxicity in Selinexor. We need to further do was done at different Selinexor treatment time-points in an ALL PDX sample. The protein expression of the cell started changing as soon as 24 hours post Selinexor treatment (**Figure 4A**). The analysis also showed upregulation of oxidative phosphorylation in sensitive cells (**Figure 4B**). Proteomic analysis of resistant ALL PDX samples in current ongoing.

Sensitive and resistant ALL cell lines were identified using (A) Drug response profiling (see legend of figure 1) (B) Annexin-Propidium iodide apoptosis assay

(C) Immunoblots of XPO1 and p53 in ALL cells lines (NALM6 and REH)

(D) Mitochondrial flow-cytometry based assays to measure reactive oxygen species (ROS), superoxide dismutase (SOX), mitochondrial activity and mass using (Thermo-Fischer Scientific kits)

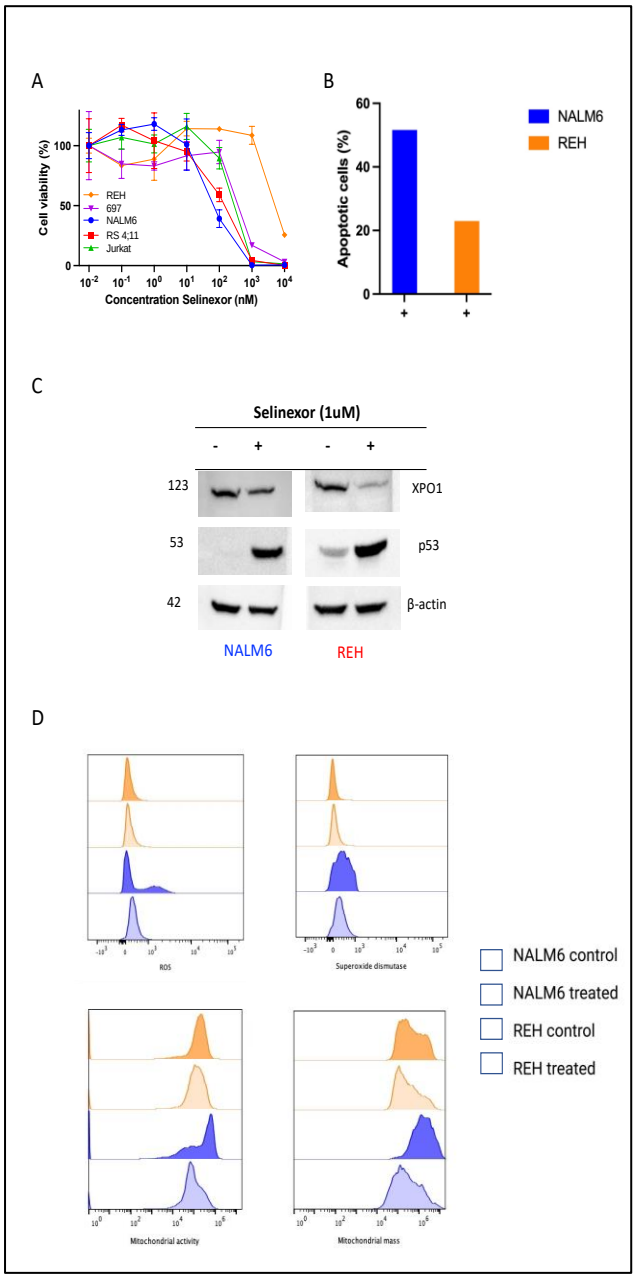

Figure 3. Selinexor induces apoptosis and upregulation of oxidative phosphorylation in ALL

(A) Heatmap depicting change in protein expression of cells over serial time-points. Each time-point has been tested in triplicates
(B) List of upregulated pathways after 24hour treatment with selinexor

Future plans:

Short-term goals:

- Widening of drug panel, combination drug screening and automation of drug response profiling pipeline at TTCRC
- Reporting use of DRP and venetoclax use in ALL therapy
- Analysis of proteomic data to identify biomarkers of response to Selinexor/combination therapy

Long-term goals

- Systematic evaluation of DRP as potential strategy for very high-risk patients through PATH01-2023 study.

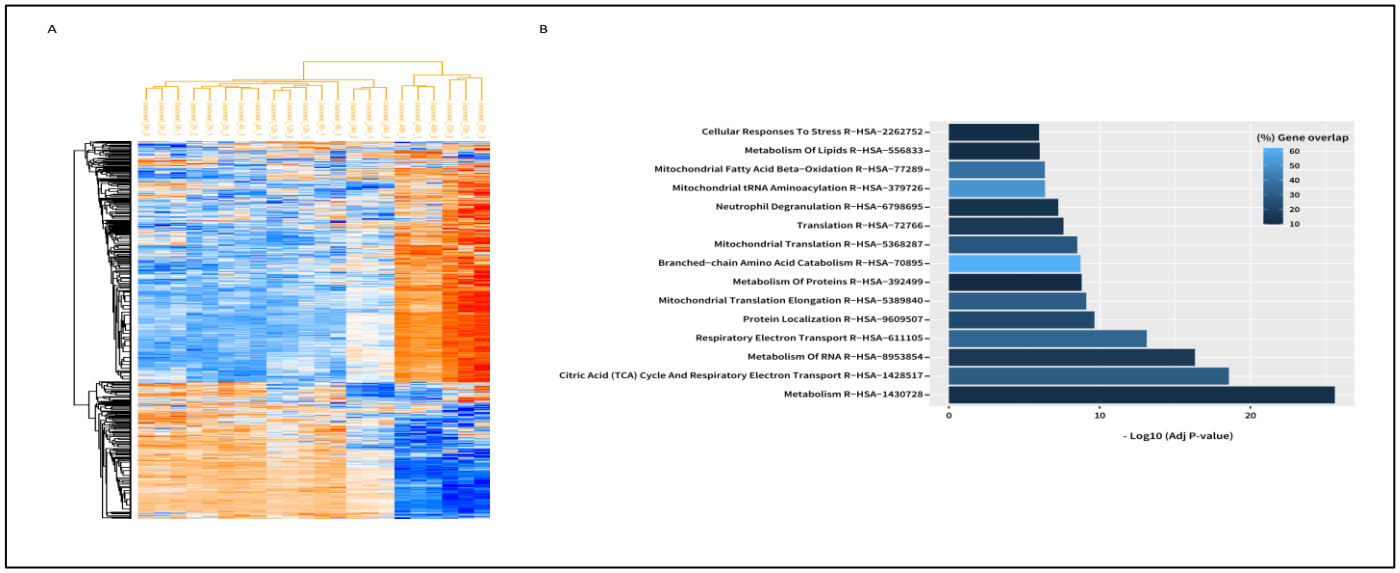

Figure 4. Proteomic analysis of Selinexor-sensitive ALL PDX sample (LK2020.43)

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Gall Bladder Cancer Group

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Title: Pre-clinical *ex vivo* modelling for Gallbladder Cancer

Summary: Gallbladder cancer (GBC) project was initiated in the mid 2019 with the goal of understanding the disease pathogenesis as well as testing new therapeutic approaches to improve the outcome in patients. The group primarily focuses on developing patient derived organoid (PDO) models of gallbladder diseases and create a well-annotated living organoid biobank. The biobanked organoids and the corresponding molecular derivatives and the associated clinical and epidemiological data together would serve as valuable resources for gallbladder cancer research globally. The other objective the team is working towards is to set up a PDO-based drug response profiling platform with the aim of providing personalized precision medicine to the cancer patients.

The team:

TTCRC Biology research team members:
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Background and rationale: In the north and north-eastern region of India incidence of GBC is among the highest globally. At Tata Medical Center (TMC), Kolkata, there were 698 cases of confirmed GBCs between 2017-2019 with the median age of 58. Of them, 91% patients were at stage III or IV at presentation and the 2-year overall survival of these patients were only 18% and 2%, respectively.

Aims:

(1) Develop a suitable pre-clinical study model (patient derived organoids) for GBC and creating a well-annotated living PDO biobank.

(2) Investigate the disease pathogenesis using the PDOs and identify new biomarkers and alternative therapeutic targets.

(3) Drug response profiling using the PDOs and thereby help provide personalized medicine for the patients.

Hypothesis: We hypothesize that inflammatory conditions in the gallbladder tissue caused by a combination of stress factors (e.g., high biliary cholesterol, environmental toxins like trivalent arsenic, or *Salmonella* infection, etc.) result in oxidative DNA damages. Repeated oxidative DNA damage events in chronic inflammatory condition combined with dysfunctional DNA damage repair pathway lead to accumulation of tumorigenic mutations in the epithelial cells eventually promoting neoplastic changes and gallbladder cancer. Lack of suitable pre-clinical study model has further limited the understanding of the disease pathogenesis as well as hindered testing of alternative therapeutic modalities. To address the poor outcome, this project works towards achieving the following aims.

Table 1: Number of PDGCOs developed from different gallbladder pathologies		
Broad pathological variety	**Detailed pathology**	**Number of patients for whom successful PDGCOs have been developed**
Malignant	Adenocarcinoma	7
	Intra-cholecystic Papillary Neoplasm (ICPN)	3
Inflamed	Xanthogranulomatous Cholecystitis (XGC) +/- AoC +/- cholelithiasis	6
	Acute on Chronic Cholecystitis (AoC)	2
	Chronic calculous cholecystitis or cholelithiasis +/- hyperplasia	2
	Papillary epithelial Hyperplasia +/- RA sinuses +/- cholesterolosis	2
	Cholesterolosis	3
	Chronic cholecystitis (CC)+/- cholesterolosis	21
	Adenoma	2
Normal	Unremarkable or normal	11
Total		59

Experimental approaches and results:

a. Development of patient derived gallbladder cholangiocyte organoids (PDGCOs):

Our group at TTCRC has developed patient-derived Gallbladder cholangiocyte organoids (PDGCOs) from patients who have undergone cholecystectomy for cancer in gallbladder or in a neighbouring organ. The pathologies of the gallbladder tissues from which the organoids have been developed include malignant (invasive and non-invasive), inflamed (with a wide range of inflammatory conditions) and pathologically normal variety [**Table-1**].

Wnt pathway plays an important role in organoid development as well as cancer growth and progression. We have optimized the PDGCO development protocol using two alternative culture conditions – one, where canonical Wnt is activated (CA) using glycogen synthase kinase 3 (GSK3) inhibitor and the other, where canonical Wnt pathway is inhibited (CI) using dickkopf 1 (DKK1) [**Figure 1**].

b. Refinement of culture maintenance method prolonged the viability of the PDOs in culture and improved the yield enabling downstream experiments:

We observed that some of the organoid cultures started to degenerate after 2-4 passages. We hypothesized that for these cultures, instead of mechanical harvesting, where dissociation is incomplete producing large clusters, enzymatic digestion would be able to dissociate the organoids better during passaging. Thus the separated viable cells in smaller clusters would be able to form new organoids preventing the PDO line to be degenerated **[Figure 2A].** Indeed, we found that the modified protocol **[Figure 2B]** helped to rescue several degenerating cultures **[Figure 2C, D]** and thereby made long-term culture propagation possible. This improved the average yield of organoids generated from

Table 2: Distribution of histologically analysed PDGCOs		
Pathology	**Total number of PDGCO lines (grown in any culture condition) analyzed**	**Paired (CA and CI)**
Adenocarcinoma (AdCa)	3	1
Intracholecystic papillary-tubular neoplasm (ICPN)	1	
AdCa on the background of ICPN	4	2
Xanthogranulomatous cholecystitis	3	
Cholesterolosis or Chronic cholecystitis (CC) +/- cholesterolosis	4	1
Papillary Epithelial Hyperplasia	2	
Acute on CC	1	
Normal	2	1
Total	20	5

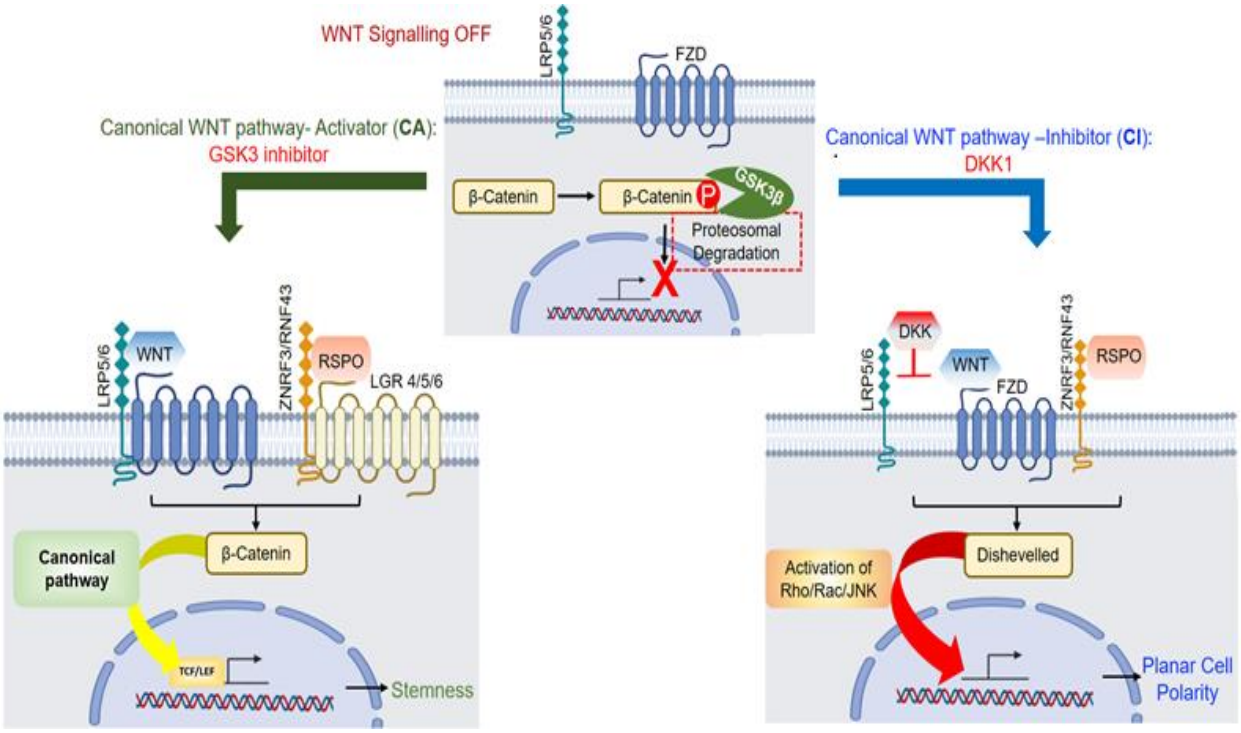

Figure 1. Schematic diagram of the pathways for the organoid culture conditions used**.**Canonical Wnt pathway is dependent on beta-catenin dependent downstream activation of genes. Glycogen synthase kinase 3 (GSK3) inhibitor stabilizes beta-catenin by inhibiting GSK3 mediated phosphorylation and proteosomal degradation of beta-catenin, thus activating canonical Wnt pathway. Dickkopf 1 (DKK1) is a known inhibitor of canonical Wnt pathway that competes with WNT ligand and binds to LDL-receptor related protein -5 or -6 (LRP5/6). Canonical Wnt activation is reported to promote stemness in the cells. CA, Canonical Wnt Activator; CI, Canonical Wnt Inhibitor; FZD, Frizzled receptor; RSPO, R-Spondin1.

each parent tissue **[Figure 2E]** enabling us to perform more downstream experiments.

c. Characterization of the developed PDGCOs:

To assess how well the developed PDGCOs recapitulate the corresponding parent tissues it is important to characterize them. Following are the different characterization approaches that we have been pursuing.

i) **Histological analysis shows PDGCOs maintain histo-pathological fidelity of their parent tissues:**

This year we have performed histopathological analysis of PDGCOs developed from a wide variety of gallbladder pathologies [**Table-2 and Figure 3**].

