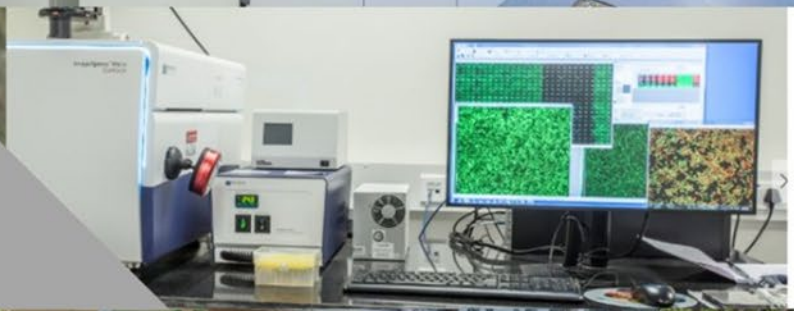
ANNUAL SCIENTIFIC REPORT 2019

TATA TRANSLATIONAL CANCER RESEARCH CENTRE



Kolkata, India

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The Director's Desk

After the inception of the center in 2018, this has been a year of consolidation. The completion of the academic center has allowed the building to take shape, though we are yet to install the IBMS. The laboratories are now fully functional with the activation of the mass spectrometer. Biobank has doubled its foot space. A number of visitors came by. Tanja Gruber from St. Jude, Memphis spent a week as resident visiting faculty at the end of November. Arunabha Chakrabarti returned from his year in Manchester, Pritha Paul is currently abroad working with Patricia Muller. Anindita Dutta went on a UICC fellowship to Cambridge and Manchester and Shivani Bhagwat visited the National DNA Bank in Salamanca, Spain. Prakriti Roy, Satyam Banerjee, Avishek Banerjee and Soumasree Tapadar moved on and I thank them for their hard work and wish them all the very best for their future. Asima Mukhopadhyay has moved with her team to CNCI along with the fellowship in what promises to be an exciting venture. As I write, Susri Ray Chaudhuri will also leave to join TMC in February 2020. She played a crucial role in establishing TTCRC and I am sure that she will be greatly appreciated at TMC.

The Clinical Trial Unit, led by Shekhar Krishnan, has expanded its portfolio, published its experience with relapsed ALL and a number of other papers are now in the pipeline. Genomics has streamlined pipelines for DNA and RNA analyses with NGS. There are interesting observations in high hyperdiploid ALL. In collaboration with DKMS, NGS MRD is now moving to the second phase. Oxford Nanopore Technology is in use for RNA analyses. The organoid laboratory, a concept last year, has now successfully established itself with a focus on gallbladder cancer.

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Summary

The Clinical Research Unit at TTCRC (CRU) was established in January 2014 and is involved in the design, development, management, analysis and reporting of academic clinical studies in cancer. In 2019, the unit's services were reorganised with appointment of new personnel in administration (ND), data management (PD) and informatics (TM). The year saw expansion of the CRU's portfolio of clinical studies in paediatric acute lymphoblastic leukaemia (ALL) and involvement in a newly-initiated multi-disciplinary institutional study in gallbladder cancer. The CRU continues to function as the pivot for translational laboratory studies at TTCRC aimed at developing effective affordable anti-cancer therapeutics (asparaginase in ALL) and therapies (minimal residual disease monitoring & maintenance therapy in ALL). In 2019, the unit joined the initiative to develop a network of academic CRUs in India, an effort led by Prof Usha Menon and colleagues at the Clinical Development Services Agency of the Translational Health Science and Technology Institute (https://thsti.res.in/cdsa/).

Clinical studies in paediatric acute lymphoblastic leukaemia

Acute lymphoblastic leukaemia, first presentation

The 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). At the end of December 2019, the study will complete 38 months of enrolment, the last 22 months involving all study sites. Patient enrolment and randomisation are satisfactory (~1500 patients enrolled, >95% eligible patients randomised) and the trial is expected to continue accrual for a further 24 months. Interim trial findings indicate continuing decrease in treatment-associated mortality (currently at ~6%) with standardisation of care and risk- adapted therapy. Pre-trial observations underscore the impact of risk-stratified management on clinical outcomes, with the lowest frequency of events (relapse and treatment-related mortality) observed in patients categorised as having low-risk disease. Risk-stratified treatment also reduces direct treatment costs (**Figure 1**). The cost of specialised laboratory studies required to identify patients with lower risk disease (~50% of ALL patients) is more than offset by the reduced cost of lower-intensity treatment administered to these patients. 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. The CRU has worked to support institutions in this initiative, including JIPMER Puducherry, Kidwai Memorial Institute of Oncology Bengaluru, St Johns

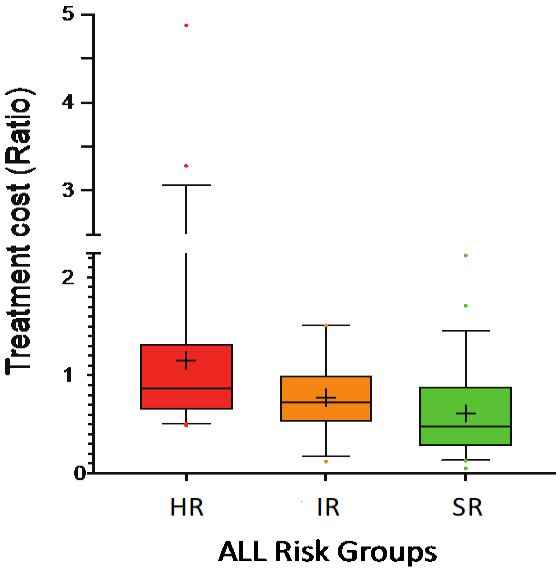

Figure 1: Higher direct treatment costs with increased intensity of ALL treatment
Box-whisker representation comparing direct treatment costs in patients treated as Standard Risk (SR), Intermediate Risk (IR) and High Risk (HR) ALL at the Tata Medical Center. Boxes denote interquartile ranges [IQR (25%-75%)], horizontal lines and 'plus' symbols within boxes indicate median and mean values respectively, whiskers represent 1.5 × IQR, dots represent outliers

National Academy of Health Sciences Bengaluru and the Nil Ratan Sircar Medical College Hospital Kolkata. The impact of the ICiCLe ALL initiative was also featured in the University of Manchester magazine in its Summer 2019 issue (https://tinyurl.com/ICiCLe-ALL).

Relapsed acute lymphoblastic leukaemia

In June 2019, the CRU published its experience with a risk-adapted treatment protocol as a feasible affordable option for standardised management of children with untreated first relapse of ALL at the Tata Medical Centre[1]. The protocol, TMC ALLR1, is a modification of the international ALLR3 protocol (ISRCTN45724312), uses a combination of conventional (immunophenotype, timing, site of relapse) and biological (cytogenetics, minimal residual disease) variables to identify patients with relapsed ALL who can be cured with chemotherapy alone (**Figure 2A**). The observations additionally highlight the impact of disease-directed therapy at extending survival and maintaining well-being in patients with relapsed ALL, even in the absence of curative allogeneic haematopoietic stem cell transplantation (**Figure 2B**). MRD analyses for risk stratification is done both by flow cytometry and RQ-PCR.

This approach has been introduced as a multicentre Indian Paediatric Oncology Group clinical study (InPOG-ALL-19-02; CTRI/2019/10/021758) coordinated by the CRU, with Tata Medical Center Kolkata (TMC) as the first participating centre (principal investigator, Dr Niharendu Ghara). Data management will be carried out using a customised electronic data capture system developed using the Integrated Data Management database (v 4·0) developed by Tata Consultancy Services.