We found that all the organoids derived from the non-malignant gallbladder tissues displayed systematic array of single layer epithelial cells and mucin glands **[Figure 3B, C]**. The cells displayed normal morphology and nuclei. Some of the organoid lines developed from inflamed tissues showed features of reactive atypia. Organoids derived from the xanthogranulomatous cholecystitis (XGC) tissues showed low level of dysplasia, some nuclear atypia and focal multilayering. Both papillary hyperplasia and XGC derived organoids showed some cribriform structures and high nucleus to cytoplasm ratio, indicating high cellular proliferation rate. However, overall features were benign in nature **[Figure 3A]**. In contrast, organoids derived from the malignant tissues, viz., intracholecystic papillary neoplasm (ICPN) or adenocarcinoma (AdCa) on the background of ICPN, retained several features of malignancy similar to what we had reported for AdCa last year. The malignant features included high nucleus to cytoplasm ratio, pleomorphic cells and nuclei, nuclear disarray, clumped chromatin, irregular nuclear membrane, hyperchromatic cells, high number of mitotic cells, cribriform structures, multi-

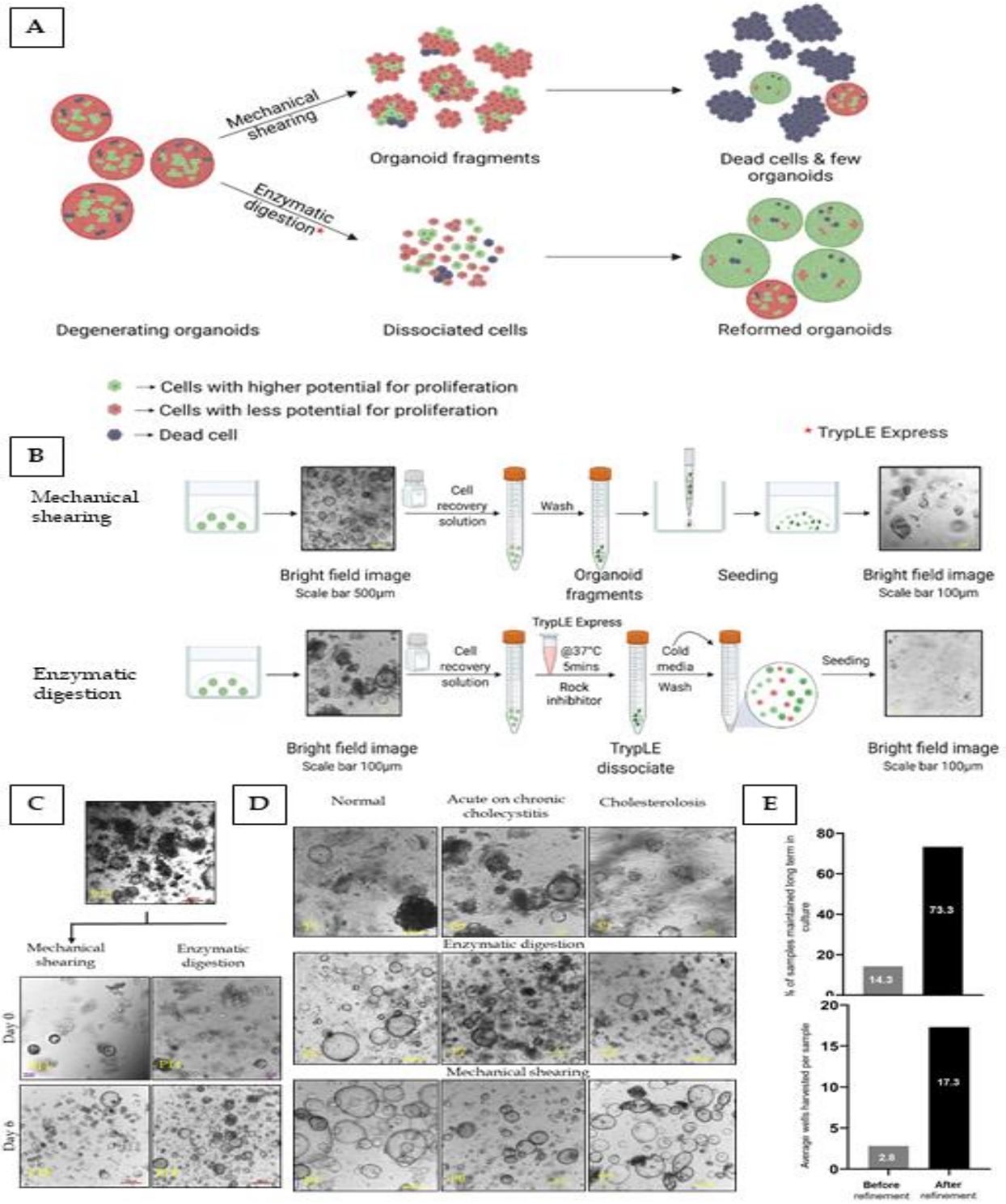

Figure 2. Refinement of organoid culture maintenance method improved organoid yield.

(A) Hypothetical concept of the two organoid passaging methods. **(B)** Schematic workflow of organoid passaging methods by mechanical shearing and Enzymatic digestion. **(C)** Bright-field microscopic images of the organoids showing the benefit of enzymatic (TrypLE Express) dissociation mediated passaging compared to the mechanical shearing mediated passaging for denerating organoids. Organoids were grown from cholesterolosis gallbladder tissue. Slow growing degenerating organoids (top) were passaged by enzymatic digestion (right panel) shows improved growth compared to those passaged by mechanical shearing (left panel). **(D)** Recovery of the degenerating organoids (top panel) by passaging with enzymatic digestion from different gallbladder pathologies (middle panel). Images of recovered organoids maintained for multiple passages by mechanical shearing (bottom panel). **(E)** Bar plot depicting the percentage of samples reaching passage ≥ 4 (top) and an average number of confluent wells (24-well dish) of organoids harvested per organoid line (bottom) in the period of 12 months. P, passage number.

layering of epithelia, exophytic growth and filled lumen **[Figure 3A-C]**.

Analysis on paired organoid lines developed in both CA and CI culture conditions derived from the same parent tissues (n=4) revealed that irrespective of the culture conditions PDGCOs preserved the histological features equally well **[Figure 3]**. There was one exception though, where one organoid line derived from a malignant tissue (AdCa on ICPN background) and grown in CA culture condition did not retain the malignant features [figure not shown]. One possible explanation for this could be, heterogenous cell types during culture initiation followed by selection of normal cell derived organoids in culture over tumour cell derived organoids.

ii) Functional characterization shows developed PDGCOs are functionally active:

Previously we had reported optimization of the functional characterization experiments to assess whether the developed organoids were functional or not. Now, we have performed the following functional assays for paired (CA and CI) organoids to test whether the organoids grown in both the culture conditions are functional or not **[Figure 4]**. All these experiments have been performed in biological triplicates.

We observed that irrespective of the culture conditions (CA or CI), the developed PDGCO retained epithelial barrier function **[Figure 4A]**, pump activity **[Figure 4B]** and enzymatic activity **[Figure 4C]**.

iii) Reverse Transcriptase- quantitative Polymerase Chain Reaction (RT-qPCR) demonstrates cholangiocyte marker genes are expressed in the developed PDGCOs:

RT-qPCRanalysis confirmed thecholangiocyte/biliary marker gene expression in the developed PDGCOs. The genes included cytokeratin-7 or CK7 (*KRT7*), cytokeratin-19 or CK19 (*KRT19*) and gamma-glutamyltransferase 1 (*GGT1*). We found all the marker genes were expressed in all the PDGCOs tested

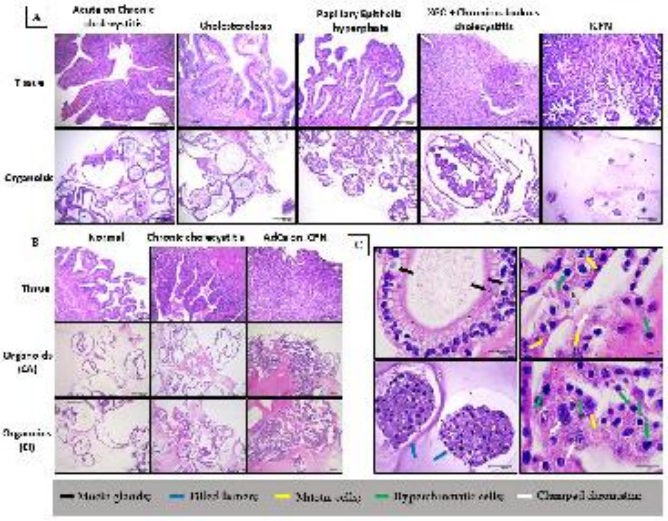

Figure 3: PDGCOs preserve the hisopathological features of their parent tissues.

(A) Haematoxylin-Eosin (HnE) staining of formaldehyde-fixed paraffin embedded (FFPE) tissue (top panel) and the derived PDGCO (bottom panel) sections from various non-malignant (left 4 panels) and malignant (right most panel). Detailed pathologies are mentioned above the corresponding panels. XGC, xanthogranulomatous cholecystitis; ICPN, intracholecystic papillary-tubular neoplasm. Scale bar denotes 200µm. **(B)** HnE staining of FFPE sections of the tissues (top panel) and the paired organoids grown either in CA (middle panel) or CI (bottom panel) conditions. The pathologies are mentioned above each respective panel. Scale bar denotes 200µm. **(C)** Representative images of different cytological features observed in the HnE stained FFPE sections of the PDOs. Mucin glands (black arrow), filled lumen (blue arrow), mitotic cells (yellow arrow), hyperchromatic nuclei (green arrow) and clumped chromatin (white arrow) have been indicated. Scale bar denotes 20µm.

without any significant difference between the two culture conditions (CA or CI) **[Figure 5]**. Experiments were performed in both biological and technical triplicates for each analysis.

d. Transcriptomic analysis:

To further characterize the models as well as to understand the disease biology better, we are also performing molecular characterization of the primary gallbladder tissue samples and the PDGCOs derived from the tissues. We have started with the transcriptomic (Illumina TruSeq Stranded

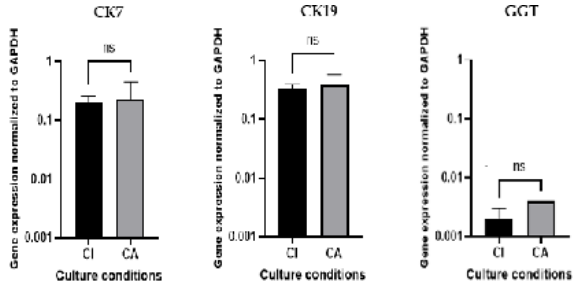

Figure 5. Cholangiocyte marker genes are expressed in PDGCOs developed in both the culture conditions.Reverse transcriptase –quantitative polymerase chain reaction (RT-qPCR) confirming the gene expression of key cholangiocyte marker genes (*CK7, CK19* and *GGT)* for the PDGCOs developed in both the culture conditions; n = 3 biological replicates. Values are normalizedagainst the reference gene *GAPDH* and represented in $\log_{10}$ scale. ns, non-significant (non-parametric paired Student's t-test). Error bar: Mean ±SD.

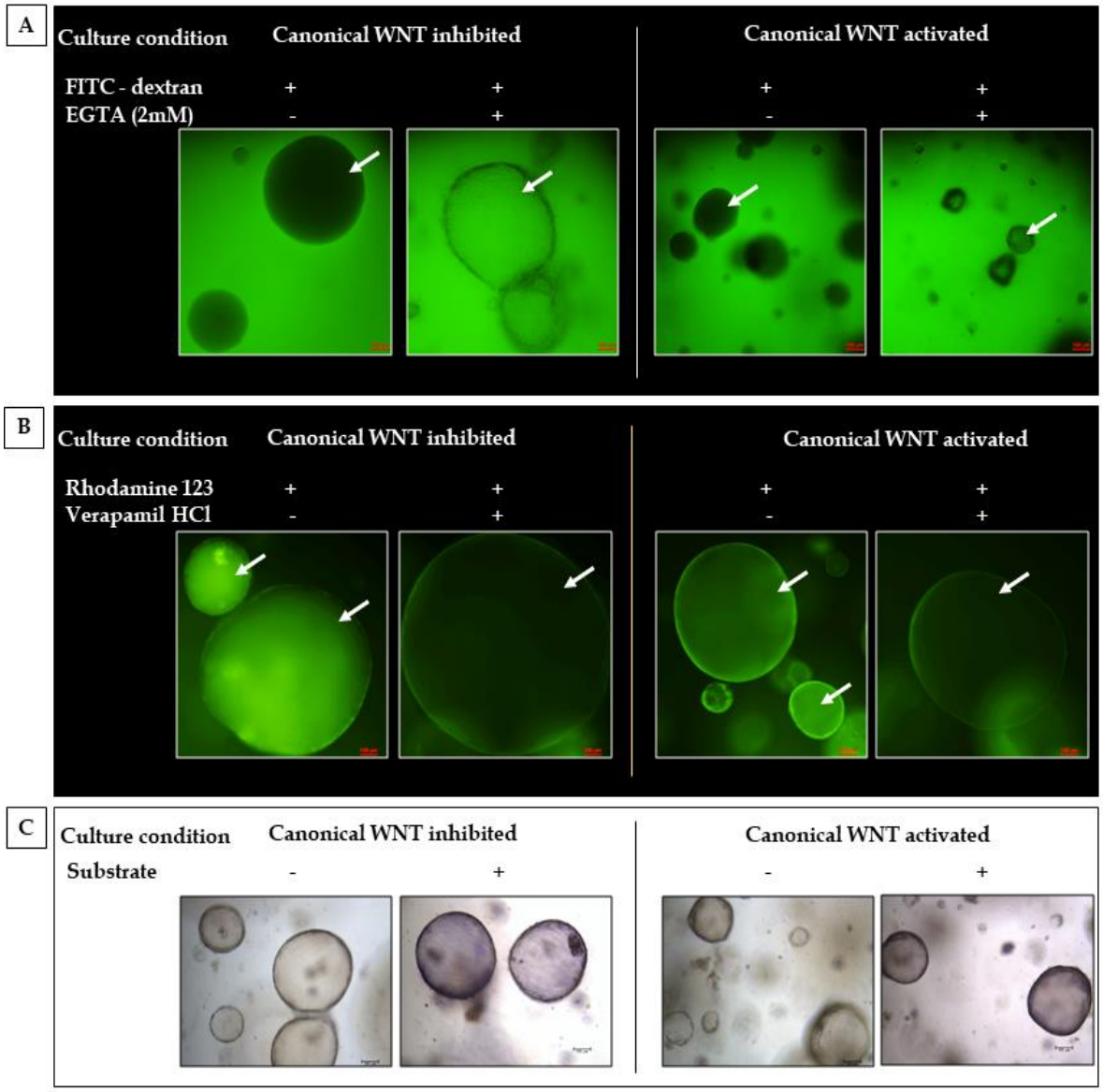

Figure 4. Functional characterization shows the PDGCOs developed in both the culture conditions (CA and CI) retain key functional activities of the cholangiocytes.

(A) Representative fluorescence images showing tight junction activity of the PDGCOs. FITC labelled dextran was used to test the integrity of the epithelial tight junction barrier. Images were taken before and 30 minutes after addition of calcium chelator EGTA (2mM), indicated by '-'and '+' symbols, respectively. The white arrow indicates the lumen of the organoids. Scale bar - 100µm. **(B)** Representative fluorescence images exhibiting MDR1 pump activity of the PDGCOs. Rhodamine123 was used as substrate for the MDR1 pump. Active efflux of fluorescent substrate Rhodamine123 in the lumen which is inhibited in presence of MDR1 pump blocker Verapamil HCl (10µM) exhibits the presence of an active MDR1 pump in the PDGCOs. The white arrow indicates the lumen of the organoids. Scale bar - 100µm. **(C)** Representative bright-field microscopic images demonstrating the alkaline phosphatase (ALP) activity of the PDGCOs. Purple blue tetrazolium formation in presence of the substrate BCIP/NBT signifies ALP releasing activity of the organoids. Scale bar - 100µm. EGTA, Egtazic acid; MDR1, Multi-drug resistance pump1; BCIP, 5-Bromo-4-chloro-3-indolyl phosphate; NBT, Nitro blue tetrazolium.

mRNA-seq) analysis in collaboration with the Genomics team. TCS life Sciences team has supported us for the bioinformatics analysis. Previously we had reported developing the mRNA-seq data analysis pipeline. The analysis pipeline was validated using external dataset as we had too few samples at that time.

Primary tissues clustered primarily based on the presence of absence of malignancy

We have now sequenced a total of 37 primary tissue samples with various pathological status that spans from malignant (n=10) to different varieties of inflamed (n=23) and pathologically normal (n=4) samples. Hierarchical clustering (with 500 highly variable gene expression) and principal component analysis **[Figure 6A, B]** showed that malignant samples and non-malignant samples had formed separate primary clusters with oneexception, where one adenocarcinoma tumour sample (ts_m18) clustered with the inflamed samples. This discrepancy could possibly have resulted from any difference in the pathological states of the tissue part used for the mRNA-seq study as opposed to that used for the pathological diagnosis purpose. Also interestingly, two samples (ts_i41 and ts_i42) that belonged to a rare highly inflamed pathological variety, known as xanthogranulomatous cholecystitis) (XGC), clustered with the core biopsy malignant samples, suggesting some similarity in gene expression in the XGC samples with that of the malignant samples.

Although primary clustering correlated well with the presence or absence of malignancy of the tissues, sub-clustering within the non-malignant or malignant clusters were not correlated according to the detailed sub-varieties of the pathologies. This could be attributed due to possibly very subtle differences at the gene expression level between the sub-varieties.

Differential expression of genes (DEG) analysis revealed expressions of 1727 and 2067 genes to be upregulated and downregulated, respectively, in the malignant samples compared to the non-malignant samples (log2 fold change >1, p<0.05, outliers were excluded from this analysis) **[Figure 6C]**. Proteomic analysis would be conducted on similar groups to validate and further understand the GBC disease biology.

Non-malignant PDGCOs clustered based on the culture condition

Transcriptomic analysis of the PDGCOs so far has been limited to only the non-malignant tissue derived samples. We had a total of 29 PDO lines (18 in CA and 11 in CI condition) that were sequenced and analysed. We observed that the primary clustering here was based on the culture conditions (CA and CI) **[Figure 7A, B]**. Similar to the primary tissues, for PDGCOs too we did not see any correlation of the sub-clustering pattern with the sub-varieties of the non-malignant pathologies. Geneset enrichment analysis (GSEA) confirmed that PDGCOs grown in the CA condition has significant enrichment (Normalized Enrichment score 1.39, False discover rate.q. value 0.09) of the reported direct target genes that are known to be upregulated upon activation of canonical Wnt pathway. The list of the genes used for this GSEA can be found along with the references here:https://web.stanford.edu/group/nusselab/cgi-bin/wnt/target_genes.

PDGCOs preserved the single nucleotide polymorphisms (SNPs) of their patient-specific sources:

Using the mRNA-seq data, we also assessed whether the PDGCOs preserved the SNP signatures of their particular source tissues. Indeed, we found that the PDGCOs (irrespective of their culture conditions, cellular dissociation methods or passage numbers) preserved the patient-specific SNP signatures present in the source tissues. All the PDGCOs had the maximum percentage of shared SNPs with the corresponding parent patient tissues. The overlap was 62.8- 96.3% for the SNPs with allele frequency (AF) > 0.001 and 53.6-84.2% for AF <0.001 **[Figure 8A, B]**.

We conclude that we have successfully developed functionally active PDGCOs in two different culture conditions, both of which recapitulate the histo-pathological features and molecular markers of the tissues. To understand the disease biology and progression of the disease we are currently performing high throughput molecular characterization of

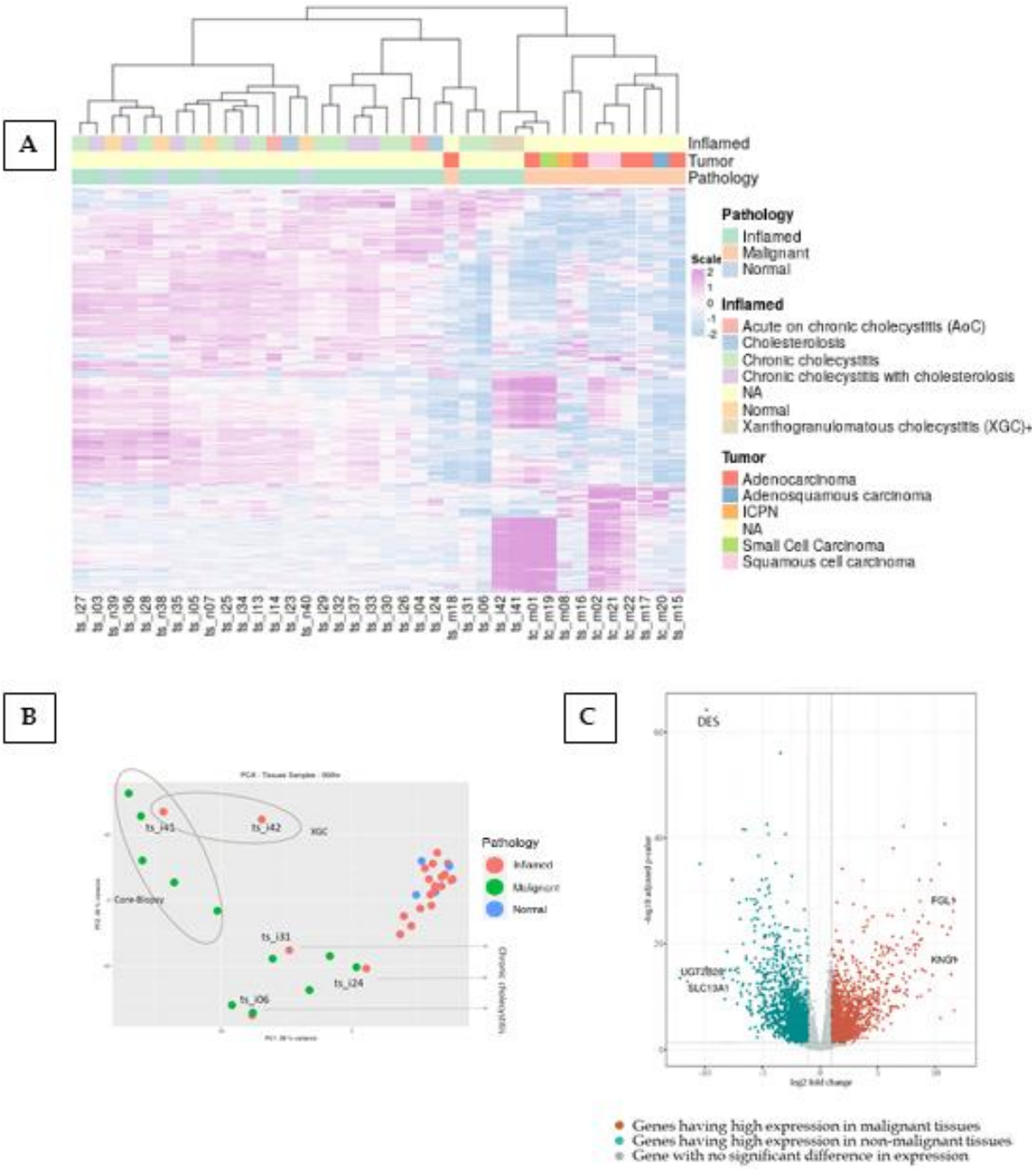

Figure 6. Transcriptomic analysis of primary gallbladder tissues demonstrate separate gene expression clusters for malignant versus non-malignant pathologies.

Primary gallbladder tissues from donor patients with different pathologies were used as a source of RNA for performing mRNA-seq analysis (Illumina TruSeq stranded mRNA-seq) using Nextseq 550 or NovaSeq 6000 (paired end 150bp or 100bp, respectively). **(A)** Heatmap depicting unsupervised hierarchical clustering (Pearson distance, Clustering centroid) with 500 highly variable genes. Pathological and sub-pathological varieties are indicated on the right hand side in the figure. The respective sample identities are indicated below the heatmap. **(B)** Principal component analysis shows broadly malignant and non-malignant samples formed separate clusters, with a few outliers. Xanthogranulomatous cholecystitis (XGC) samples (ts_i41 and ts_i42) have clustered closer to the core biopsy malignant samples (indicated by the elliptical enclosures in the figure). **(C)** Volcano plot showing the differential gene expression (using DESeq2 tool) between malignant versus non-malignant samples. A total of 9 malignant and 25 non-malignant samples (including 4 pathologically normal tissues) were used for this analysis. The counts were filtered with genes having >= 10 read counts in at least 25% of the samples. |Log2Fold Change|>1 and P value (Hochberg-Benjamini adjusted) <0.05 were used as cut offs.

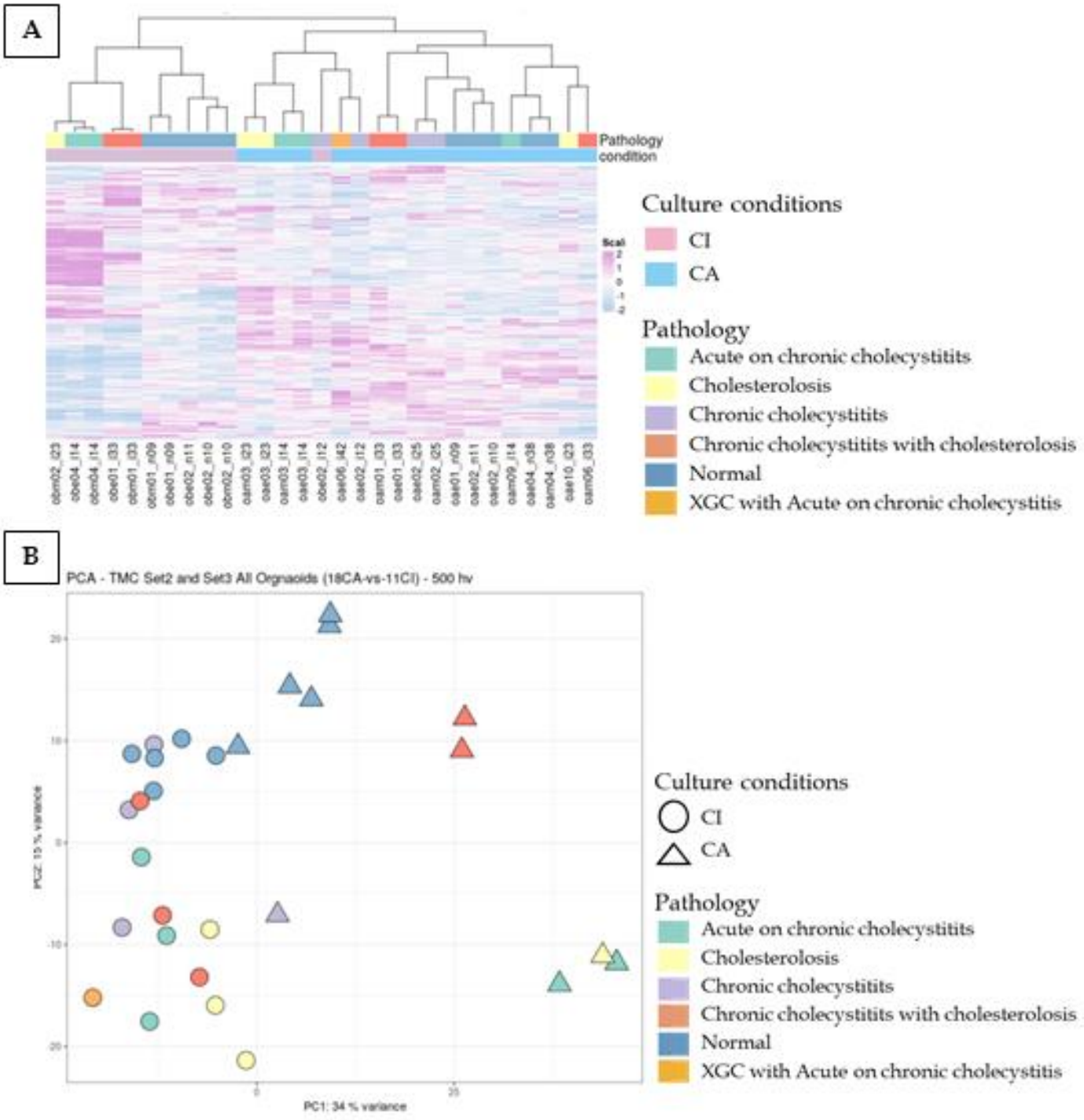

Figure 7. Transcriptomic analysis of the non-malignant PDGCOs primarily clustered based on the two culture conditions.

mRNA-seq analysis (Illumina TruSeq stranded mRNA-seq) using Nextseq 550 or NovaSeq 6000 (paired end 150bp or 100bp, respectively) was performed on RNA extracted from the non-malignant PDGCOs. (A) Unsupervised hierarchical clustering and (B) Principal component analysis showing primary clusters based on culture conditions - canonical WNT activated (CA) and canonical WNT inhibited (CI). Sub-clustering did not correlate with the pathological sub-varieties. Pathological sub-varieties are represented by different colors as indicated in the figure.

HQ - GQ > 30 and PASS For allele frequency <0.001

	ts_i42	ts_n38	ts_i23	ts_i14	ts_i25	ts_i33
obe04_i14	5.1	8.1	8.5	76.2	7.6	9.2
oae03_i14	6.2	8.5	9.4	77.2	7.6	10.6
obm04_i14	4.8	7.9	8.3	74.2	7.9	10.3
oam03_i14	6.5	8	10.2	80.1	9.2	12.3
oam09_i14	5.3	8.4	8.4	79	7.7	9.6
obe01_i33	4.4	7.8	8.6	10.4	8.2	76.7
oae01_i33	6.9	9.2	11.6	11.3	9.8	81.5
obm01_i33	6.2	8.3	10.4	9.5	5.4	84.2
oam01_i33	8.2	7.6	12.7	10.3	8.2	82.5
oam06_i33	5.7	7.6	9.1	9.9	6.5	77.6
oae10_i23	6.5	7.8	59.4	8.8	9.4	11.8
oae03_i23	6	8.4	70.7	10.1	10.1	11.1
obm02_i23	7.2	7.2	70.7	10.7	8.5	9.6
oam03_i23	8.1	9.6	71.8	9.6	11.7	12.9
oae04_n38	6.7	67.2	10.1	10.1	11.3	15.3
oam04_n38	9.2	74.9	11.2	10.4	10.1	13.5
oae02_i25	6.6	8.8	8.8	8.6	65.9	13.1
oam02_i25	6.4	8	8	8.4	72.5	10.7
oae06_i42	53.6	6.8	8.9	7.8	9.6	10.7

Figure 8. Single Nucleotide Polymorphism (SNP) analysis showed PDGCOs preserve the patient-specific SNP signatures of their parent tissues irrespective of the culture conditions, cellular dissociation methods or passage numbers.

Genome Analysis Toolkit 4 pipeline was used for SNP calling from mRNA-seq data. Similarity matrices showing shared SNPs between parent tissues (columns) and the developed PDGCO lines (rows). Tissue from each patient and the organoid lines derived from that particular tissue have been shown in the same colored boxed. The numbers in the matrix table indicates the percentage of shared SNPs. For nomenclature of tissue and organoids please see Table 3 below.

A. Tissue sample nomenclature:

Position from left	**Used code letter**	**Interpretation**
1 (type of sample)	t	Tissue
2 (method of tissue collection)	s	Surgical resection
	c	Core biopsy
3 (separator)	_	
4 (pathology)	i	Inflamed
	n	Normal
	m	Malignant
5 and 6	Double-digit numeral	Patient ID

B. Organoid sample nomenclature:

Position from left	**Used code letter**	**Interpretation**
1 (type of sample)	o	Organoid
2 (culture condition)	a	Grown in CA media
	b	Grown in CI media
3 (cellular dissociation method for organoid seeding)	e	Enzymatic dissociation
	m	Mechanical dissociation
4 and 5	Double-digit numeral	Passage number
6 (separator)	_	
7 (Pathology)	i	Inflamed
	n	Normal
	m	Malignant
8 and 9	Double-digit numeral	Patient ID

Table 3: Nomenclature of samples

the PDOs and their source tissues. This would also help to find potential targets for alternative therapeutic strategy for GBC. Finally, we are also establishing a well-annotated living organoid biobank of PDGCOs with different pathological varieties which would serve as a valuable research resource globally for further investigation in the field of gallbladder disease biology, biomarker study or drug discovery.

Future direction:

Our ultimate goal is to improve the outcome of the cancer patients. With that in mind, following are our plans of action going forward.

- We would complete the high throughput molecular analysis to assess to what extent the disease tissue genome, transcriptome or proteome are recapitulated in the derived organoids.
- Cryo-preservation technique will be optimized for the living PDGCOs and primary cells for (re)growing organoids at a future time point.
- We would set up a drug response profiling platform for the PDOs with the goal of providing personalized treatment to the patients.
- We want to expand the application of this PDO model development technology to other relevant solid cancers, e.g., breast cancer.

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Application of High-Throughput Workflows for Personalizing Therapies in ALL

In TTCRC, we have successfully established a Illumina-based workflow for transcriptome sequencing using patient samples which can be used to identify cryptic gene arrangements, mutations as well as high risk CNVs through one single assay. To examine the feasibility and utility of incorporating transcriptome sequencing in a clinical setting we need to consider two aspects of this application, one is the cost and another is the turnaround time (TAT), which play an important role in considering the utility of this approach in the clinic. Through ALL 2022 strategy in TTCRC, we are aiming to streamline this workflow for providing personalized therapy to the ALL patients in Tata Medical Center (TMC), Kolkata. Using the information from transcriptomic data, we have been able to identify patients with ABL-class fusions who can be provided with targeted therapy as well as patients eligible for augmented therapy/ Blinatumomab. Through this approach we are addressing additional research areas such as the pathways enriched in a subset of ALL patients who are represented with a persistent high MRD and not responding to the current treatment strategy for ALL and/or further characterizing the patients who have experienced early or very early relapse during the course of treatment. We are also collaborating with TCS bioinformatics team for analysing the CNV data from RNA-Seq.

In addition to finding out mutations and fusions in ALL, transcriptome sequencing has provided a fundamental support to the "correlative biology" projects executed by the other investigators in TTCRC such as ALL drug-response profiling (DRP) as well as studying and characterising a gall-bladder organoid model. Transcriptome sequencing was used in the molecular characterisation of *IKZF1* deleted patients in the cohort of BCP-ALL at TMC. We identified known targetable fusions as well as novel fusions in the patients. Additionally, we investigated the transcript variant profile of *IKZF1*, expressed in our patients (Figure 1). *IKZF1*-001 represents the full-length transcript, and is expressed in both wild-type and *IKZF1* deleted patients. In *IKZF1* deleted patients, this is expressed either by the normal allele or by the background normal cell population. As evident from Figure 1, *IKZF1*-202/IK6 (representing splicing or loss of ∆4-7) is not only significantly over-expressed in cells with an intragenic deletion (IGD) but also expressed in whole gene deletions (WGD) and wild-type (wt) at similar levels seen for *IKZF1*-001. I*KZF1*-007 and *IKZF1*-009, both representing loss and alternate splicing of ∆4-8 are also expressed at high levels by wt, IGD and WGD. While *IKZF1*-009 is non-coding, *IKZF1*-007 is a coding isoform. The expression level of the IKZF1 variants identified from RNA-Seq data have been validated by RT-PCR and fragment analysis on Tape Station. High expression of these two dominant negative variants (*IKZF1*-007 and *IKZF1*-202), in patients not carrying IKZF1 intragenic deletion, is suggestive of a putative role at the protein level but requires further proteomic validation.