Infant acute lymphoblastic leukaemia

In December 2019, the CRU formulated an approach for standardised management of infants (age ≤ 365 days) with newly-diagnosed ALL at TMC. The approach was developed in discussions with Dr Tanja Gruber, chief investigator of the multicentre Total Therapy for Infants with Acute Lymphoblastic Leukaemia I study protocol (TINI, ClinicalTrials.gov identifier NCT02553460), from the St Jude Children's Research Hospital during her visit to TMC in late Nov 2019. Management is stratified based on the presence of *KMT2A* rearrangements, and subsequent

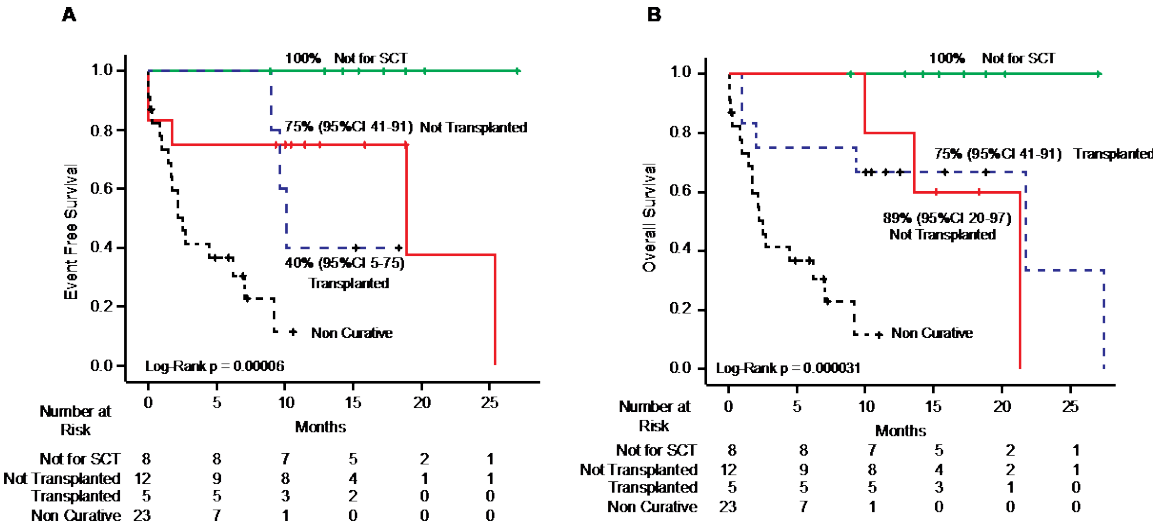

Figure 2. One-year Kaplan–Meier estimates of (A) event-free survival and (B) overall survival in patients with relapsed acute lymphoblastic leukaemia (ALL) treated at Tata Medical Centre. Not for SCT = patients identified by risk stratification or time point 1 minimal residual disease as not suitable for allogeneic stem cell transplant (green line). Patients not transplanted are those who are risk-stratified for SCT but who either died before transplantation or refused (red line). Non-curative shows outcomes of patients who chose supportive care only (grey dashed line).

treatment is based on early treatment response using PCR-based monitoring of minimal residual disease. If determined to be feasible, this approach would be proposed as an InPOG multicentre study for standardised management of infant ALL.

Allied research studies in acute lymphoblastic leukaemia

While risk-stratified standardised management has improved clinical outcomes in paediatric ALL, pre-trial observations indicate that these improved outcomes still lag behind those reported in developed economies. The CRU is part of research studies at TTCRC to understand the basis for these inferior outcomes. These studies, including comprehensive genetic characterisation, sensitive monitoring of treatment response and hypothesis-based biological studies, are discussed in other sections in this annual report. The CRU has a central role in two other research approaches aimed at improving outcomes in ALL. One is to address the variable quality of cytotoxic drugs used for the treatment of ALL, specifically the therapeutic enzyme L-asparaginase, a key agent in ALL treatment. The other is on developing integrated strategies to standardise the management of the maintenance treatment phase in ALL.

Optimising asparaginase therapy in ALL

With limited availability of the standard *E. coli* L-asparaginase used hitherto in ALL therapy, a variety of alternative *E. coli* asparaginases (EcASNase) are marketed commercially in India and overseas. The CRU is carrying out studies to monitor post-treatment asparaginase activity and drug-associated clinical toxicity during treatment with these alternative EcASNases. The findings indicate that drug activity is inadequate when the alternative EcASNases are administered every 72 hours, suggesting the necessity for shorter dosing intervals with these agents. The findings also draw attention to the need for ensuring affordable access to quality-

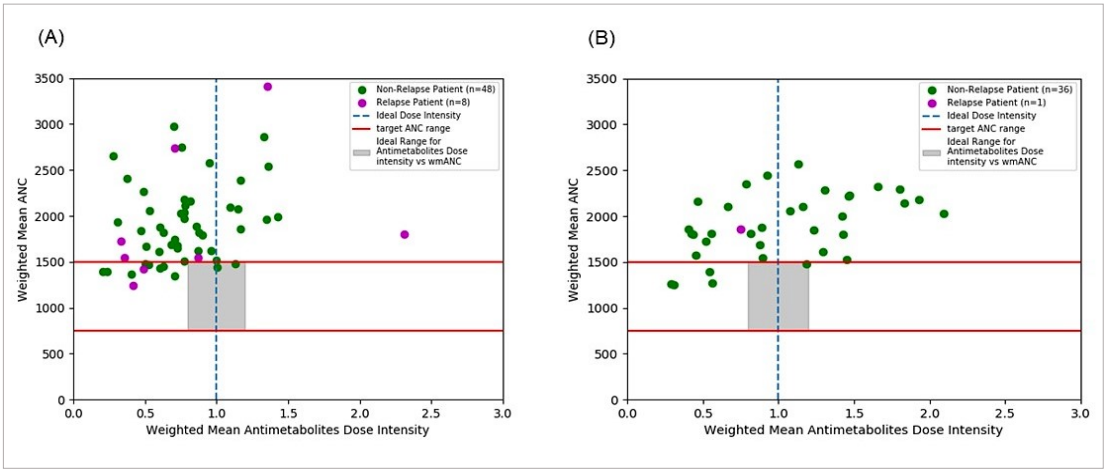

Fig 3: Scatter-plot analyses of adaptive dosing practice during the ALL maintenance treatment phase. Treatment during the ALL maintenance phase aims to maintain antimetabolite dose intensity (product of weighted means of ratios of prescribed and recommended weekly doses of 6-mercaptopurine and methotrexate) between 0•8 and 1•2 (vertical grey bar) while ensuring suitably low absolute neutrophil counts (ANC) through 96 weeks of maintenance treatment (weighted mean ANC between 0•75 and 1•5 x 10^9/L, horizontal red lines). Each dot represents summary information for the two parameters in a patient. Analysis in an initial cohort (A, 56 children, start of maintenance treatment in 2016) indicates that treatment objectives for both parameters were met poorly in the majority of patients (31/56, 55%) while improved antimetabolite dose intensity was observed with more rigorous supervision in a later cohort (B, 22/37 patients, 59%; start of maintenance treatment in Jun 2017).

assured long-acting asparaginases for patients in low-income countries.

Integrated strategies for the standardised management of ALL maintenance treatment

Optimum intensity of treatment during the maintenance treatment phase contributes critically to improved outcomes in ALL. This phase of treatment involves outpatient management and requires stringent monitoring of blood counts, drug dosing and clinical tolerance to ensure optimal dosing of the antimetabolite drugs 6-mercaptopurine and methotrexate through the 24 months of maintenance therapy. The CRU operates the weekly paediatric ALL maintenance clinic at TMC (average, 45 patients). The CRU additionally runs a weekly e-mail clinic (average, 20 patients) to advise patients on antimetabolite dosing, thus decreasing the requirement for fortnightly clinic visits. Despite these strategies, an audit of maintenance treatment practice (56 patients, Feb 2016 – Sep 2018) indicated that treatment intensity was sub-optimal for the majority of patients (**Figure 3A**).

With improved stringency in drug dose titrations, a second audit (**Figure 3B**) indicated improved dosing intensity in a later patient cohort (37 patients, June 2017 – January 2018, analysis to mid-February 2019).

Bayesian-based joint modelling to investigate the influence of both conventional prognostic variables as well as longitudinal variables during the ALL maintenance phase (serial leucocyte, neutrophil and platelet counts; 6-mercaptopurine and methotrexate doses) confirmed an independent impact of 6-mercaptopurine dose intensity on ALL outcomes, with the greatest impact

observed ~6 months from completion of maintenance therapy (263 patients; Prof Kiranmoy Das, Indian Statistical Institute). These observations provide the basis for CRU's focus on developing a comprehensive integrated strategy to standardise the management of maintenance therapy in ALL.

Data Science

In partnership with the Tata Consultancy Services (TCS), the CRU has focussed on strengthening data science to support clinical research. Electronic data management in the multicentre ALL studies has been developed by customising TCS' Integrated Data Management platform. BA was seconded from TCS to support design and customisation of the study databases as well as advise on clinical data management in the studies, focussing specifically on data quality. SP was seconded from TCS for support with data analytics and data-driven exploratory studies, including data extraction (e.g. from electronic medical records, trial database), creation of study cohorts of interest (using i2b2), data analysis (using R) and data visualisation (R, Kibana). TM joined the team in Dec 2019 and will partner SP to extend R-based data analysis as well work on developing an assisted dose advice system for antimetabolite dose management in maintenance.

Other activities

An emerging core activity of the CRU is the coordination of the newly-initiated multi-disciplinary translational research programme in gallbladder cancer at TMC. This work is described elsewhere in the annual report. Members of the CRU have also been involved in published work reporting outcomes of late bone marrow relapse in the ALLR3 international trial[2], the adverse prognostic impact of high leucocyte count at presentation in Philadelphia-chromosome positive ALL[3] and the pharmacokinetics of dexamethasone in induction therapy of childhood ALL[4].

Publications

Efficacy and safety of a bortezomib and reduced-intensity cytarabine-based protocol, TMC ALLR1, for relapsed childhood ALL in India. Roy P, Islam R, Saha D, Gogoi M, Kumar Mishra D, Arora N, Parihar M, Krishnan S, Saha V. *Br J Haematol*. 2019;186(6):861-5.

Outcomes of patients with childhood B-cell precursor acute lymphoblastic leukaemia with late bone marrow relapses: long-term follow-up of the ALLR3 open-label randomised trial. Parker C, Krishnan S, Hamadeh L, Irving JAE, Kuiper RP, Revesz T, Hoogerbrugge P, Hancock J, Sutton R, Moorman AV, Saha V. *Lancet Haematol*. 2019;6(4):e204-e16.

Long-term follow up of pediatric Philadelphia positive acute lymphoblastic leukemia treated with the EsPhALL2004 study: high white blood cell count at diagnosis is the strongest prognostic factor. Biondi A, Cario G, De Lorenzo P, Castor A, Conter V, Leoni V, Gandemer V, Pieters R, Stary J, Escherich G, Campbell M, Attarbaschi A, Li CK, Vora A, Bradtke J, Saha V, Valsecchi MG, Schrappe M. *Haematologica*. 2019;104(1):e13-e6.

Impact of dose and duration of therapy on dexamethasone pharmacokinetics in childhood acute lymphoblastic leukaemia-a report from the UKALL 2011 trial. Jackson RK, Liebich M, Berry P, Errington J, Liu J, Parker C, Moppett J, Samarasinghe S, Hough R, Rowntree C, Goulden NJ, Vora A, Kearns PR, Saha V, Hempel G, Irving JAE, Veal GJ. *Eur J Cancer*. 2019;120:75-85.

TiMBR- The Biorepository

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Biobank Technologist

During 2018-19, the research infrastructure of the biobank and resource sharing has been considerably expanded. New projects include that of gallbladder cancer and an extension of HYPORT-B Adjuvant (Radiation oncology). Each year, there has been an increase in the numbers of patients recruited. In past three years more than 5,200 patients across 12 projects in seven departments have been recruited (Figure 4).

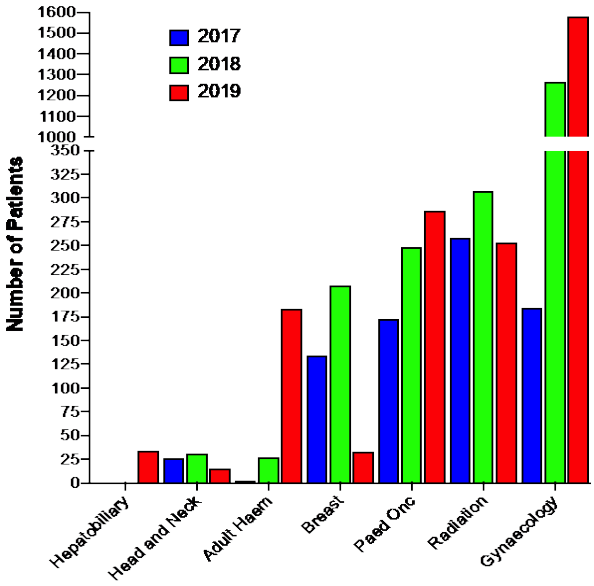

Figure 4. Number of patients recruited by each study group in the last 3-years

The major sample sources in the biobank are plasma/serum, blood, viable patient primary cells and cervical scrape (Figure 5). Protocols for the storage and extraction of nucleic acids from snap frozen tissue, FFPE (Formalin fixed paraffin embedded tissues) and biopsy samples are ongoing. Much of the work this year has been towards drafting health & safety guidelines, internal audits, quality controls and streamlined sample disbursement. TiMBR is now a member of International Society for Biological and Environmental Repositories (ISBER) and Asian Network of Research Resource Centres (ANRRC). Based on ISBER's guidelines to achieve excellence in our work we have started periodic user's assessment, training schedules, induction to the facility & equipment and internal audits for Laboratory Information Management System (LIMS) and sample storage. The LIMS system has been upgraded from LV 6.0.1 to LV 8.2. Customization of LV system based on needs gives the opportunity to track pre-analytical variables for all samples received in biobank for processing. The interactive dashboard of LV provides us real-time status of samples and their disbursement. The LIMS covers almost every aspect of the biobanking from samples, equipment, calibrations report, incident and consumable inventory and provides an opportunity for a paper-free environment. The LIMS now needs to expand to capture the sample life cycle under each project. Quality assessment records/reports need to be maintained project wise. Freezers need external monitors where alarm can be linked to concerned person to respond in case of emergency. There is a continuous process of implementation of quality management and self-assessment surveys to allow the bank to meet international standards.

Dr Bhagwat obtained hands-on training at the National DNA Biobank (Banco adn) in University of Salamanca, Spain. Banco adn was established in 2004 and is associated with 60 centres across the country including hospitals, universities and regional transfusion centres and stores more than

39,000 donor samples. It is an ISO certified bank and works with Genome Spain Foundation and part of the biosafety committee in ISBER. Training was received

on robotics, workflow of sample received, documentation, processing, storage and sample disbursement. Dr Bhagwat is establishing a collaboration with Banco adn for the exchange of protocols, experiences and troubleshooting.

For a resource-oriented research infrastructure it is crucial to know what the stakeholders need. User group and oversight committees enable mapping to be undertaken in greater detail, including requirements and needs of patient recruitment, policy making, infrastructure and operational inputs. It is pivotal to recognize the needs of researchers which includes pre-analytical quality control, new sample processing and storage conditions. As a prospective project-based repository, the challenge is to work on long term sustainability. The team is now working to conceptualize financial reserves and budgetary needs for the next five years. To equilibrate with technological innovations & requirements, a concrete plan is necessary which would cover cost of depreciation and process upgradation. Another challenge is to formalize health and safety guidelines and train users to work with liquid nitrogen and basic safety rules. Many challenges lie ahead but our efforts are towards providing high quality samples, biosafety rules, stringent audits, accreditation, International harmony and sustainability.

In the past year one team member, Ms Soumasree Tapadar moved to the mass spectrometry laboratory to learn more sophisticated and high throughput platform. She has now left to get married and we thank her for all her hard work and wish her all the best for the future.

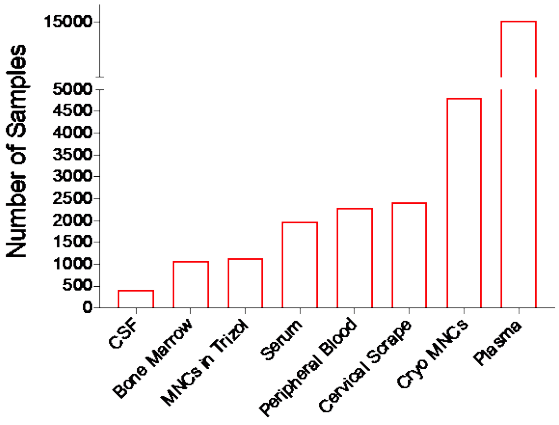

Figure 5. Number of fractionated samples processed and stored in the last 3-years

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Genomics Laboratory

The genomics team conducts hypothesis based research, aids in patient care and provides corelaboratory works for other groups at TTCRC. The laboratory works in close cooperation with the Department of Cytogenetics at TMC. Our areas of research are listed below.

Minimal Residual Disease (MRD) monitoring in Childhood ALL using RQ PCR for Ig/TCR

Our current approach for for treating childhood ALL, as outlined in the previous section, is based on risk stratification of therapy based on the early response to therapy measured by MRD levels at the end of induction therapy. The ICiCLe network uses flow cytometry (FCM) to detect MRD. FCM is rapid, cheap and available at all ICiCLe centres. Analyses of FCM data suffers from subjective variation and the assay requires large numbers of fresh cells. This limits its utility as the assay is difficult to standardise or centralise. Many centres in India do not have access to accurate FCM based MRD limiting their ability to offer risk stratified therapy. An alternative approach is to quantify the Ig/TR rearrangements in the mononuclear DNA samples obtained from bone marrow aspirates. This is an objective, real-time quantitative PCR based analyses, not reliant on fresh samples. The assay is easily standardised across multiple centres. Our laboratory is a member of the European MRD Network and regularly takes part quality control assessments. We and other have shown previously that Ig/TCR is more sensitive than FCM (see previous section). This assay is now routinely used at TMC for relapsed ALL patients treated on TMC ALLR1 and in frontline patients who have persistent levels of MRD detectable by FCM (Figure 6).