In parallel to the conventional next-generation sequencing approach (Illumina), we have optimised and established the pipeline for whole Transcriptome sequencing 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 make a transition to Nanopore sequencing for real-time reporting of samples in a clinical setting. As first step, we have optimised the RNA-Seq workflow using few ALL cell-lines (SupB15, NALM-6 and REH), serving as reference positive control, and checked for their concordance with Illumina data using our in-house analysis pipeline. The schematic workflow alongside shows the steps involved in library preparation (Figure 2). Recently we have developed a research collaboration with St. Jude's Children's Research Hospital and University of North Carolina, Chapel Hill for genetic classification of ALL patients based on gene expression profiling from

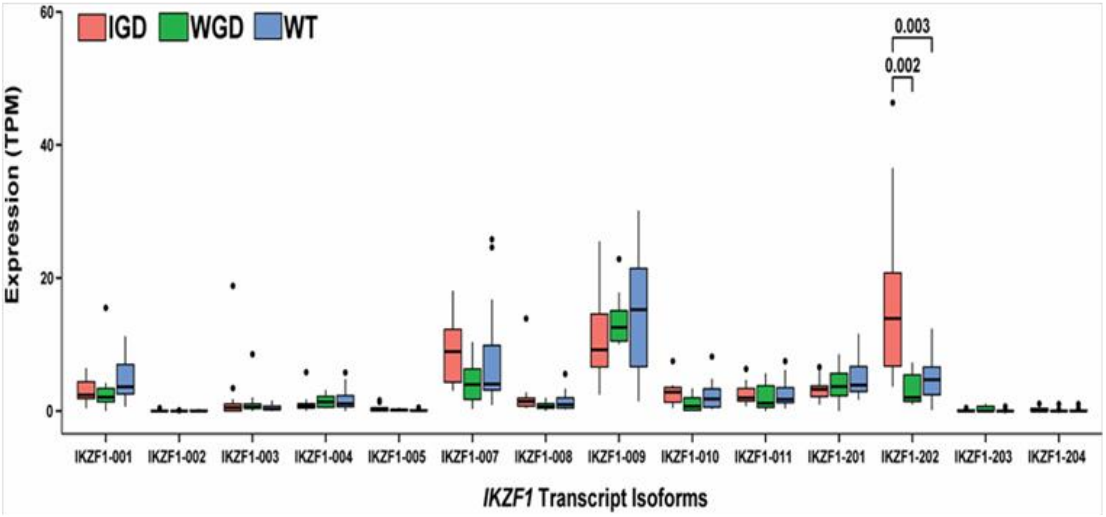

Figure 1. Relative abundance of IKZF1 splice variants in BCP-ALL patients from Transcript Variant Analysis

transcriptome sequencing data. As a part of this collaborative effort, we have designed a pilot study of 12 ALL patients (listed below) as a proof-of-principle experiment which were sequenced using the Nanopore and the analysis is currently ongoing.

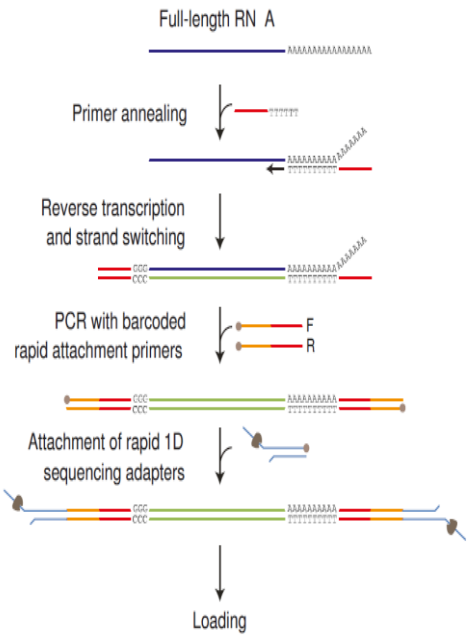

Figure 2. Library Preparation for cDNA Sequencing

In addition to RNA Sequencing workflow, we have also optimised candidate gene-specific amplicon sequencing using Nanopore for rapid mutation analysis. *TP53* mutations are enriched in ALL relapses and are independently predictive of poor response to therapy.

Sl No	Cytogenetics	A priori Genomic Information
1	B-Other	IKZF1-WT, PAX5-deletion, CDKN2A/2B-homozygous deletion
2	B-Other	IKZF1-whole gene-hetero-del, POR-hetero-deletion
3	B-Other	PAX5-AUTS2 fusion,IKZF1-whole gene-hetero-del, JAK2-hetero-del, CDKN2A-homo-deletion, CDKN2B-hetero-del, PAX5-hetero-partial-deletion
4	TCF3-PBX1	IKZFZ1-WT, JAK2-hetero-del, CDKN2A/2B-homo-deletion, PAX5-whole gene-hetero-del
5	T-ALL	ABL1-RANBP2 fusion (ABL1 Rearranged in 56% of cells by FISH)
6	B-Other	IKZF1-del2-7-hetero-del, ETV6-hetero-del, ZNF384-TAF15 fusion by FISH
7	B-Other	IKZF1-WT, CDKN2A-homo-del, CDKN2B-hetero-del, EBF1 gain
8	B-Other (Ph-like)	ZMIZ1-ABL1 fusion (ABL1 Rearranged in 84% of cells), IKZF1-WT, CDKN2A/2B-hetero-del, ETV6-partial-hetero-del
9	B-Other	IKZF1-WT, EBF1-gain-hetero-duplication, PAX5-homo-partial duplication, CDKN2A/2B hetero deletion
10	B-Other	PAX5-ETV6 fusion (ETV6 Break apart probe shows deletion of 5 prime end of ETV6 gene in 100% of cells), IKZF1-whole gene-hetero-del, JAK2-hetero-del, CDKN2A/2B-hetero-del, PAX5-hetero-partial del, ETV6-hetero-partial del
11	B-Other	IKZF1 del4-8-hetero-del, PAX5-hetero-partial del, CSF2R-hetero-del, IK3RA-hetero-del, P2RY8-hetero-del
12	B-other	Persistent positive MRD till end of consolidation phase

We developed a strategy for long-range PCR including the entire *TP53* gene, as shown in schematic figure alongside. (Figure 3). The amplicons generated are purified and librararies are prepared, followed by long-read sequencing (Figure 3). The gel image (Figure 4) shows amplification of specific 8kB fragment in NALM6 (*TP53* WT) and KOPN8 (*TP53* mut p.R248Q) cell-lines.

The data acquired in Minion is readily demultiplexed and basecalled by Guppy, which is integrated in MinKnow (operating interface for Nanopore). The basecalled reads are aligned using minimap2, against hg19. The resulting BAM files is sorted and fed to bcftools. Bcftools mpileup uses mapping scores to perform variant calling. The variants called are then annotated using ANNOVAR including various databases like dbSNP, COSMIC etc.

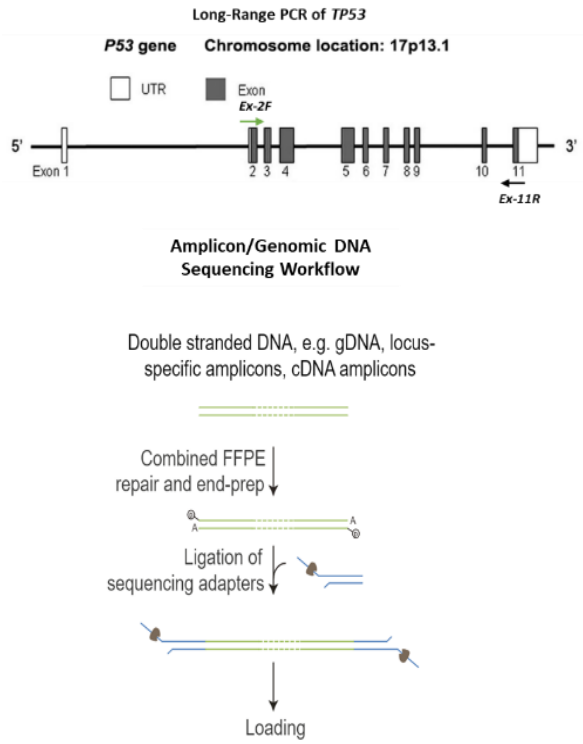

Figure 3. Amplicon Sequencing Workflow in Nanopore

We have also run two patient samples with known *TP53* mutations earlier identified in our targeted gene-panel sequencing using

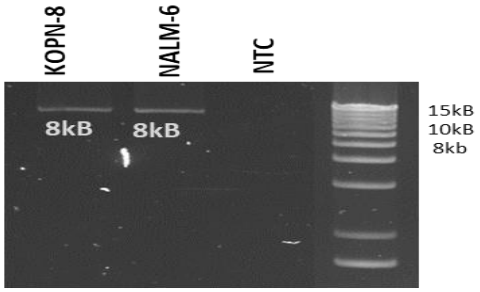

Figure 4. Gel showing amplification of 8KB fragment in cell lines

Illumina for validating the current approach.

The current analytical pipeline for identifying mutations from Nanopore sequencing data was able to successfully identify the known TP53 mutation in one patient sample.

In order to detect the limit of sensitivity of the current approach, we performed a limiting dilution assay with six different spike-in concentrations using KOPN8 and NALM6 cell lines at 50%, 40%, 30%, 20%, 10% and 5% variant allele frequencies. We have also included a paired frontline and relapse diagnosis patient sample for mutation analysis. No mutation was detected in frontline sample but relapse sample shows the presence of p.R248Q mutation at 83.7% VAF with the other allele deleted. We are working towards continuous refinement of the analytical pipeline for low-level detection of mutations.

Going forward, we are hoping to optimise workflows for comprehensive genomic characterisation of ALL patients involving whole genome sequencing, copy number analysis and methylation profiling.

Minimal Residual Disease Group

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Minimal Residual Disease Laboratory (MRD Lab) at TTCRC is focussed on tracking the therapy response of acute lymphoblastic leukaemia patients based on their personalised Ig/TR gene rearrangements.

Finally, 2022 – a year with no lockdowns and shutdowns! We started the year albeit with a few setbacks. Our dear colleague Debparna Saha found her next level career opportunity and though the MRD team shall miss her I am sure she will prosper in her new responsibility. Three members of the team went down with covid in the very first week of the year, fortunately they had mild illnesses and recovered quickly. Sreyasree and Atreyi (our intern) managed to escape the epidemic and manned the fort successfully ensuring MRD reporting went as planned.

As we went along, the year started turning eventful for the MRD team in a number of important ways. After a successful NGS pilot run (by EuroClonality method) at the end of 2021, the team was charged up to focus more on high-throughput technique. We realized that the prospective tracking of our ALL patients should move on in parallel. Therefore, this year we put more effort onto expediting the prospective rounds along with more emphasis on reducing one-target and non-informative reporting. To enhance our team strength while making up for the loss created by Debparna's exit, we recruited a research assistant in the MRD team by the end of the first quarter. Atreyi joined in this position as she comes equipped with all the skillsets she gathered while working with MRD team during her last internship. Thereafter, the team started working hard and successfully reduced each MRD round from 8 weeks to 6 weeks! CRU team was glad to find the timely support in the form of the prospective reporting done at a faster pace. In the meantime, we received samples (though after a lot of delay!) for the EuroMRD quality assessment (QA). While balancing between the QA (QA-40) and prospective rounds, Uzma and Anushka came up with great achievements - PhD offers from universities abroad. There was no time to fret over another two losses in manpower, and hunting for new members started soon, while wishing Uzma and Anushka good bye. Eventually, Aishwarya and Sukalpa joined the MRD team by August. The new members gelled quite well and this reflected in the performance of team when we started noticing further improvement in the reporting status and prospective documentation. Collated data of 2022 shows that the MRD Team had reported 90 follow-up (FU) timepoints from 45 frontline and relapse acute lymphoblastic leukemia (ALL) patients. As compared to previous data, this year we had only three non-informative patient (6.7%) along with 57.8% two-target reporting and 35.6% one-target reporting (Figure 1).

Apart from the prospective reporting, the team focused on next round of EuroMRD QA (QA 41) in late August parallelly with the new technique development to make the MRD workflow faster, better, and cheaper!

This year witnessed our ongoing collaboration attaining new heights. Our partners in this collaboration are University of Charité (Berlin) and DKMS LSL (Dresden), Germany. The aim of this collaboration is to establish the high throughput MRD tracking grounded on unique molecular identifier (UMI) based next generation sequencing (NGS) method. The workflow was initially established by DKMS LSL and we are implementing this here at TTCRC. As part of this collaboration, we began with sample curation (for which PCR-MRD data is existing) and study design. Till date we have successfully curated 39 patients and 137 FU timepoints altogether (Figure 2).

The execution of the strategic plan involves hands-on training and direct interaction among the collaborators. To facilitate this knowledge transfer, two members of the MRD group were selected to visit DKMS LSL and University of Charité (Germany). The purpose of the visit is twofold. First, the hands-on training would allow us to learn the UMI-NGS workflow; second, the in-person interaction would help us master the existing PCR-MRD workflow to finetune our current standard operating procedure (SOP). Pritha Dasgupta and Sayantani Mitra visited DKMS LSL and University of

Charité in November-end for a four weeks training. A successful UMI-NGS pilot run had been done by Pritha during the training time with two cell lines and six patient samples. Results of the cell lines (REH and NALM-6) successfully exhibit reproducibility in the data. Patient sample results demonstrate concordance with NGS-MRD data generated by Sayantani at University of Charité (Figure 3).

Finally, when we come to a year end, in retrospect, I feel proud of the achievements the **young** MRD team has been able to accomplish despite multiple adversities. It brings us great satisfaction to say that we qualified the EuroMRD QA rounds (QA 40 and QA 41) with flying colours. First pilot run on UMI-NGS was successfully done at DKMS LSL, Dresden. We thank Dr. Cornelia Eckert, University of Charité and the DKMS LSL team for their continuous input and support to the MRD Team at TTCRC. We look forward to a promising time ahead with hope and high spirit, particularly with a strong emphasis on implementing UMI-NGS MRD at TTCRC.

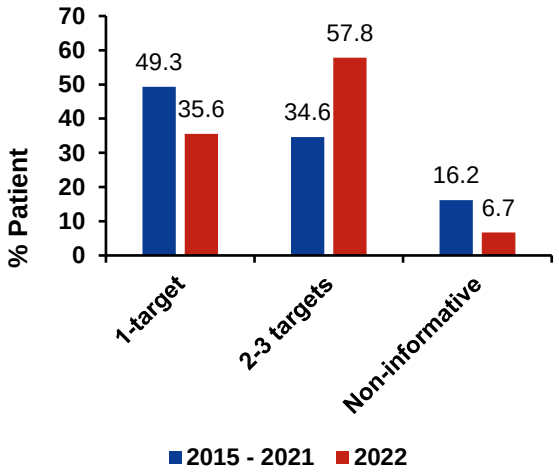
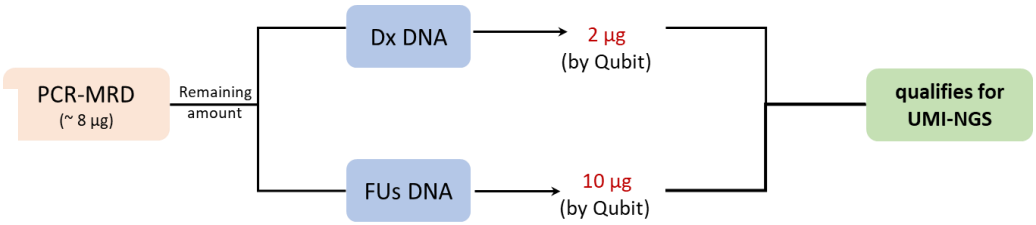


Figure 1. Comparative study of patient reporting (from 2015 till 2022). 43 patients are reported in 2022. As compared to 2015-2021 cohort, two targets reporting has increased from 34.6% to 57.8% with a decrease of one target reporting from 49.3% to 35.6% and non-informative from 16.2% to 6.7% in the year 2022.

2A)



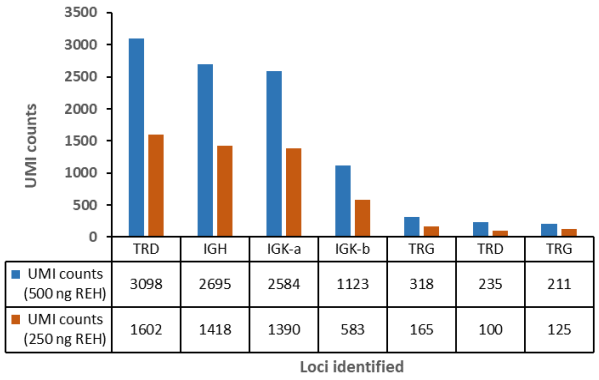
2B)

Status	No. of patients	Dx samples	FU1 (EOI)	FU2 (EOC)	FU3 (any other FUs)	Total samples
Projections	70	70	70	70	-	210
Available	39	39	39	29	30	137
Pending	31	31	31	31	-	73 (expected)

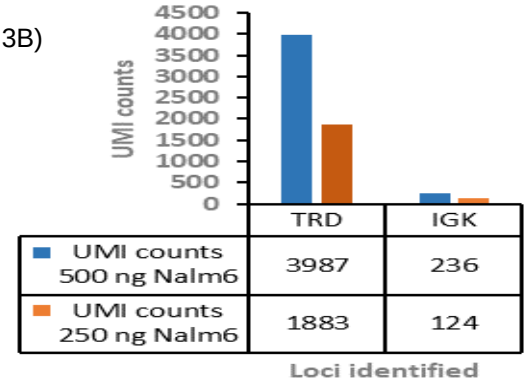
Available: already curated
Pending: PCR-MRD to be done

Figure 2: Patient samples selection for UMI-NGS based MRD. (A) Schematic representation of the samples qualified for UMI-NGS. Samples are qualified for UMI-NGS only if the remaining Dx DNA and FUs DNA amount is above 2µg and 10 µg respectively after PCR-MRD workflow. (B) UMI-NGS study is projected for 70 patients with a total of 210 FU samples (with existing PCR-MRD data) of which 137 FU samples have been curated from 39 patients. 73 more FU samples from at least 31 patients are to be curated. (UMI: unique molecular index, Dx: diagnostic, FUs: follow-up sample).

3A)



3B)



3C)

Patient	Sample	Target	Hybrid Approach	NGS MRD	UMI-NGS MRD
Patient 1	FU1	VH6D2JH6	1.20E-03	4.10E-04	7.77E-04
	FU2		4.00E-04	2.00E-04	1.35E-04
	FU1	VH6D5JH6	1.40E-03	3.40E-04	7.05E-04
	FU2		3.60E-04	1.80E-04	3.15E-04
	FU1	Vg9Jg1/2	--	7.20E-05	8.64E-05
	FU2		--	2.00E-05	3.00E-05
	FU1	Vd2Dd3	not identified	not identified	1.55E-03
	FU2				1.05E-04
Patient 2	FU1	Vd1Jd1/2	8.40E-05	1.30E-04	1.5E-03
	FU2		Negative	Negative	Negative
	FU1	Vb7Jb2.3	3.50E-04	Negative	Negative
	FU2		Negative	Negative	Negative
	FU1	Vg10Jg1/2	1.00E-04	5.90E-05	1.5E-05
	FU2		Negative	Negative	Negative
	FU1	Vg3Jg1/2	1.10E-03	2.30E-04	3.0E-04
	FU2		Negative	Negative	Negative

Concordant Discordant

Figure 3. UMI-NGS MRD detection for cell lines and patients. Graphical representations of UMI counts obtained from 500 ng and 250 ng of REH (A) and Nalm6 (B) for the targets identified by UMI-NGS method of MRD detection. (C) MRD detection by hybrid approach (screening by NGS and quantification by qPCR), NGS and UMI-NGS methods showing concordant and discordant quantification of follow-up (FU1 and FU2) time points from two different patients.

p53 Group

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Title: Investigation of aberrant TP53 regulation in Acute Lymphoblastic Leukaemia (ALL)

As Douglas Adams wrote, “I may not have gone where I intended to go, but I think I have ended up where I intended to be.” – the work p53 Leukaemia Cell Biology team did for the last 5 years may not have been exactly what the India Alliance project proposal stated, but we did end on a note very similar to what we hoped for back in 2016; to understand how despite a functional p53, some leukaemic blasts survive by evading chemotherapy-induced stress.

Analyses of the genomic landscape of matched diagnostic and relapsed acute lymphoblastic leukaemia (ALL) reveal that though 5-6 pathways are commonly altered in resistant clones, no single gene can explain multidrug resistance [1,2]. These studies suggest that very early relapses (on treatment) arise from pre-existing drug-resistant clones early and late relapses (off-treatment) possibly arise from persister clones that survive initial therapy and later expand to give rise to multidrug-resistant clones. We have previously demonstrated that stress adaptation mediated by the bone marrow microenvironment supports survival of clones during ALL therapy [3]. Two master regulators of cellular stress adaptation are p53 [4] and MYC [5]. While alterations in *MYC* are rare in paediatric ALL, relapse-specific alterations that predict non-response to ALL treatment [6] are enriched in *TP53* [1,2]. Drugs commonly used in ALL treatment induce apoptosis by generating genotoxic stress or by altering mitochondrial metabolism, implicating a central role for wild-type functional p53 in eradicating ALL blasts during treatment. Presence of residual cells and eventual relapse on- or off-treatment, despite wild-type p53 in >98% cases of childhood ALL at initial presentation, suggest a broader mechanism involving subversion of p53-dependent signalling pathways is at play. We hypothesise that wild-type p53 induces DNA damage, cell cycle arrest and apoptosis in BCP-ALL cells under conditions of cytotoxic stress, but some cells are able to escape via mitochondria-regulated stress adaptation.

Aims to be achieved:

Our aim was to investigate the role of aberrant signalling pathways, regulated by aberrant *TP53* downstream targets, involved in apoptosis, redox adaptation and metabolism that occur as a result of stress adaptation in childhood ALL. Our hypothesis is that this protects ALL cells from cytotoxicity. Briefly this can be broken down into the following:

1. What are the *TP53*mut downstream targets in ALL cells?
2. What are the main stress and metabolic changes that result from these mutations?
3. Are these changes also seen in *TP53*wt early relapses?
4. What strategies can be used to target the processes involved in drug resistance?

From 2018-2021, we focused on:

(a) Establishing and characterising TP53 knockout isogenic BCP-ALL cell lines.

(b) Loss of wild-type p53 induced resistance to topoisomerase II inhibitors in BCP-ALL cells.

In 2022, we aimed to understand:

(a) How loss of wild-type p53 induced resistance to topoisomerase II inhibitor-mediated DNA damage in BCP-ALL cells.

(b) Whether loss of wild-type p53 induced resistance to topoisomerase II inhibitors via mitochondria-mediated stress adaptation in BCP-ALL cells.

Loss of wild-type p53 induced resistance to topoisomerase II inhibitor-mediated DNA damage in BCP-ALL cells.

A recent study identified a transcription-independent role for p53 in the DNA damage response cascade, where loss of p53 reduced phosphorylation of H_2AX in cells with DNA damage [7]. We next examined whether the p53 status determined DNA damage recognition and

repair in ALL cells treated with topoisomerase II inhibitors. When double stranded (DS) DNA breaks occur, the histone H_2AX is rapidly phosphorylated (within 20 seconds) and initiates DNA repair. Accumulation of phosphorylated histone variant H_2AX, referred to as pH_2AX (Ser139), can be detected by immunoblotting, flow cytometry or microscopy [8] and is widely used as surrogate marker to detect and quantify dsDNA breaks.

Topoisomerase II inhibitors induce DS DNA breaks (DSB). Immunoblotting-based detection of pH_2AX (Ser139) suggested a time-dependent increase in DSBs in NALM-6 p53WT cells treated with topoisomerase II inhibitors (**Figure 1A**). NALM-6 p53KO cells demonstrated no appreciable upregulation of pH_2AX

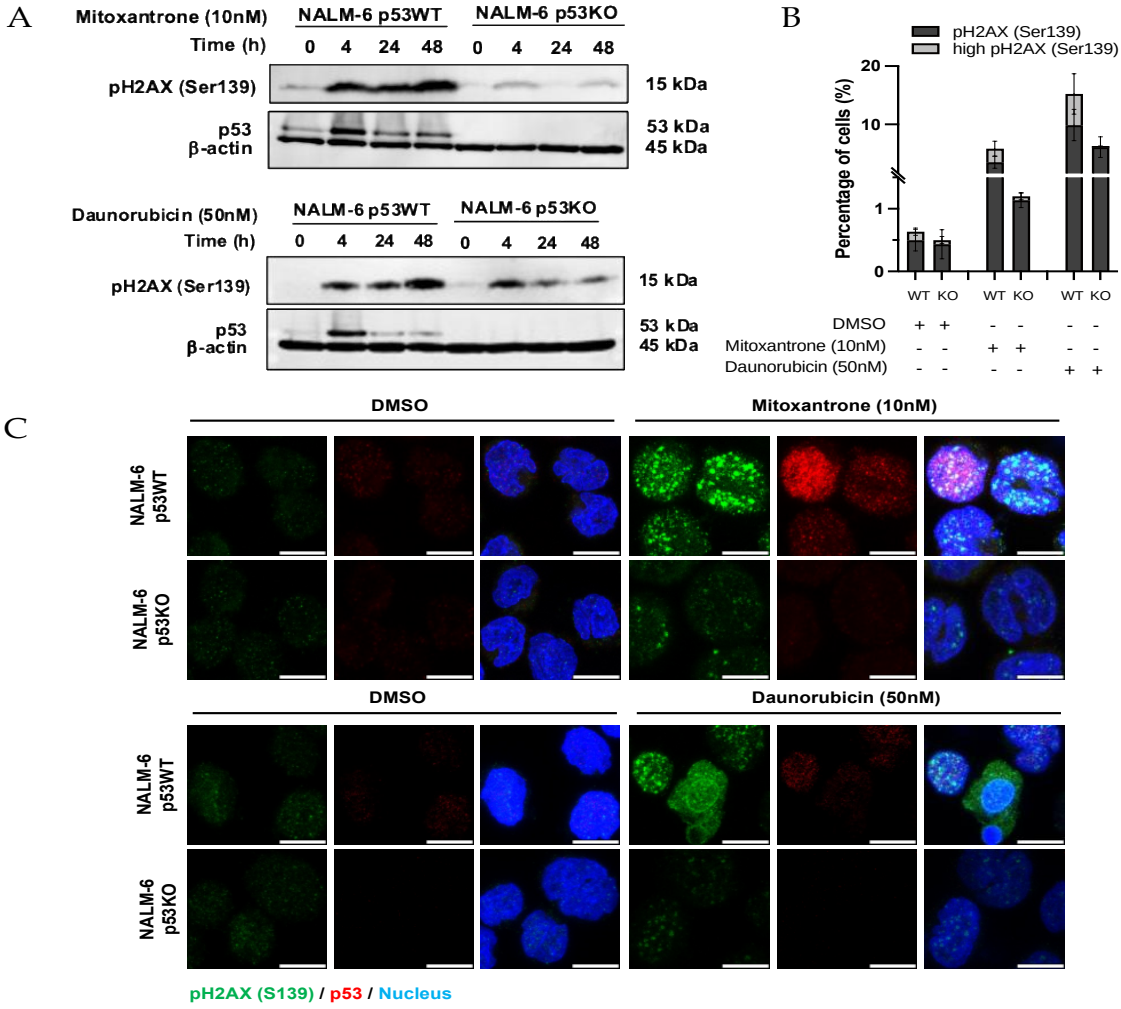


Figure 1. Loss of wild-type p53 dampened recognition of DNA damage in stressed BCP-ALL cells. A, Treatment with topoisomerase II inhibitors, mitoxantrone and daunorubicin, increased p53 in a time-dependent manner in NALM-6 p53WT cells, with expression peaking at 4h for p53. Drug-treated NALM-6 p53KO cells did not express p53. pH_2AX (Ser139) expression peaked at 48h post-drug treatment in NALM-6 p53WT cells, whereas expression of this surrogate marker of double-strand breaks showed no appreciable time-dependent difference in drug-treated NALM-6 p53KO cells. **B**, Increased pH_2AX (Ser139) foci formation in drug-treated NALM-6 p53WT versus isogenic p53KO cells after 4h. **C**, pH_2AX (Ser139) foci formation and p53 expression in DMSO or drug-treated NALM-6 p53WT confirmed diffused expression of pH_2AX, whereas small sized foci were observed in drug-treated isogenic p53KO cells. Cells were treated with either DMSO or topoisomerase II inhibitors for 48h. Scale bar = 10□M.

(Ser139) or p53 expression after mitoxantrone treatment. Daunorubicin-treated NALM-6 p53KO cells demonstrated an increase in pH_2AX (Ser139) expression comparable to that of p53WT cells after 4h of treatment (**Figure 1A**). This differential expression at 4h post treatment was further investigated by flow cytometric assessment of absolute percentage of cells expressing pH_2AX (Ser139) foci. Flow cytometry, which is more sensitive than immunoblotting, identified a population of drug-treated NALM-6 p53WT cells that expressed higher levels of pH_2AX (Ser139) fluorescence intensity (**Figure 1B**). Such a population was not observed within the drug-treated NALM-6 p53KO cells. Additionally, immunoblotting revealed peak expression of pH_2AX (Ser139) around 48h post drug treatment in NALM-6 p53WT cells, but not in drug-treated p53KO cells (**Figure 1A**). Assessment of pH_2AX (Ser139) foci formation by immunofluorescence at 48h post topoisomerase II inhibitor treatment suggested formation of diffused halos of pH_2AX (Ser139) foci in drug-treated NALM-6 p53WT cells (**Figure 1C**), suggesting that the level of DSBs had exceeded the cellular capacity to repair [8].

Overall our data suggests that topoisomerase II inhibition induces DSBs irrespective of p53 status. In p53WT cells, the sustained accumulation of pH_2AX (Ser139) could be a result of p53 recruitment and localisation to DNA damage sites [7].

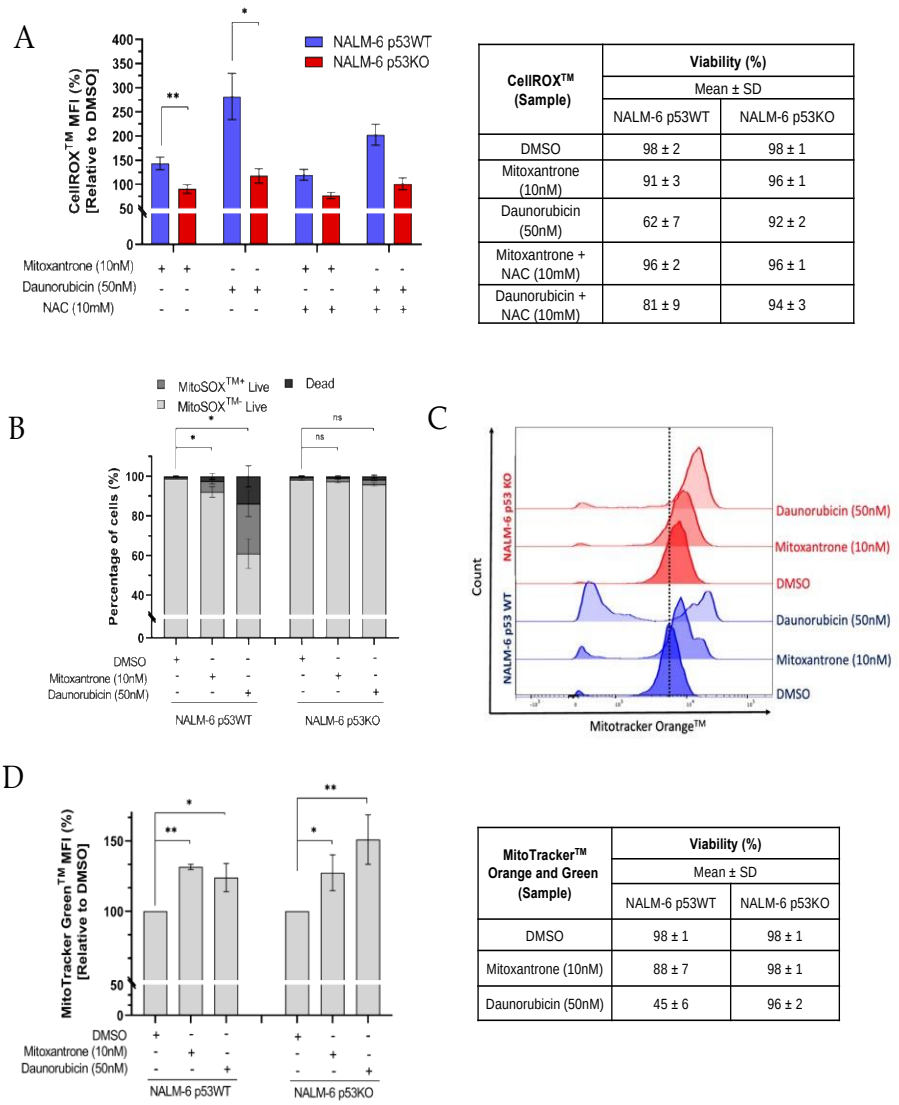
Loss of wild-type p53 induced resistance to topoisomerase II inhibitors via mitochondria-mediated stress adaptation in BCP-ALL cells.

We noted a difference in pH_2AX (Ser139) expression in cells exposed to mitoxantrone and daunorubicin. Although, drug-treated NALM-6 p53KO cells lacked high pH_2AX (Ser139) expression, overall levels of pH2AX (Ser139) were comparable in NALM-6 p53WT and isogenic p53KO cells after 4h of daunorubicin treatment (**Figure 1A**). Daunorubicin induces an oxidative stress implicated as a critical trigger for cell cycle arrest and eventual apoptosis[9,10]. Mitoxantrone does not readily generate free radical species[9].

Global reactive oxygen species (ROS) levels were assessed using CellROX™ Deep Red reagent by flow cytometry and cells were counterstained with a viability dye (eFluor 450 fixable live dead dye). Daunorubicin induced ROS accumulation to a higher extent than mitoxantrone after 24h. ROS levels were noticeably higher in viable NALM-6 p53WT cells than in viable isogenic p53KO cells after 24h of daunorubicin treatment (**Figure 2A**). NALM-6 p53WT cells treated with mitoxantrone demonstrated a mild increase in ROS levels when compared to mitoxantrone-treated isogenic p53KO cells after 24h (**Figure 2A**). Scavenging cellular ROS using N-acetylcysteine (NAC; 10mM) pre-treatment for 2h followed by topoisomerase II inhibitors reduced ROS levels, improving cell viability of the drug-treated NALM-6 p53WT cells. Cell viability remained unchanged in isogenic p53KO cells without or with topoisomerase II inhibitor treatment, though a mild decrease in ROS levels were noted in NAC-pre-treated p53KO cells subjected to topoisomerase II inhibition (**Figure 2A**).

DNA damage is not the only source of ROS production in stressed cells with mitochondria as the main site of ROS generation. MitoSOX™ Red Mitochondrial Superoxide indicator by flow cytometry, suggested an increase in the percentage of NALM-6 p53WT cells with elevated levels of mitochondrial ROS post treatment with topoisomerase II inhibitor. A minor increase in the percentage of p53KO cells with elevated mitochondrial ROS levels was observed post treatment with topoisomerase II inhibitors (**Figure 2B**).