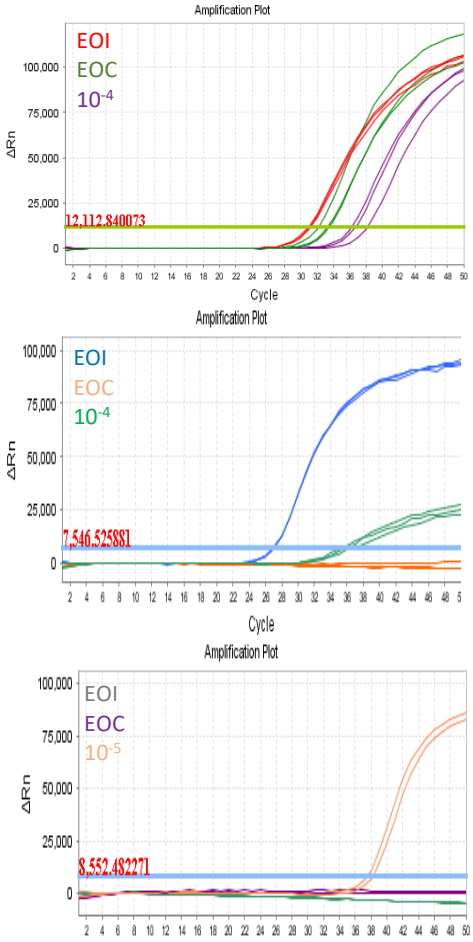

Figure 6. RQ-PCR traces of MRD estimation. EOI = end of induction; EOC = End of consolidation. Assay sensitivity ranges from 10-4-10-5. Upper panel, MRD positive at both time points; middle panel, MRD positive at EOI and negative at EOC and bottom panel, negative at both time points

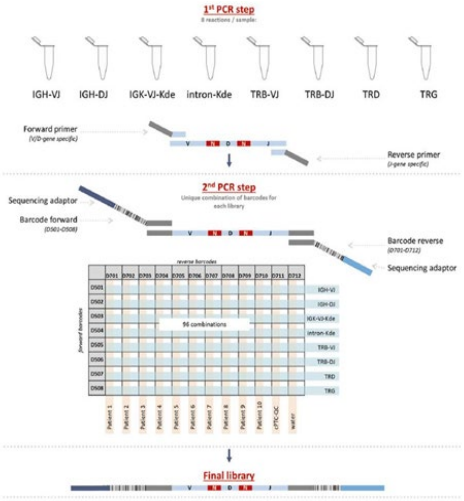

Figure 7. Amplicon based NGS MRD workflow

Disease (MRD) monitoring in Childhood ALL using NGS

Ig/TCR is sensitive and reproducible. As it depends on DNA, it can be centralised. Our experience is that samples sent to referral centres are stable even up to 5 days. Thus this technique would allow most centres to be able to offer MRD based risk stratified therapy for children with ALL. However this technique takes a few weeks, is expensive and laborious and too expensive for wide use in India. With the help of EuroMRD we are developing a NGS based approach for MRD. Potentially this is more rapid and allows the detection of a wider number of clones than RQ PCR (Figure 7).

The Genomic Landscape of High Hyperdiploid ALL in Indian ALL patients

We have previously reported on the higher prevalence of the High Hyperdiploid (HeH) subtype of ALL in children presenting to our centre (PBC paper). We have used a combination of high density whole genome SNP array and RNA sequencing (RNA-Seq) followed by validation of mutation by targeted gene panel sequencing (GPS) in a cohort of HeH patients. Overall our results suggest unique chromosomal copy number alterations and loss of heterozygosity in our patient cohort. Further validation has been performed using whole genome sequencing (WGS) and Sanger sequencing platforms.

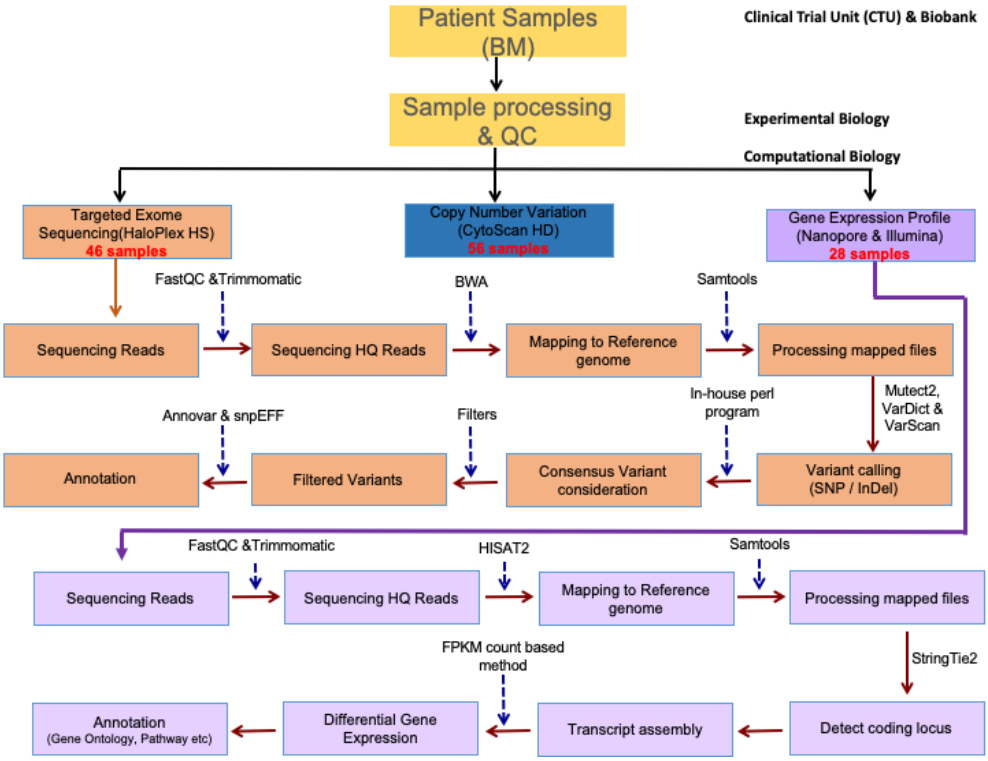

Figure 8: Schematic of Analyses of Patients with High Hyperdiploid ALL

Germline mutations have been identified and removed using patient-matched remission samples. Somatic mutations are further considered for the downstream analysis and various filtering criteria have been imposed to identify likely pathogenic mutations. The analyses includes identification of recurrently mutated genes like *KMT2D*, *CREBBP, KRAS, NRAS, NCOR1, NOTCH2, TYK2 and FLT3.* The mutational landscape of ALL patients harbour non-synonymous mutations which are further classified into indels, stop-gain, frame-shift and non-frame-shift mutations. In order to ascertain whether chromosomal gain in HeH patients is associated with gene dosage effects we are analysing the transcriptome data in collaboration with the computational biology team of Division of Vaccine Discovery La Jolla Institute for Immunology (Figure 8).

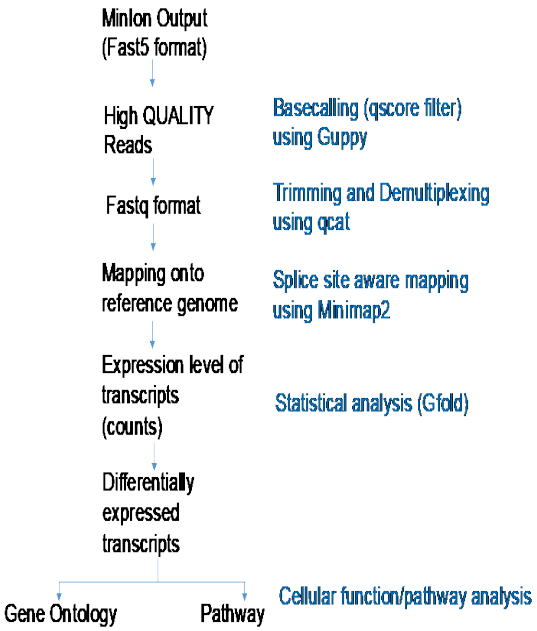

Figure 9. Schematic of the ONT informatics workflow

Oxford Nanopore

In addition to the conventional next-generation sequencing approaches by Illumina, we have explored the feasibility of the third generation sequencing technology from Oxford Nanopore technologies. Primarily we have been using this for RNA analyses. We have used the sequencer for PCR-cDNA and direct cDNA analyses. Direct RNA analyses is ongoing. Gene expression in set of cell lines analysed with the Minion has shown good correlation with RNAseq data obtained with the NextSeq550 sequencer. Figure 9 shows the schematic of the approach taken by us in analysing the data output.

Computational Biology

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Abhishek H. Iyer
TCS

The computational biology group at TTCRC is a cluster between the TTCRC bioinformatics group and the computational biology group of TCS, Noida. The department was established in 2018 and currently it is well equipped with the high end servers, workstations and the storage facilities. Further, in order to obtain better understanding onto the advanced computational research, we have set-up active collaboration with international groups which provides a vivid insight onto the different field of bioinformatics.