We and others have demonstrated a role for mitochondrial adaptation in cell survival during leukaemia therapy [3,11-13]. Additionally, Complex I of the electron transport chain (ETC) has been identified as the mitochondrial site for anthracycline reduction and for generation of hydroxyl radical by daunorubicin [14], but to a much lesser extent by mitoxantrone [15]. Higher levels of ROS accumulation in drug-treated NALM-6 p53WT cells might be due to



CellROX™ (Sample)	Viability (%)	
	Mean ± SD	
	NALM-6 p53WT	NALM-6 p53KO
DMSO	98 ± 2	98 ± 1
Mitoxantrone (10nM)	91 ± 3	96 ± 1
Daunorubicin (50nM)	62 ± 7	92 ± 2
Mitoxantrone + NAC (10mM)	96 ± 2	96 ± 1
Daunorubicin + NAC (10mM)	81 ± 9	94 ± 3

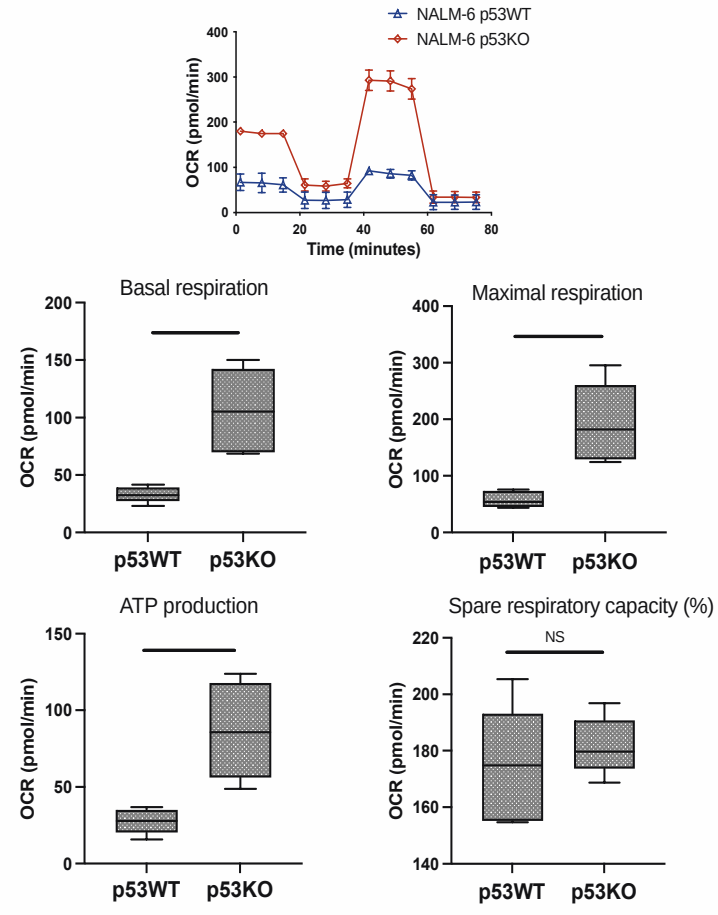
MitoTracker™ Orange and Green (Sample)	Viability (%)	
	Mean ± SD	
	NALM-6 p53WT	NALM-6 p53KO
DMSO	98 ± 1	98 ± 1
Mitoxantrone (10nM)	88 ± 7	98 ± 1
Daunorubicin (50nM)	45 ± 6	96 ± 2

Figure 2. Mitochondria-mediated stress adaptation resulted in chemoresistance of BCP-ALL cells lacking *TP53*. A, Cellular ROS levels were enhanced in drug-treated NALM-6 p53WT cells that remained viable post treatment. Such a phenotype was not observed in drug-treated, but viable, NALM-6 p53KO cells. NAC pre-treatment for 2h improved cell viability in drug-treated NALM-6 p53WT cells. No appreciable difference was observed in NAC-pre-treated NALM-6 p53KO cells. The table on the right highlights average cell viability without or with 24h drug treatment for n=3 experiments. **B**, Percentage of NALM-6 p53WT cells with high mitochondrial superoxide levels increased post drug-treatment for 24h, but no such increase in mitochondrial superoxide levels was observed in drug-treated NALM-6 p53KO cells. Percentage of dead NALM-6 p53WT cells post treatment was higher than in drug-treated isogenic p53KO cells. **C**, A shift in MitoTracker Orange™ fluorescence intensity suggested increased mitochondrial activity in both NALM-6 p53WT and isogenic p53KO cells that remained viable post 24h drug-treatment. **D**, Increase in MitoTracker Green™ fluorescence intensity suggested increased mitochondrial mass in both NALM-6 p53WT and isogenic p53KO cells that were mitochondrially-active post 24h drug-treatment. Image is representative of n=3 experiments.

increased mitochondrial activity in these cells [14,15]. Contrary to expectation, oxygen consumption rate (OCR), used as a surrogate marker for mitochondrial activity [16], was higher in NALM-6 p53KO cells under steady state, with a higher ATP production, when compared to unstressed isogenic p53WT cells (**Supplementary Figure 1**). Increased mitochondrial activity in steady state NALM-6 p53KO cells when compared to steady state isogenic p53WT cells was confirmed using Mitotracker Orange™ dye that specifically assesses active mitochondria in target cells (**Figure 2C**). Cells stressed with topoisomerase II inhibitor exhibited higher mitochondrial

activity irrespective of *TP53* status (**Figure 2C**). Non-viable NALM-6 p53WT cells exhibited complete loss of mitochondrial activity post drug-treatment, which was not observed in drug-treated isogenic p53KO cells. To determine whether increased mitochondrial activity post drug treatment was due to changes in mitochondrial biogenesis, NALM-6 p53WT and isogenic p53KO cells were co-stained with Mitotracker Orange™ and Mitotracker Green™ dyes. Mitotracker Green™ dye allows for the assessment of mitochondrial mass/content in live cells. Treatment with topoisomerase II inhibitors for 24h increased mitochondrial mass in both p53WT and p53KO cells that remained mitochondrially-active post treatment (**Figure 2D**). Taken together, the data suggests that while NALM-6 p53WT cells demonstrate sensitivity to topoisomerase II inhibitors by upregulating ROS production, mitochondrial activity and mitochondrial mass, drug-treated isogenic p53KO cells adapt to increased mitochondrial activity and mass without a correlative increase in ROS production.

Production of antioxidants is regulated by p53. With physiological stress, p53 has an anti-oxidant effect, however with cytotoxic stress, p53 has a pro-oxidant effect along with expression of pro-apoptotic genes. This is a potential explanation for cell death in drug-treated p53WT cells. Isogenic p53KO cells remained viable despite drug-treatment by maintaining low levels of overall ROS. This suggests that despite extensive DSBs, in the absence of p53, DNA repair continues supported by increased metabolism without the p53-regulated switch from anti to pro-oxidant [17]. This is in line with existing knowledge regarding a central role for p53 activation in induction of oxidative stress, degradation of mitochondrial components, and eventual apoptosis [18]. Further experiments will



Supplementary Figure 1. Loss of wild-type p53 enhanced mitochondrial activity in BCP-ALL cells**.** Steady state mitochondrial respiration indicated significantly enhanced maximal and basal respiration, and ATP production in NALM-6 p53KO cells when compared to the isogenic p53WT

validate the role of the antioxidant systems in drug-treated leukaemic cells.

Moving Forward

What strategies can be used to target the processes involved in drug resistance?

Our preliminary observations indicate a potential commonality between cancer cells lacking functional wild-type p53 and cancer cells with dampened activation of p53-dependent pathways in a wild-type p53 background – mitochondria-mediated stress adaptation under conditions of DNA damage. Mitochondrial vulnerability has been targeted to overcome challenges of drug resistance in multiple cancers. Perturbing mitochondrial ROS can be detrimental or beneficial for cancer cells. Our preliminary data suggests that a "goldilocks zone" exists for mitochondrial activity. One mechanism by which cancer cells undergo pre-programming for chemoresistance might be by mitochondrial rewiring due to genetic/epigenetic or micro environmental changes. But enhanced mitochondrial activity requires cellular controls, for instance an active antioxidant system, such that the resultant increase in mitochondrial superoxide is not lethal to these cells. This is plausibly how unstressed leukaemic cells lacking p53 maintain high levels of oxidative phosphorylation that remains almost unchanged despite conditions of DNA damage. Leukaemic cells with intact p53 are primarily glycolytic under steady state, but demonstrate enhanced mitochondrial activity when DNA is damaged. These stressed cells exhibit a much higher level of mitochondrial superoxide suggesting lack of parallel activation of the antioxidant system, eventually resulting in cell death.

In line with our previous observation [3], we hypothesise that mitochondrial adaptation allows residual cancer cells to evade cytotoxic stress under conditions of loss of wild-type *TP53* at the gene level or aberrant activation of p53 direct targets and p53-dependent pathways. Moving forward, we would target mitochondrial vulnerability in such cells and sensitize them to conventional therapeutic strategies. Using a high-throughput screening platform, we have identified drugs that target mitochondrial activity (such as venetoclax, IACS-010759) that render cells lacking p53 susceptible to conventional chemotherapeutics.

Our data suggests that p53 is activated early in DSBs and is physically recruited to DSB sites. In collaboration with Dr Sagar Sengupta (National Institute of Immunology) we are also investigating at what stage of the DSB repair cascade is p53 recruited.

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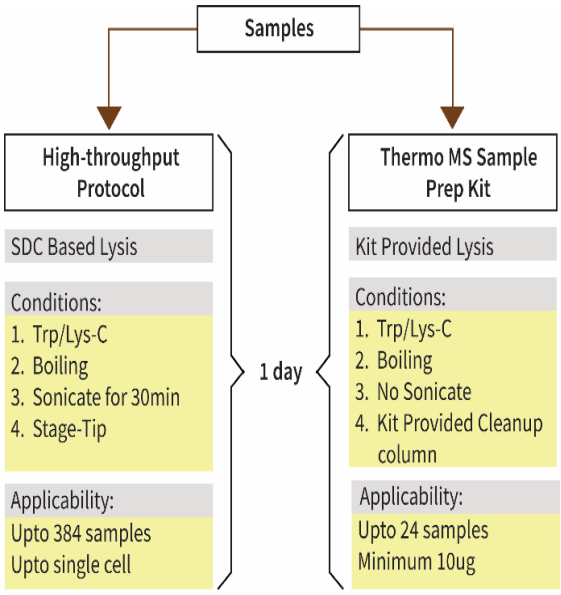
Currently, our facility is equipped with TripleTOF 6600 mass spectrometer (AB Sciex), connected to a nano-LC system (Eksigent) coupled with a nanoelectrospray ion source for bottom-up MS-based proteomics.

1. High Throughput MS Sample Prep Setup

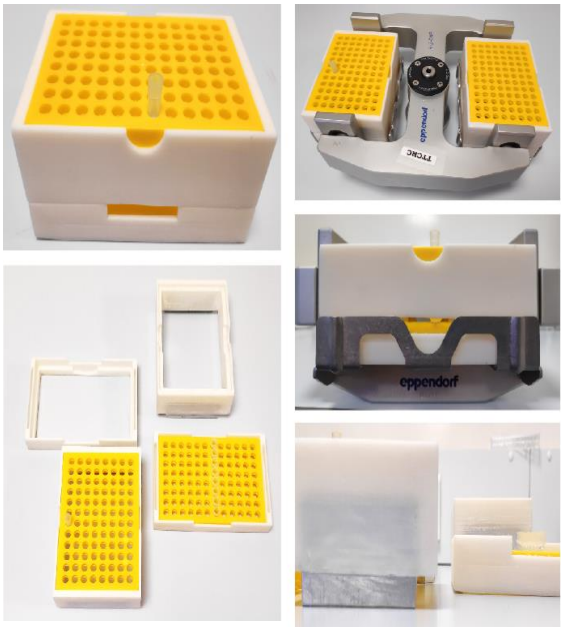
Our rigorous efforts have allowed the proteomics unit to overcome the several hurdles and limitations faced concerning the sample preparation workflow and instrument optimization.

Sample preparation in MS-based proteomics is a very important part of the workflow. It determines the overall sensitivity, reproducibility, and robustness of the entire proteomic analysis. In particular, this is critical for complex sample mixtures from a low sample input amount. The process consists of a multistep procedure that begins with the extraction and solubilization of proteins followed by denaturation, reduction, and alkylation of cysteines, and protease enzymatic digestion. The generated peptide mixtures from protease digestion need to be desalted and cleaned up for LC-MS/MS. Starting up with the sample preparation workflow, we moved away from using SDS and TEAB as cell lysis buffers, to using sodium deoxycholate (SDC), an acid-labile surfactant for lossless sample preparation with the highest efficiency in the extraction of proteins. SDC-based buffer allows complete inactivation of endogenous protease and kinases, maintains pH in the buffer for efficient Trypsin enzymatic action, and as well as serves as a better detergent for extracting the membrane-bound proteins which are often difficult to extract (1-2). This move also impacted the life of the column, by reducing the incidences of increase in the back pressure due to clogging. In this new HT protocol, reduction and alkylation of the proteins are done during lysis. Samples are boiled instead of allowing them to remain in cold, to better denature the proteins. Further sonication of the samples drastically improved the protein recovery from the cells in particular the



nucleosome bound. This aids in the action of Trypsin and Lys-C enzymes for tryptic peptide generation, which is crucial for peptide ionization and MS data processing.

The next development in the sample preparation and clean-up is making the procedure high throughput for parallel processing. We currently aim to establish 186 sample preparation systems per day, and this could be scaled to 384 samples per day. For this, we are in the process of preparing the Stage Tips (3) which require multiple small components to all be in place. The tips are prepared using the CDS analytical - Empore SDB-RPS discs. Then they are placed in the 3D



printed tip and tube holder for the samples to spin down through the packed bed of the column and collect into the tube placed at the bottom (Figure 2). We require fabricating a syringe plunger to load the SDB-RPS discs into the tips, which is the only remaining part of the stage tip-sample purification workflow.

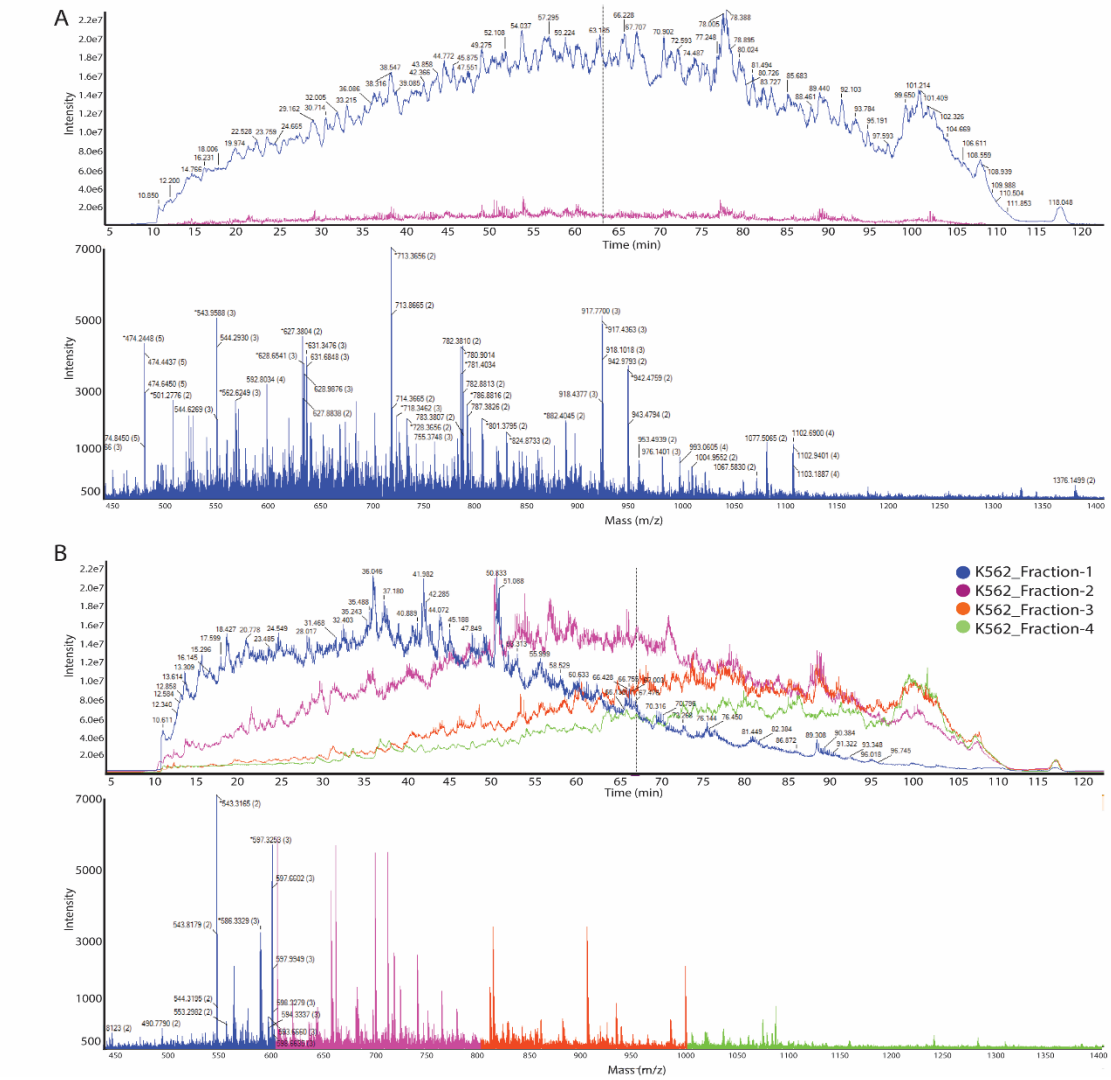
While we were developing and implementing the HT protocol, we also came across the EasyPep MS Sample Prep Kit from ThermoFisher Scientific to bridge the gap until the HT protocol is fully established. The kit temporarily solved our issues by addressing the bottleneck we faced with the HT Protocol, of not having a working sample clean-up platform. The kit came with a simpler and faster lysis method and solution, alkylating and reducing buffers, a sample clean-up resin bed, and an elution buffer, along with Trypsin/Lys-C enzyme mix.

The sample prep protocol is merely 4-5 hours long and it mostly gives reproducible results. The kit comes with a sample range of 1x106 to 5x106 cells, with an upper limit of 100ug protein load per column for purification.

The only downside to using this kit is the cost per sample, which is around Rs. 2500.When it comes to the number of proteins the kit can isolate from a particular number of cells, it stands in the range of 1.5-2.5ug/ul proteins. We hope that once the HT protocol is fully established and standardized, we would surpass these numbers with ease, that too at nearly 10th the cost of the commercial protocols.

Instrument Optimization

Moving ahead from last year's data acquisition methods, we attempted to enhance the depth of acquisition at the



DDA mode. For this, we designed 4 Gas-Fractionated windows for covering the full mass range (4). The full mass range of acquisition is set at 400-1500Da, where we segregated the 4 gas-fractions as FR1(400-600Da), FR2(600-800Da), FR3(800-1000Da), and FR4(1000-1500Da). This improves the quantity of data acquired in each fraction, and when these four fractions are combined, we end up covering the entire mass range, with more data points being contributed by each fraction. On average around 800-1000 more proteins are identified upon performing the gas fractionation, when compared to a single full mass range scan.

Constant monitoring of the tuning and calibration parameters and updating the acquisition methods with the current instrument condition make sure that we get the most accurate and precise data possible. We are expanding our possibilities by exploring newer column architectures to make sure we get the most out of our samples. By performing regular maintenance and checks on both LC and MS, we make sure our data is proofed.

2. The computational pipelines that complement the high-throughput analysis

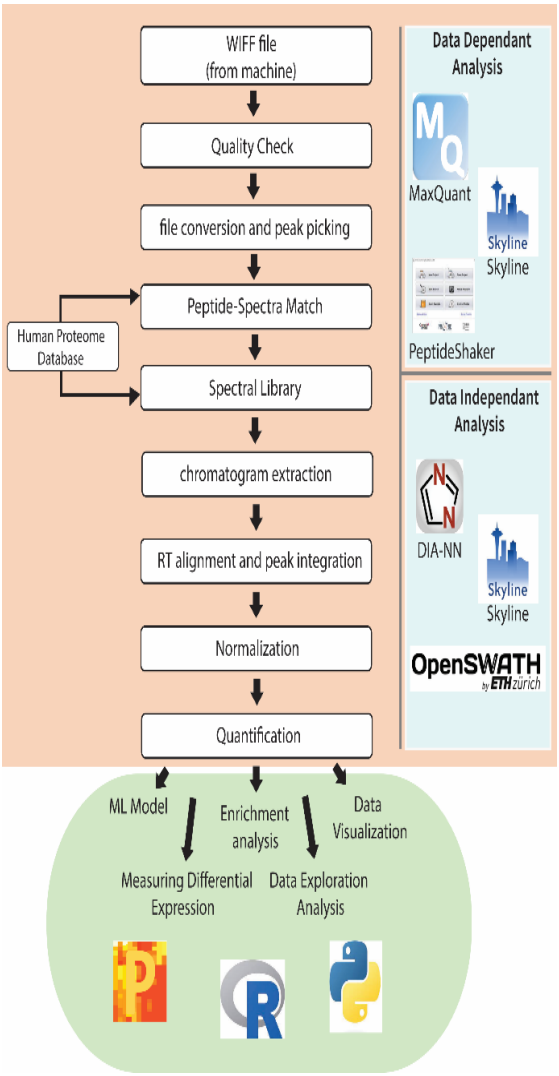
The central dogma of biology describes the flow of information through the transcription of DNA into RNA and then, after processing the RNA into mRNA, the translation of mRNA into protein. These proteins dictate cellular structure and activity, provide the mechanisms for signaling between cells and tissues, and catalyze chemical reactions that support metabolism. As we develop our proteomic facility to answer questions in context to our disease type, the computational solutions needed to emerge to complete the bottom-up proteomics analysis in compliance with global standards as well researched and explicit. We are developing pipelines to analyze two kinds of data i.e. Data Dependent Acquisition (DDA) and Data Independent Acquisition (DIA).

We have developed a pipeline around Skyline for DDA-based data analysis (5). The data files are received in our server as WIFF files which are converted to MGF, an open-source format that facilitates us to perform peptide search in PeptideShaker (6). This is a java based tool that can perform peptide spectral match using all algorithms available in the community and combine the result at 1% FDR. We have standardized XTandem, Comet, and MSGF+ to perform peptide spectral match and combine the result at 1% FDR (7-9). This search result is served to Skyline, which uses the results to build a spectral library and next takes in the chromatograms. The software facilitates a rich graphical interface that can be used to control features like inter-sample peptide retention time normalization, peak integration, peak detection at isolation scheme, etc. The result can be exported as a profile format or as a result of group-wise comparison wherein the normalized peptide quantification is analyzed using limma based pipeline (10).

For DIA-based analysis, we employ DIA-NN software suit (11). DIA-NN is java based application that uses deep neural networks and signals correction strategies for the processing of data-independent acquisition (DIA) proteomics experiments. DIA-NN takes in the DIA file and then works by extracting the chromatograms followed by scoring and choosing the best peaks. These interfering peaks are removed and the best-scoring peaks are subjected to an ensemble of DNNs that calculates the q-value. These best peaks are used for peptide spectral match thereby enabling protein discovery.

Both the Skyline and DIA-NN-based pipelines export results that are analyzed downstream using R and Bioconductor packages. We have standardized the use of Protti, an R package for performing differential protein expression, and then use ggplots to visualize the results (12). Going forward we are awaiting the release of MaxQuant which can analyze WIFF files from Sciex systems (13). The

entire DDA analysis will be further standardized on MaxQuant and the results shall be analyzed downstream using Perseus while for the DIA analysis the plans are to base on DIA-NN. The analysis team has helped in analyzing data for 3 projects viz Asparginase



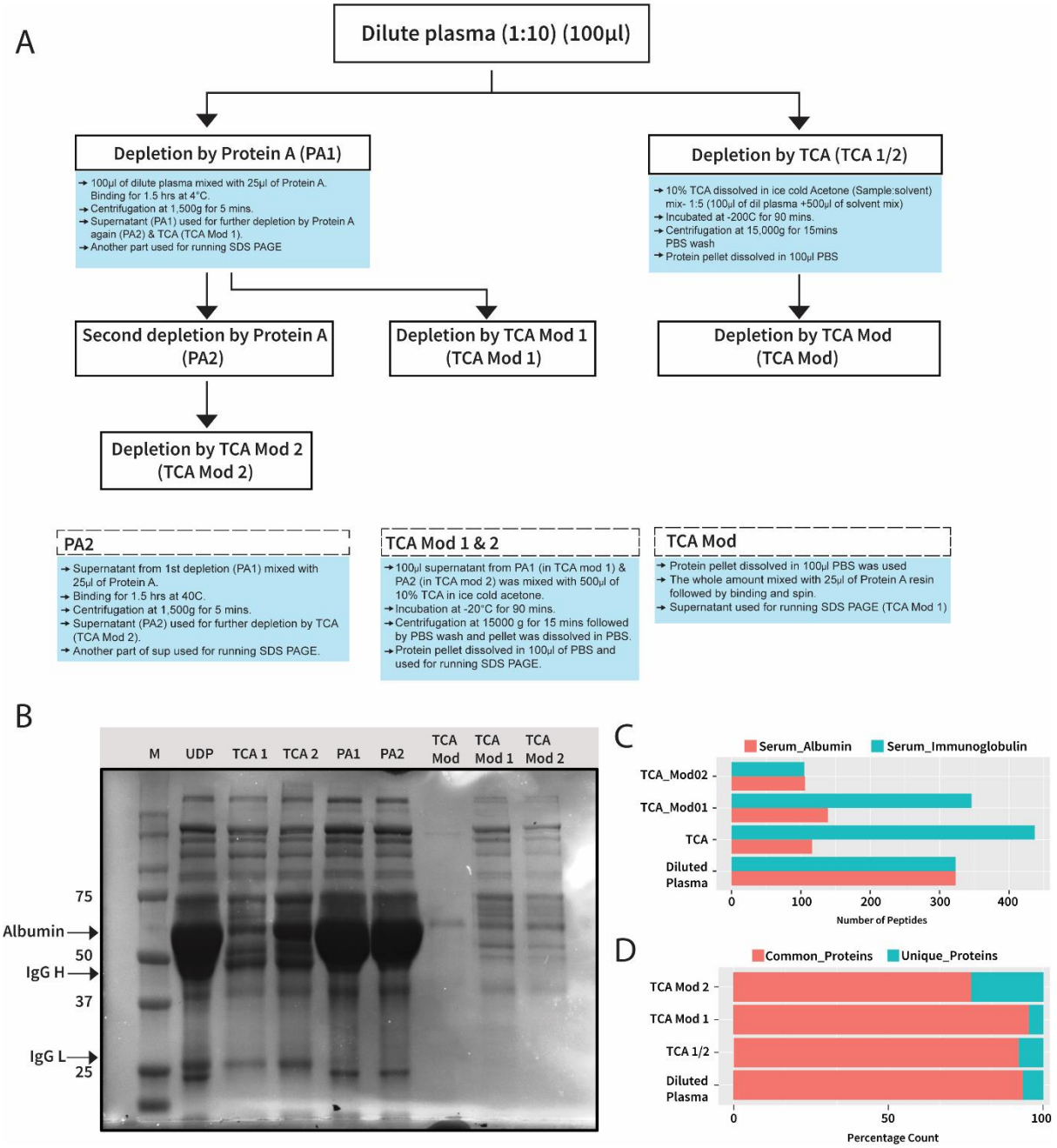
detection using plasma samples, validating chemo-resistance in NALM6 p53-KO models proteome, and Drug Response Profiling in NALM6 Cell Line. The team is in efforts to automate basic analysis steps like machine status check using K562 cell lysate, sample QC, etc. through automation scripts and then check the visualization in R shiny app.

3. Plasma Proteomics and Biomarker discovery

Human plasma/serum is a rich and attractive source of biochemical molecules indicative of the physiological status of an individual. For instance, serum-based hormones, enzymes, etc provide a plethora of information about the normal state as well as the disease-like cancer-induced altered state of a patient (14-15). Thus, it has a tremendous potential to provide a platform for biomarker discovery. Mass spectrometry (MS) based label-free approach for the detection of the biomaskers in serum has been widely used or its robustness and extensive proteome coverage (16). It has recently developed as a powerful tool for high throughput screening and unbiased detection of potential biomarkers underpinning the diseased state of the patient. Regardless of the wide utility of the technique in biomarker discovery, the protein profiling of especially low abundant proteins is often masked due to the presence of some highly abundant proteins like albumin (~50-70%), immunoglobulins (10-25%), 1-antitrypsin, transferrin, and haptoglobins, etc (17-18).

A large number of diagnostically significant proteins exist in lower concentrations, e.g. biomarkers for early-stage squamous cervical cancer Thus removal of these abundant serum proteins enhances the detection of such less abundant marker proteins indicative of the disease state (15-16). Affinity-based separation with Protein A / Protein G on sepharose or agarose matrices is often used for Immunoglobulin removal. Pre-fractionation, alcohol precipitation, and salting out methods are also some of the other plasma depletion methods (17). Current commercial affinity-based depletion kits are expensive and not cost-effective (18).

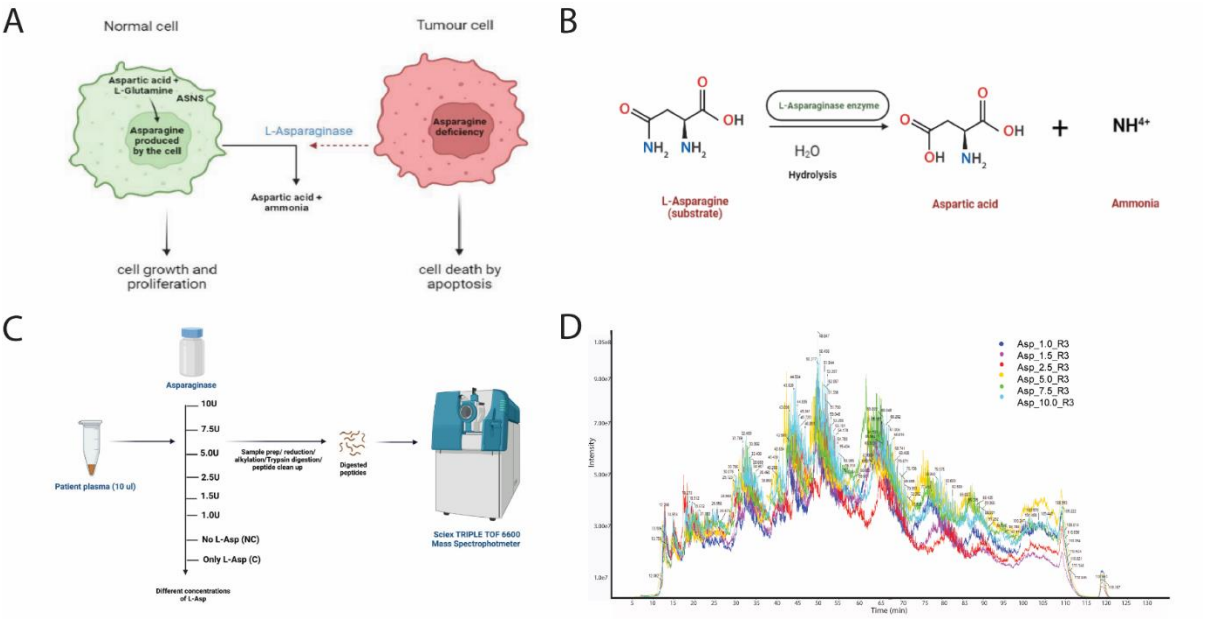
We are developing a cost-effective plasma depletion strategy to enhance the detection limit of such low-level biomarkers through mass spectrometry. We are benchmarking depletion protocols for plasma proteomics using the commercially available Protein A and Protein G sepharose beads which resulted in the partial removal of



immunoglobulins and albumin that are highly abundant. As a significant move, we also introduced a protein precipitation technique using Trichloro Acetic Acid (TCA) and Acetone in combination for the removal of highly abundant albumin from plasma (19). This step should greatly improve the dynamic range of proteins quantified by the MS. Interestingly, it resulted in almost 60-70% removal of albumin from plasma. Currently, we are optimizing a modified protocol based on both TCA-acetone precipitation followed by affinity-based separation via Protein A/G.

Supporting TTCRC research groups

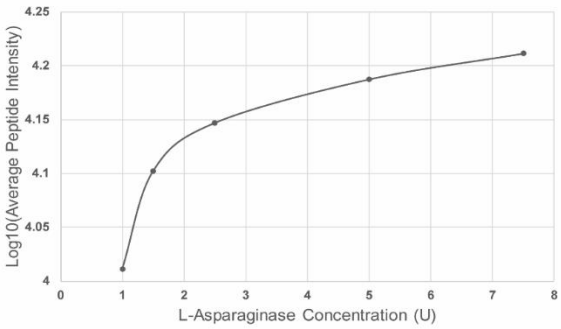
With the confidence to accurately identify and quantify proteins, we accept samples for proteomic analysis from all the research groups at TTCRC. We streamlined an online sample submission form and shared it with the entire facility to encourage sample submission and perform a preliminary run to check if the proteins they are looking for were identified or if they can use the proteomics data to correlate with the existing data. We currently support projects from the p53 biology group, the GBC group as well as the DRP group, ranging from 6 to 90



E

Unique L-Asp peptides	Retention Time (mins)	Peptide Intensity
SSRVPTGATTQDAEVDDAK	13.5	2.8 X10^7
SVNYGPLGYIHNGKIDYQR	38.8	4.6 X10^7
ALVDAGYDGIVSAGVGNGNLYK	46.8	7.4 X10^7
ASANRGVLVVMNDTVLDGR	50.4	1.0 X10^8
KHTSDTPFDVSKLNELPK	59.4	4.5 X10^7
TALAALVMGFSGAALALPNITIL ATGGTIAGGGDSATK	64.5	6.8 X10^7
VGVENLVNAVPQLKDIANVK	70.6	5.5 X10^7
INTDCDKTDGFVITHGTDTMEET AYFLDLTVKCDK	77.4	3.8 X10^7

F



samples per project, for answering their individual research questions.

Detection of L-Asparaginase by LC-MS/MS

The enzyme L-Asparaginase plays a very significant role in the treatment of childhood Leukemia as it is an essential component of the combination chemotherapy for childhood Acute Lymphoblastic Leukemia (ALL). Asparagine is a nonessential amino acid that aids in normal cell growth but is considered crucial for leukemic cells as they lack their asparagine synthesizing machinery and depend on exogenous sources of asparagine for growth and survival. L-Asparaginase (recombinant protein in the PEGylated form is used as a drug) treatment depletes the asparagine level in blood and directs the malignant lymphoid cells toward apoptosis, thus forming a cornerstone for this multidrug therapy (20). Regular intervals of drug administration throughout the induction and intensification treatment phase necessitate the detection and quantification of the levels of Asparaginase in plasma. The most common method of detection is the colorimetric method which quantifies the presence of enzyme products like aspartic acid or ammonia (Fig 6).

Here, at TTCRC proteomics, we embarked on a highly sensitive and consistent method of Asparaginase detection using mass spectrometry. We began by directly testing the L-Asparaginase in plasma through LC-MS/MS using our step-up to confirm ionization and detection properties of L-Asparaginase peptides for reliable quantification. In the next series of experiments, we prepared patient-derived plasma samples (10µl) with different concentrations of spiked in L-Asparaginase.

The spiked levels range from higher (10U) to lower (1U) concentrations with two controls (with and without L-Asparaginase). These samples were processed for MS/MS analysis using the EasyPep sample preparation kit (Thermo Fisher Scientific). We measured 2µl of each sample in technical triplicates amounting to about 1-1.2µg peptides per injection, on a 120 min active HPLC gradient (total gradient time 135 mins) and data were acquired on DDA mode using TripleTOF 6600. Raw MS files were deconvoluted by MaxQuant software version 1.6.0.17. Proteins were identified by searching the MS/MS peak lists against a target/decoy version of a modified human UniProt protein database (UP000005640 release 2014) that contains recombinant L-Asparaginase protein sequence (manually listed) using default settings. Interestingly, our instrument was able to capture most of the L- Asparaginase peptides even at the lowest concentration of the drug. Thus our established method can pave way for highly sensitive detection of L-Asparaginase in patient plasma, which however would warrant further optimization for reproducible and robust quantification.