The TTCRC computational biology group mostly focused in understanding the patient specific genomic variance using different sophisticated tools and techniques. The study includes the identification of the mutational marker using the targeted sequencing technique to identify the single nucleotide variance among the sample cohort. Moreover, our interest expands in analysing the RNA data derived from the patient samples. Our study most focused in understanding the gene expression analysis, followed by mutational identification and fusion detection using Illumina sequencing platform. To undertake the comparative analysis among different sequencing platforms, the group has successfully standardized the gene expression data workflow yield from Nanopore MinION platform and compared the same data with Illumina platform with high confidence.

SNV detection using Targeted Sequencing Approach

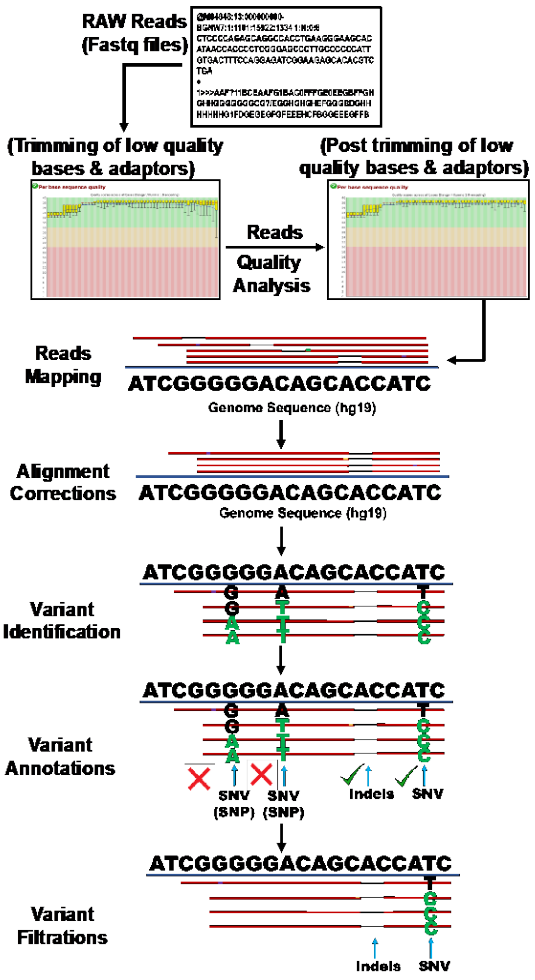

Figure 10. Somatic mutation discovery pipeline

The targeted sequencing of 95 genes panel across the samples cohort yields a better understanding in detecting the mutational landscape using the single nucleotide variance (SNV). The automated analysis pipeline has been created in order to overcome the manual intervention within the analysis. The standardised workflow includes the multilayers analysis process which includes quality check of the sequencing reads to mapping of them onto the reference genome. The workflow includes the both pre-and-post alignment

process techniques to obtain the high mapping coverage. Different variant calling tools are considered independently in predicting the genomic variant to obtain the most consensus variants through this analysis. Further each and every predicted variant is passed through the rigorous filtering criteria to most important probable mutations among the sample cohort (Figure 10).

RNA Sequencing

RNA-Seq derived from our patient cohort has passed through various levels of quality parameters to ensure that most of the data captures from protein-coding genes. The high quality reads are first mapped onto the reference human genome (Grch37 assembly) using hisat2 alignment program. Aligned bam files obtained from the program are further used to generate the hisat2 specific counts for annotated human Ensembl Grch37 genes using feature Counts program. Finally, the count files are then used to identify a common set of differentially expressed (FDR < 0.1) genes from EdgeR and DESeq2 program for downstream analysis. The RNAseq data are further used to predict the probable Fusion-transcripts within the sample cohort. The fusion transcripts are first identified by STAR-Fusion program and further rigorously revaluated using Fusion Inspector program to obtain the most confidence fusion transcripts with the sample data. The RNA-Seq data is further being used to detect the mutational variants to support the mutational data predicted from genomic DNA in SNV analysis.

The team has also established the automated gene expression analysis pipeline for the data produced from Nanopore MinION platform. The comparative analysis between two cross platforms like Illumina and MinION reveals the high specificity among them.

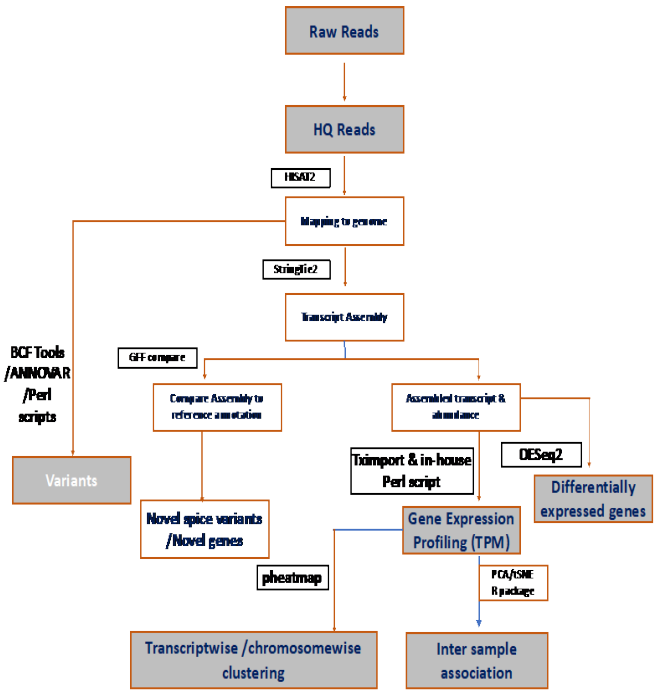

Figure 11. RNA sequencing analyses pipeline

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Dr Arunima Maiti
Research Assistant

Cell Biology

Aim

Gallbladder cancer (GBC), although rare in most part of the world, has higher incidence in North and North-east India with 5-year survival rates of 0-10%. At Tata Medical Centre, ~300 patients are diagnosed as GBC per year. Due to lack of cell lines and biological models representing the human gallbladder, the occurrence and development of the disease in poorly understood, and limits pre-clinical therapeutic intervention. Our aim is to develop pre-clinical models representing normal and diseased gallbladder to understand the disease pathogenesis and therapeutic intervention.

Hypothesis

We hypothesise that exposure to environmental stresses leads to chronic inflammation in gallbladder. This results in gallbladder stones and mutations in the DNA damage repair pathway which progress towards cancer.

Work in Progress

To test the hypothesis, a representative model recapitulating either normal or diseased gallbladder is required. Organoids, representing the same basic intrinsic patterning events i.e. organ-like organization, are valuable tools for disease modelling. Development of tissue specific organoids mostly relies on the fact of pluripotent nature of the tissue resident adult stem cells or embryonic stem cells.

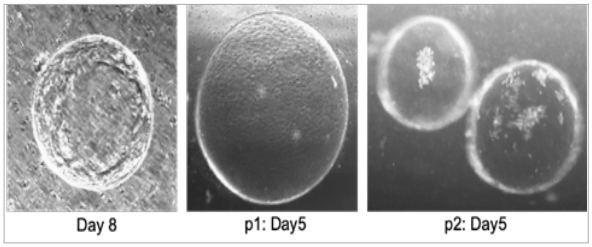

Figure 12. Adult stem cell derived organoid. Normal gallbladder was collected from patients undergoing Whipples surgery after informed consent, Tissue resident adult stem cells were enriched in culture condition and seeded with Matrigel. Organoids began to form on day 5. A full grown organoid was observed on day 8 (left panel). Organoids were split and full grown organoids obtained on serial passage – passage 1 middle panel and passage 2 right panel.

At TTCRC, a dedicated organoid facility has been established. Two different approaches to develop gallbladder organoids from the primary tissue have been developed. Tissue resident adult stem cells are enriched in culture conditions and allowed to grow on matrigel droplets to obtain stem cell derived organoids (Figure 12). Parallel to this approach, mature cholangiocytes obtained from tissue are cultured to generate mature-cell derived organoids on matrigel (Figure 13). Both

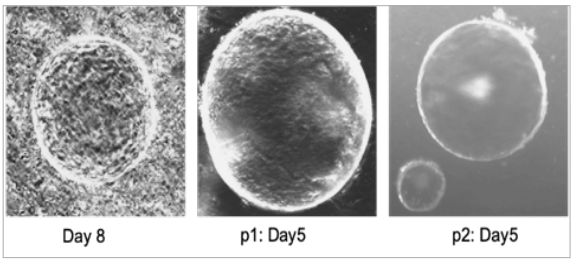

Figure 13. Mature cholangiocyte derived organoid. Normal gallbladder was collected from patients undergoing Whipples surgery. Mature cholangiocytes were isolated and seeded in Matrigel. A full grown organoid was observed on day 8 (left panel). Organoids were split and full grown organoids obtained on serial passage – passage 1 middle panel and passage 2 right panel.

approaches have successfully generated organoids from both normal and tumour gallbladder tissue (Figure 14).