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TCS @ TTCRC

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TTCRC Annual report 2022

Introduction

TCS continues to collaborate and contribute to accelerate research at TTCRC. We work with TTCRC team to analyse scientific data, as well as implement digital solutions for and provide technology backbone and software development. Some of the key projects where we are collaborating for scientific data analysis include Differential gene expression analysis of TP53 knockout in Acute Lymphoblastic Leukaemia as well as exploratory data analysis of mRNA Sequence data of gall bladder patients. Synopsis of the analysis are mentioned in this section while detailed results of analysis have been captured in the respective project sections. Analysis of data of other projects like Oral Cancer and ALL biomarkers are underway and will be updated periodically. TCS has been implementing digital solutions in the area of multi-center clinical trial management wherein a new study has gone live, Clinical decision support solution for clinicians during Adaptive Maintenance Therapy of ALL patients, Analytics of data from EMR and clinical trial and other systems. Translational Research solutions for meta-data extraction, validation and tagging from NGS, imaging and other scientific instruments are some of the other key highlights

A. Differential gene expression analysis of TP53 knockout in Acute Lymphoblastic Leukaemia

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 line (NALM6) 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 and, 4) TP53 knocked out ALL cells treated with Mitoxantrone. The quality of the sequencing reads was first evaluated, followed by mapping of reads to reference genome (GRCh38) to obtain read counts. The knockout and wild type samples formed distinct clusters in the t-SNE plot analysis for the protein coding genes, indicating significant differential expression for the two groups. The differential gene expression analysis for the two groups was performed by univariate analysis of log fold change and multivariate analysis using characteristic direction method. The gene set enrichment analysis (GSEA) for the differentially expressed genes 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.

B. Gall Bladder Cancer

mRNAseq data from primary tissue of malignant, inflamed and normal pathologies of Gall Bladder samples and organoids grown with different culture conditions and passages were assayed with our bioinformatic pipelines in detail. Post in-depth quality checks, mRNAseq data with adequate high quality reads underwent Exploratory Data Analysis using PCA, Hierarchical Clustering and tSNE to evaluate various hypotheses integrating external study data when relevant for further validation. Genes that were differentially expressed between different groups were examined and related pathways studied for insights into biological mechanisms. Method details and results are shared in the main section.

C. 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.

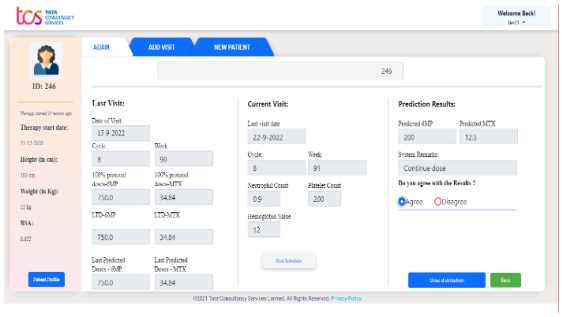
How ADAM Works:

It is a solution that facilitates a physician in a virtual clinic setting. It enable clinicians to view longitudinal patient information as well as giving rule based suggestion on the individual drug doses, thereby enabling precision medicine. The application works by predicting the doses based on the algorithm running in the backend. The application will help in saving clinician's effort and time, reducing medication errors, and overall easing the burden of clinicians.

Adaptive Maintenance Therapy Features Description:

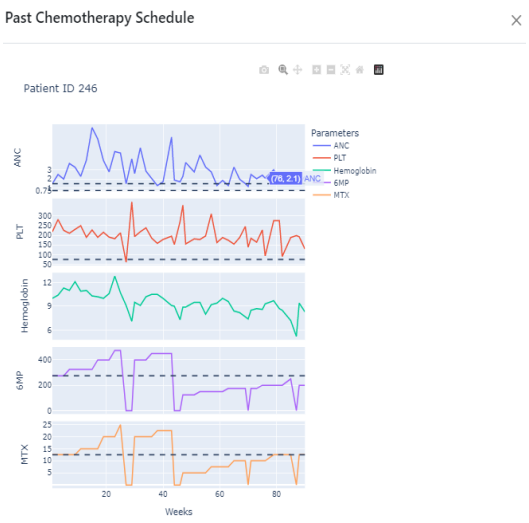
Adaptive Dose Advice Method (ADAM):

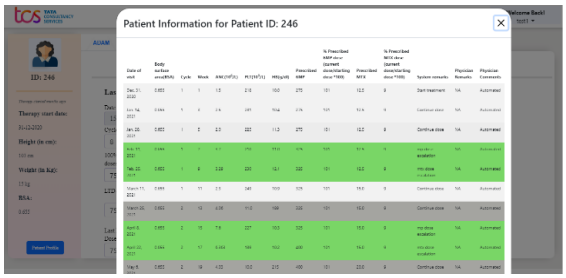
This is the landing page of the application where user can search for the patient information using his/her id, based on the search two columns (last visit, current visit) will be displayed. The application gives the facility to predict the next doses using the prediction button, prediction is based on the set of well-defined rules in the backed. The leftmost column in the app projects the basic information about the patient.



Patient Profile:

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 alternate cycles are highlighted with different colors moreover the highlighting of the escalated dose has been done. The feature to download the patient profile is also being provided if in case clinician requires the detailed information.





Patient Plot:

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

Add Visit:

This section of the app provides the facility to add new record for the exiting patient, update feature has also been provided to correct the existing record.

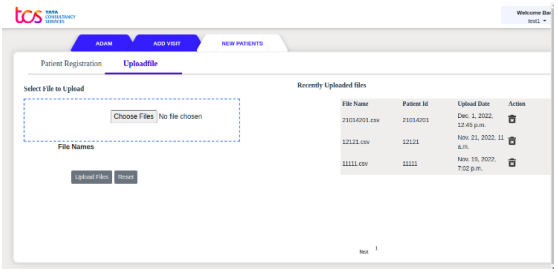
Upload File:

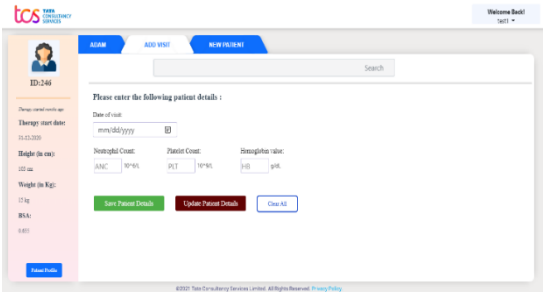
Users can upload new patient's complete maintenance therapy data in one time using this application. User can also delete the uploaded data if incorrect data is uploaded.

D. ALL R1 Study (Relapsed ALL)

ALL R1 Study objectives:

- Affordable, minimal residual disease (MRD) based strategy for the treatment of relapsed ALL in LMICs (Low Middle-Income Countries).
- Introduce new drugs for the treatment of childhood ALL





- Decrease toxicity of treatment and achieve outcomes comparable to western studies
- Manage toxicity instances experienced by patients effectively.

We have successfully completed the set-up of this complex study. This is the second clinical study built and implemented on TCS Platform. It is a one-stop, integrated, cloud-deployed, self-service solution that caters to all data management needs. It is scalable as per the requirement. It provides automated review, reconciliation, and query management. The platform is powered by a cognitive artificial intelligence engine, data-driven smart analytics.

In this development we have addressed additional complex requirements from study team to reduce errors and to generate quality data for patients enrolled. We have collaborated with functional teams to achieve study requirements. Patient onboarding into IDM application has started successfully.

E. ICICLE:

Integrated Data Management for ICICLE trial: ICICLE is the first multicentre clinical trial created on IDM for TTCRC.

This is a multicentre trial to create treatment strategy for Acute Lymphoblastic Leukaemia (ALL), which is the most common cancer of childhood. This clinical study has been improving outcome of clinical treatment. Clinical DB was set up as per requirements to collect, review and analysis of data for patients enrolled for study.

This study was built many years back and with prevalent standards. Over the period, new security standards have evolved, and it was a need to align this study with the latest standards. This is to improve data security and reduce risk of data loss. We have planned and initiated the migration of this ongoing study to latest IDM version. All data with previous application has been migrated into new IDM version successfully. This IDM version has better performance and reduced latency. There is online query management feature in application. It improves overall compliance and efficiency through intelligent decision-making.

Site users have started using latest IDM application. Additional study design parameters configuration is ongoing. There is data backup maintained for reference.

F. Translational Research Platform

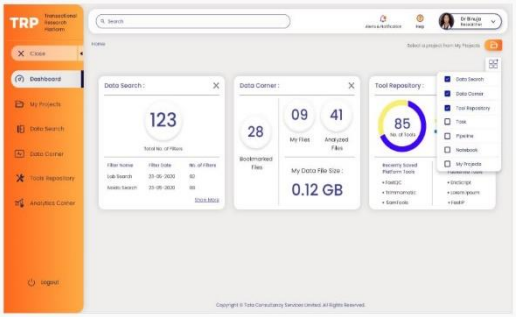
Translational Research platform is collecting and integrating clinical research data, molecular data, patient Electronic Medical Records (EMR) followed by data cataloguing and creation of research databases. The large amount of data files can be searched based on metadata to generate better insights for analysis and exploratory visualization. The platform fulfills the needs of researchers and data scientists on a larger scale to store, access and manage voluminous amounts of data across various datasets of the existing research projects.

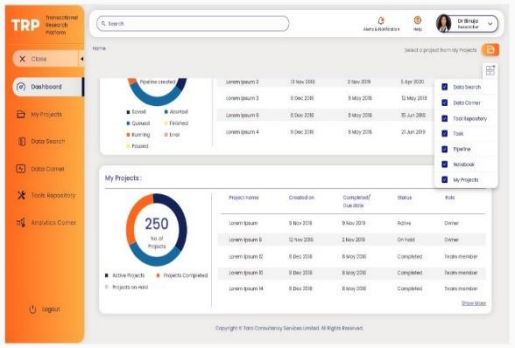
Translational Research Platform Features:

Some of the key features include Dashboard, Project management, User Workspace Management, User On-boarding, Metadata based Data Search, Data Corner, Tools Repository. Few functionalities have been enhanced in data search & data corner modules and explained below in detail

Customizable Dashboard

Users will be able to view the details from different sections in a customizable dashboard. The widgets can be added/removed or rearranged for custom view.



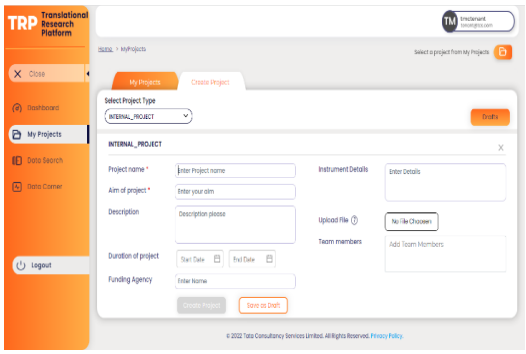


Project Management

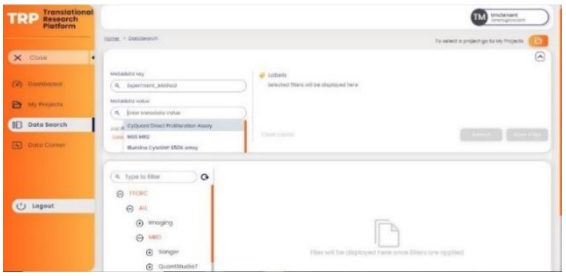
Users can define projects and capture project related information. Users will be able to access My Projects in the platform to organize and manage the data files and perform related analysis within a workspace. Each workspace can have multiple projects in it. The user who created the project will be the owner of the project, one project will have one owner. Only Owner can edit or change the status of the created project's details.

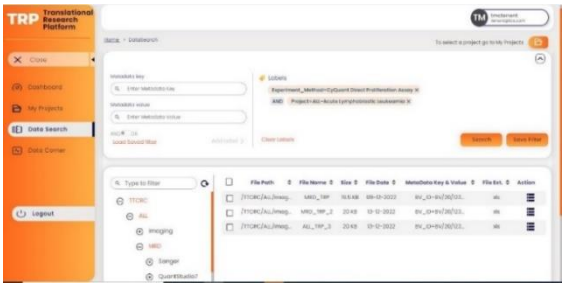
Auto Suggest while Metadata based File Search

Users will be able to search for files based on system defined metadata key value pairs e.g., file type, name, owner, Ext. etc.



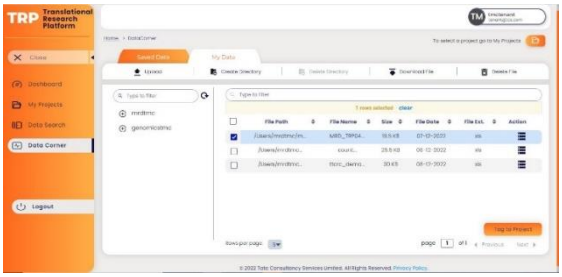
using the auto suggest list platform feature. Auto suggest list will display the keys and corresponding values list to the user while applying search criteria for files. Users can access files based on hierarchy/folder structure in data search. The user will be able to save search criteria as a filter and apply one at a time. The user will be able to view the relevant search results by clicking search.

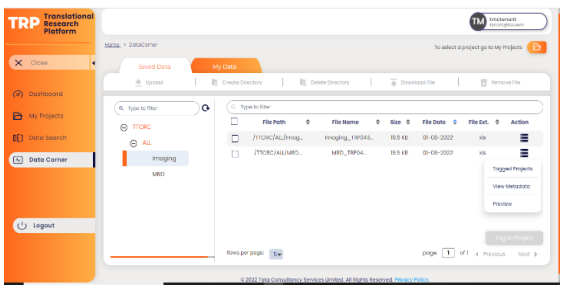


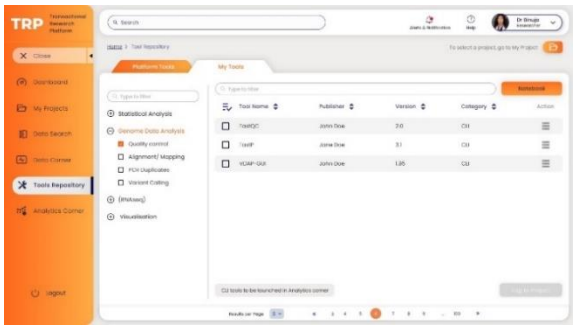


Preview Files in Data Corner

Users will be able to view & select platform files and save them into Saved Data tab to be used further. Users can expand or collapse hierarchy level name and view the corresponding files in File Details Table (FDT). Users will be able to select multiple files from FDT, can preview the files and tag files to projects. Users can upload his/her own files in My data for processing and further analytics.







GUI/CLI Tools in Tool Repository

Users will be able to view and select tools for Analysis based on roles/permissions.

Based on permission/role, a user can be assigned a group of tools or individual tools.

Each tool will be categorized based on below -

1. GUI - A tool which has it's own user interface and can be opened in browser to perform actions on that tool.
2. CLI - A tool which doesn't open in browser and need a specific environment and parameters for execution.

Rewards and Recognition

The joint submission on TTCRC by TCS and Tata Trusts was awarded the winning entry at the recently concluded Tata Innovista 2022 under the category of 'Sustainability Impact Innovations'.

- There were a total 15,000+ entries in Tata Innovista 2022
- Through the rigorous multi stage judging process out of 15,000+ only <60 teams reached the Tata Innovista 2022 final
- In the finals <20 teams received the coveted Tata Innovista 2022 trophy, in various categories, from Tata Group Chairman Natarajan Chandrasekaran and Tata Group Chief Digital Officer Aarthi Subramanian
- Entry on TTCRC was the winner in its category.

More details can be found at https://tatainnovista.com/ .

Poster Presentation at Annual Review-2022

P1	Can we predict 6-mercaptopurine intolerance prior to maintenance therapy in newly diagnosed acute Lymphoblastic Leukaemia treatment	CRU
P2	Precision approach for Treatment of Hematological Malignancies (Path 01- 2023)	CRU
P3	Biobanking at a glance: TiMBR Experience	Biobank
P4	Pediatric Leukemia Biobank: A Platform for functional precision oncology to personalize clinical treatment	Biobank
P5	Enhancement of Ig/TCR based MRD detection strategy in patients with Acute Lymphoblastic Leukemia	MRD
P6	Loss of p53 induces resistance to topoisomerase II inhibitors in an *IN VITRO Model* of Acute Lymphoblastic Leukaemia	P53
P7	Identifying sensitive alternate chemotherapeutic agents for patients with Relapsed/Refractory Acute Lymphoblastic Leukaemia	DRP
P8	Dissecting Role of Exportin Inhibitors in Acute Lymphoblastic Leukaemia	DRP
P9	Developments of Clinical Proteomics Unit at TTCRC	Proteomics
P10	Developing coordination for streamlining the procedure of patient data and clinical sample collection in a multidisciplinary translational research project	GBC
P11	Phenotypic and molecular characterization of the gallbladder cholangiocyteorganoids (GCOs)	GBC
P12	Protocol for Cryopreservation & Retrieval of Primary Cells and Organoids to Establish a Living Organoid Biobank	GBC
P13	Laboratory Management at TTCRC	Administration
P14	Administrative Support for Research Support Directorate at Tata Medical Centre	Administration

Research Publications-2022

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Picture Gallery

Legend

1-3. Moments from TTCRC Annual Review 2022 held between Jan 27-28th 2023
4-6. Visit of Prof. Nancy Rothwell-President University of Manchester, Prof Stephen Flint, Prof David Polya , Prof Aravind Vijayraghavan, Prof Keith Brennan, Richard Cotton, Alex Gaskill, Daryl McManus, Sarah Mcminn on on 26th September 2022
7. 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-Chief Executive Officer and whole-time Director at Gennova on 9th December 2022
8. The TATA Innovista 2022 Trophy
9. The TCS-TTCRC Team