Future plans

In the coming year, tissue features, histopathology and proteo-genomics

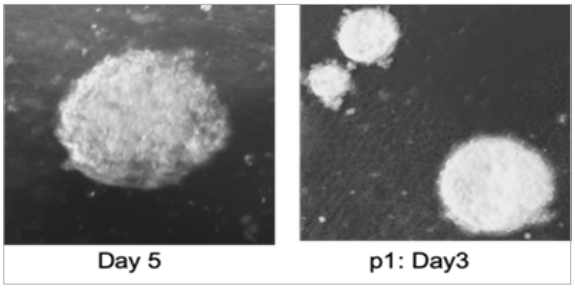

Figure 15. Gallbladder cancer derived organoid. Malignant gallbladder was collected from patients undergoing surgery. A full grown tumour-organoid (tumouroid) was observed on day 5 (left panel). Tumouroids were split and full grown tumouroids obtained on serial passage – passage 1 right panel.

Figure 14.

characteristics of the normal and tumour organoids will be correlated with their respective primary tissue counterpart. Preservation of tissue functions by organoids will be checked using organ-specific functional assays. Once confirmed, the normal organoids, genetically modified with or without specific mutations, will be exposed to environmental stresses to study the pathogenesis of gall disease.

Challenges

Variability in the system offers major challenges in organoid modelling. Preservation of major genetic features between patient and corresponding model and retention of the clonal composition of the original tumour or reflection of the tumour heretogeneity in the models are yet to be proven. Moving forward, major efforts are required to provide information of the long-term genetic stability of this specific culture system. Current model system lacks integration of other cell types in culture, such as fibroblast-like cell types, immune cells. More complex co-cultures are needed to comprehend the reciprocal influences among the various cellular components. Another major challenge to the field requires further optimisation of culture conditions, including the composition of culture-supporting matrices (Biomaterials).

Team Composition

At the Tata Medical Centre, the clinical team includes the Hepato-billiary surgeons, Digestive disease, Medical oncology, Palliative care unit, Radiology and Pathology. A dedicated clinical trial unit works in close contact with the clinical and research team to follow up and track patients reporting to the clinic and undergoing treatment. TTCRC biobank team actively collects a process samples from patients consented for the study. Downstream multidisciplinary research work includes cell biology, genomics, proteomics team and bioinformaticians at TTCRC.

The work is further supported by national and international collaborators at NII-Delhi (DDR pathway), IIT-Bombay (Biomaterials), University of Manchester (Proteomics, Biomaterials) and University of Cambridge (Organoids).

Dr Arunabha Chakrabarti

Arunabha Chakrabarti

India Alliance DBT-Wellcome Fellow

Understanding the role of Ikaros deletion towards leukaemic cell survival and poor therapeutic outcome and its involvement of the deletion isoform IK6 in disrupting different cellular and molecular pathways leading to poor chemotherapeutic response and relapse

Aim

My research goal is to investigate the mechanisms by which the imbalances in *IKZF1* isoform expression promote leukaemic cell survival under cytotoxic stress. Understanding the impact of *IKZF1* deletion isoforms, particularly IK6, on cell survival after chemotherapy and the mechanism of drug resistance in the leukemic clones carrying the deletion isoforms is the major aim of my project.

Hypothesis

Ikaros, in association with other transcription factors (e.g. PAX5) restricts the supply of glucose and energy to the pre-B cells to prevent the malignant transformation. We hypothesize that, the dominant negative isoforms of Ikaros (IK6, carrying Δ4-7), at least in part, responsible for the metabolic adaptation of the leukemic cells with its microenvironment which helps their survival and proliferation and this metabolic reprogramming of the leukemic cells is different when the cells carry insignificant deletions in IKZF1 other than IK6 (e.g. IK8). Therefore, it is important to study the metabolic adaptation of the cells when there is complete deletion of the gene or there are other focal deletions (e.g. IK6) with respect to that when IKZF1 is wild type in those cells. I hypothesize that clones carrying Ikaros deletion, are more invasive in nature and that property helps the leukemic cells to migrate from the bone

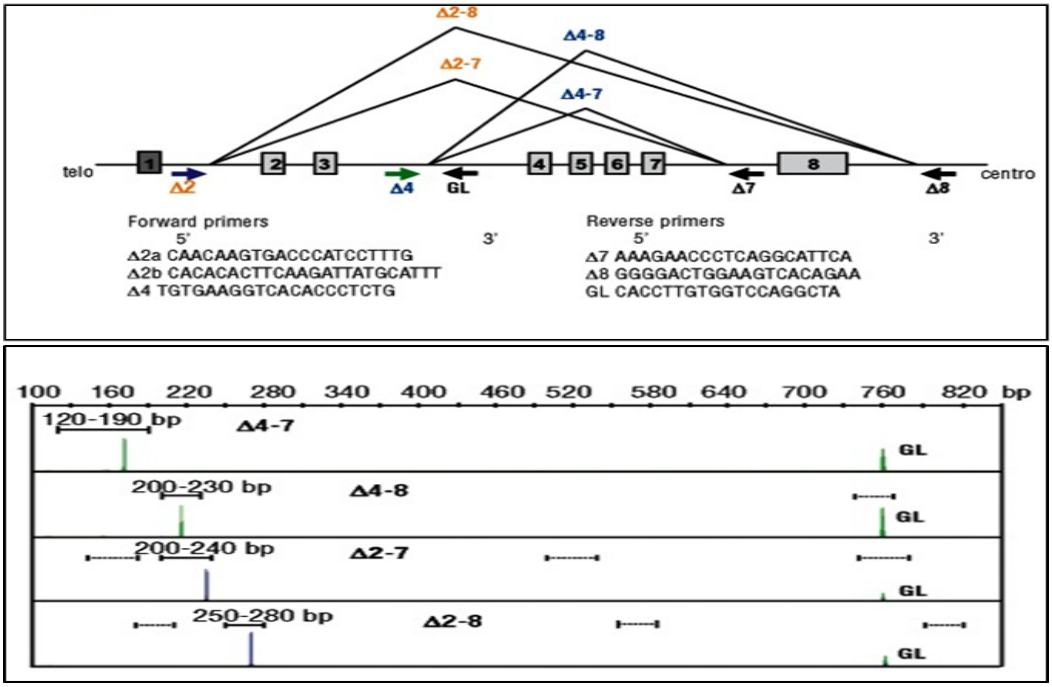

Figure 16. Multiplex fluorescent PCR design for the detection of *IKZF1* deletions in genomic DNA. Top: exon specific primer sequence and location within the *IKZF1* gene. Below: Fragment sizes generated by the specific deletions and isoforms.

marrow into peripheral blood and ultimately invade into various organs and tissues.

The wild-type Ikaros (IK1) is transported to the nucleus and has the ability to act as transcription factor since it contains all the DNA-binding domains. The IK6-Ikaros does not carry any DNA binding domain, therefore, cannot act as a transcription factor; but it carries the interaction domain which, may interfere, with IK1 in the cytoplasm to prevent its tumor suppressor function. We hypothesize that imbalance between Ik1 and IK6 which should contribute to the survival, proliferation, adherence and drug resistance to the leukemic cells.

Ongoing work

A. Patient screening for IKZF1

Screening of patients for *IKZF1* deletion has been performed using fluorescence based multiplex PCR followed by fragment analysis of the PCR products. The fluorescently labelled PCR primers throughout the 8 exons of IKZF1 and the fragments generated due to different deletion isoforms are represented in Figure 16.

Seventy B-other patients have been screened by PCR and 15 of them (21.4%) found to carry a deletion in IKZF1.

B. Generating IKZF1-knockout leukaemia cell lines

CRISPR-CAS9 mediated knock-out (ko) of IKZF1 has been performed in leukaemia cell lines NALM6 (Figure 17). This cell line expresses wild type Ikaros. The commercially available CRISP/CAS9 construct from SantaCruz Biotechnology (Sc-400530) is a mixture of 3 g-RNA plasmid specifically designed for disruption of Ikaros in the cell upon transfection. I used chemical transfection using DMRIE-C reagent from Thermo Fisher to create IKZF-null cell clones. After transfection, the cells were incubated for 48 hours and successfully transfected cells were single cell sorted for GFP+ cells in 96 well plates. The sorted cells were cultured and expanded to maintain the Ikaros-ko lines. Five of the single sorted NALM6 IKZF1-ko clones could be aseptically maintained and expanded in culture. The Ikaros-ko status

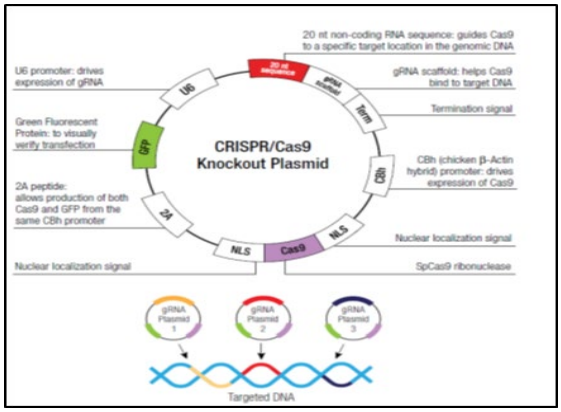

Figure 17. CRISPR-CAS9 strategy for IKZF1

were confirmed by western blot using Ikaros specific antibodies (D6N9Y; ab190691).

RNA sequencing analyses

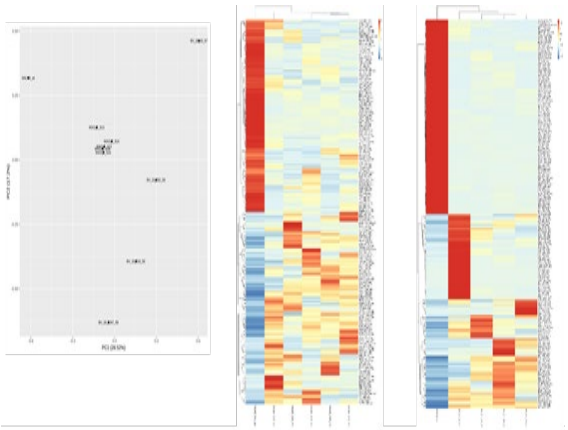

Figure 18. RNA sequencing of NALM6wt, *IKZF1*ko clones and 4 BCP-ALL patients with *IKZF1* deletion. Left panel PCA plot. Heat map of differential gene expression of (middle panel) ko clones and 4 patients and (left panel) with NALM6wt.

Whole transcriptome sequencing was perfomed using the TruSeq stranded Total RNA library prep kit (Illumina). Paired-end sequencing was performed using the Illumina Next-seq 550 platform with 75– or 150- bp reads.

Principal Component Analysis plot revealed that the five IKZF1-ko clones clustered together and well-separated from the wild-type NALM6 as well as the patient samples (Figure 18). Heat map was generated with the gene expression values of the IKZF1-ko clones compared to the wild-type and revealed a pattern of up- and down-regulated genes (Figure 18) due to disruption of functional Ikaros.

Transcriptome profile of the four B-other patients were also performed and the heat map produced distinct profile for the patients compared to the NALM6 cell line (Figure 3C), especially for the downregulated genes in the patient samples who actually carry IKZF1 deletions. Analyses of the RNA-seq data is ongoing for better understanding the pathways.

C. Creating expression constructs for IKZF1 isoforms and lentiviral transduction

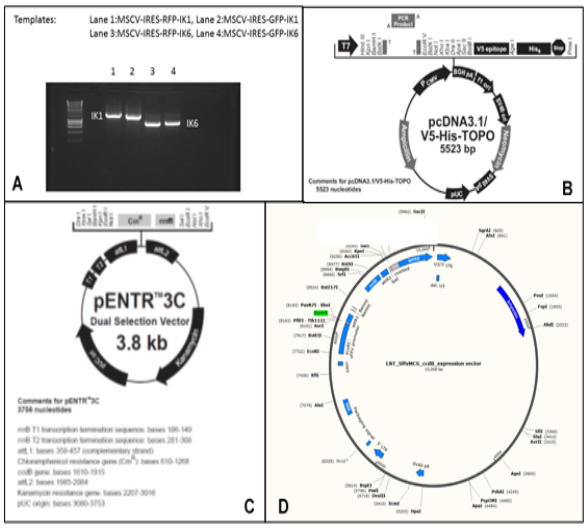

Figure 18. A. PCR amplification of IK1 and IK6. B, C and D vector maps.

Full length wild type functional Ikaros (IK1) and the exon 4-7 deletion isoform (IK6) were PCR amplified from MSCV-IRES-GFP-IK1 and MSCV-IRES-GFP-IK6 (gift from Charles Mullighan, St. Jude), cloned into pcDNA 3.1/V5-His TOPO TA mammalian expression vector and finally into the lentiviral vector pLNT-sffv for overexpression in leukaemia cell lines NALM6 and development of stable cell lines for overexpressing IK1 and IK6. GFP-2a was also inserted into these constructs for single cell sorting using GFP. The constructs (pLNT-sffv-GFP2a-IK1/IK6) were confirmed by sequencing and restriction digestion check. Vector details are provided in Figure 4.

Future plans

Lentiviral transduction for developing IK1 and IK6 overexpressing leukaemic cells pLNT-sffv-GFP2a-IK1/IK6 constructs would be used to create cell lines overexpressing IK1 and IK6. Lentiviral particles will be produced by transfecting the constructs into HEK293T cells using lentiviral packaging plasmids psPAX2 and pMD2.G (Addgene). Transduction of the virus particles collected from the HEK293T cells would be used to develop stable leukaemia cell lines for overexpression of IK1 and Ik6 into the CRISPR/CAS9 based IKZF1-ko NALM6 cells already developed. These stable cells expressing specific isoforms of Ikaros will be used for all the downstream studies.

Characterization of cell lines with different Ikaros status

Behaviour of the NALM6 cell lines with different Ikaros status would be compared with that of the wild type NALM6 cell line with respect to their viability, proliferation, metabolic status cell cycle analysis etc. IKZF1-ko NALM6 clones would be compared

with the wild type line and also with the IK1 and IK6 overexpressing cell lines after lentiviral transduction to understand the pathways which are affected due to deletion in Ikaros.

Chromatin Immunoprecipitation Sequencing (ChIP-Seq)

ChIP-seq would be performed to understand the Ikaros targets in the wild-type NALM6 as well as the IKZF1-ko constructs. This will help us to reveal how the transcriptional targets are changing due to disruption of Ikaros. This will be extended for the lentivirally transduced cell line where IK1 would be overexpressed to understand to what extent the cells regain the Ikaros transcription factor property when IK1 is reintroduced into the cell.

Transcriptomics (RNA-seq) and Proteomics (SWATH-MS)

RNA-seq analyses will be continued with the cell line constructs after lentiviral transduction of the IK1 and IK6 in NALM6 cell line. This data would help us to understand how the gene expression profile changes when IK1 is reintroduced into the IKZF1-ko lines.
For proteomics analysis with the cell line constructs with different Ikaros status (IKZF1-ko, IK6 overexpressed and IK1 overexpressed), we will perform SWATH-MS to get a whole expression profile in these cell lines. This profile would be compared to that of the transcriptomic expression in the same constructs. Overlapping genes/proteins in both the analyses would be considered for further validation in patient samples with different Ikaros status.
SWATH-MS has already been performed for the wild type NALM6 and IKZF1-ko constructs and analysis for the same is being performed using the Trans Proteome Pipelines, for SWATH data analysis.

***In vitro* drug sensitivity assays**

The cellular models developed with different IKZF1 status would be characterized for in vitro drug sensitivity using the chemotherapeutic drugs used in ALL therapy. The drug sensitivity would be performed under different types of stress (e.g hypoxia, glucose deprivation etc.) to see the effect of leukaemic cells' drug sensitivities due to disruption of Ikaros.

Details of collaboration

During my visit at the University of Manchester last year, I have developed collaboration with Dr. Annalisa Tirella and Dr. Jonny Blaker, both are involved in the research of developing biomaterials for different cancer cell types and tissues. biomaterials, we can replace the 2D co-
I also developed a collaboration with Prof. Anthony Whetton, Director, Stoller Biomarker Discovery Centre, University of Manchester. This collaboration would help me to develop SWATH based proteomics pipelines at TTCRC using the similar pipelines using Sciex Triple TOF 6600 and future proposal of a UK and India collaborative proteomics study with ALL samples.

Pritha Paul
India Alliance DBT-Wellcome Fellow

If 2018 was the watershed year for TTCRC, 2019 was for the TP53 project. While learning how to get around a wide gamut of challenges of working in a new research facility, we evolved from whiteboard scratchers to active researchers. With my work-outside-host-institute (WOHI) at University of Manchester scheduled for April 2019, a feeling of urgency marked the end of 2018 and beginning of 2019. The focus of those few months was to train Jaydeep and Rubina in high-throughput imaging and flow cytometry, respectively, along with the basic dos-and-don'ts of cell biology. These two platforms are to be extensively used for the TP53 project that primarily aims to understand how aberrant regulation of p53 signaling pathways impact key adaptations required by acute lymphoblastic leukaemic (ALL) cells to

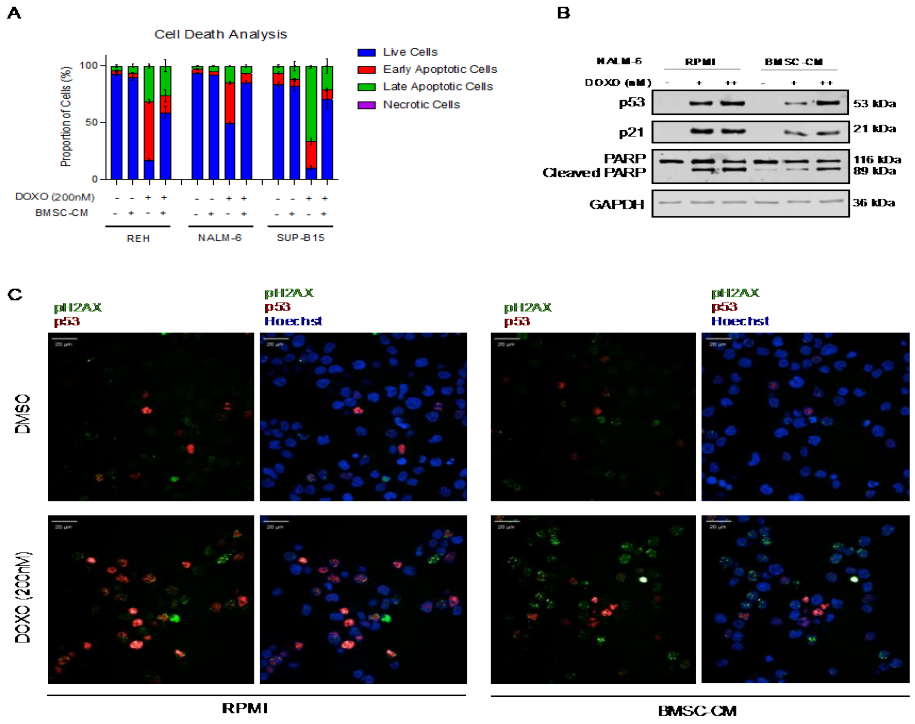

Figure 1: The bone marrow microenvironment protects ALL cells from chemotherapeutic stress *in vitro*. (A) ALL cells grown in BMSC-CM had a relatively lower percentage of cells in apoptosis (early and late) when treated with doxorubicin (200nM for 24h) in comparison to RPMI-fed ALL cells as assessed by flow cytometry using Annexin V-FITC/Propidium Iodide staining, (B) Immunoblotting-based assessment of doxorubicin treated ALL cells showed decreased expression and activation of p53 in BMSC-CM versus RPMI. Levels of cleaved PARP were decreased in BMSC-CM-fed doxorubicin treated ALL cells, indicating failure of chemotherapy to induce apoptosis, (C) Immunofluorescence staining confirmed decreased expression of p53 in BMSC-CM-fed ALL cells in comparison to RPMI-fed cells upon doxorubicin treatment. BMSC-CM, bone marrow stromal cell-derived conditioned medium; DOXO, doxorubicin.

withstand environmental and cytotoxic stress. By the end of 2018, Jaydeep, Rubina and Sanjali (our then-intern, currently pursuing a Master's degree in Public Health from Columbia University, USA), had provided an interesting insight - dampening of wild-type p53 signaling in a proportion of ALL cells could be one of the mechanisms by which the stromal microenvironment promotes survival in those cells (Figure 1).

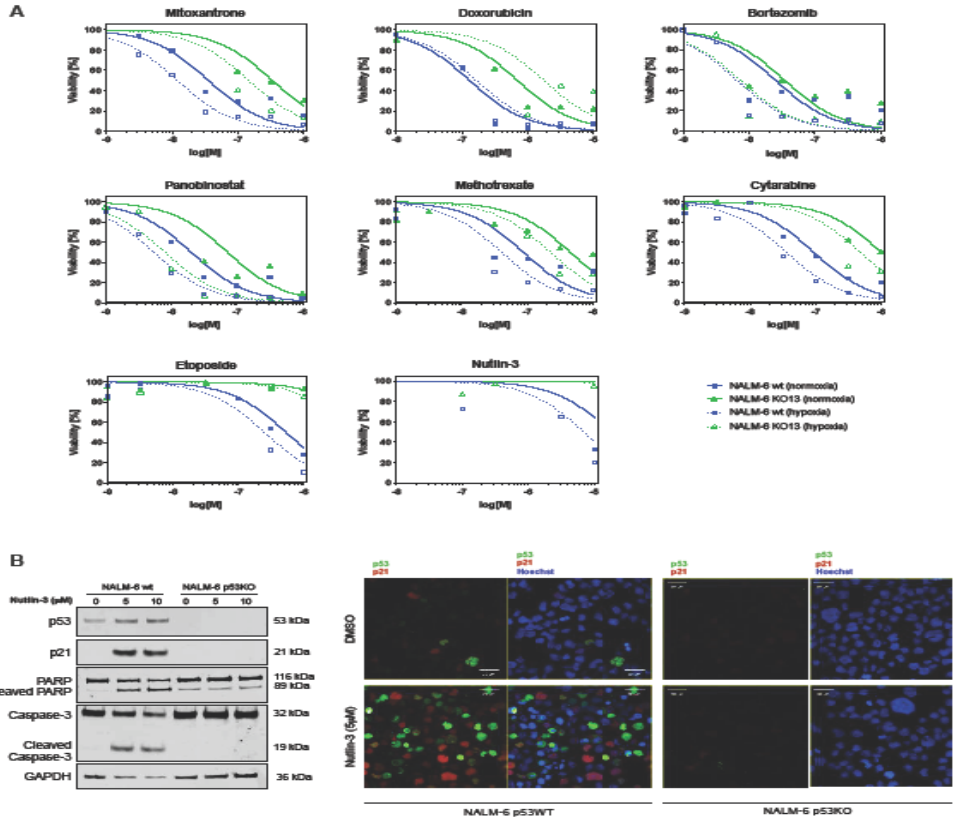

Figure 2: p53 knockdown renders ALL cells resistant to chemotherapeutic stress *in vitro*. (A) ALL cells with *TP53* knockdown showed decreased sensitivity to cytotoxics commonly used in ALL treatment as assessed by WST-1 assay. (B) *TP53* knockdown was confirmed by lack of response to Nutlin-3 treatment and immunoblotting and immunofluorescence for p53 signaling pathway. KO, knockout.

The reason why wild-type p53 induces cell cycle arrest in some cells versus apoptosis in others is incompletely understood. More importantly, how this may contribute to intratumoral heterogeneity is a relevant topic of investigation. Amidst the experimental struggles, we were privileged to host Dr JP Muliyil for a 2-day Biostatistics workshop that was eventually attended by 60 odd participants from varied departments of Tata Medical Center and TTCRC.

The last nine months focused on understanding how ALL cells adapt to the loss of TP53 in the new Childrens' Cancer Group lab in CRUK-MI, Alderley Park. This stint also provided opportunities to explore facets of the microenvironment, especially in terms of hypoxia and co-culture experiments. Loss of TP53 imparted resistance to ALL cells to majority of cytotoxics commonly used to treat ALL (Figure 2A), and transcriptomic analysis identified loss of p53 direct targets involved in apoptosis (e.g. *FAS, BAX*, B*BC3*) and cellular stress (*TP53INP1*, *ZMAT3*). It was not surprising that loss of TP53 reduced cytotoxicity-induced DNA damage as assessed by H2AX foci formation, and frequently exhibited giant, polyploid cells (Figure 2B). Whether TP53 deficiency helps cells adapt to proteomic stress induced by aberrant gene dosage would give us insights into the pathogenicity of structural aneuploidy as in low-hypodiploids with chr17 monosomy or high-hyperdiploids with isochromosome 17q.

In 2020, these preliminary observations will be extended to understand (i) how the niche supports dampening of p53 signaling in ALL cells treated with cytotoxics and interventions that can rescue this subversion – this may improve treatment efficacy, (ii) whether chemoresistance in TP53-deficient cells is dependent on the niche and can thereby be salvaged by altering its niche – this might minimise need for treatment intensification, and (iii) how acquisition of certain mutations (p.R248Q and p.G245S) alters the tumour suppressive functions of TP53 in ALL.

Dr Jasmeet Sidhu

Priyanka Bose
Research Assistant

Arka Bhowal
Research Assistant

Jasmeet Sidhu

India Alliance DBT-Wellcome Fellow

I completed my undergraduate training in medicine at the Government Medical College and Hospital in Amritsar and paediatric training at the Dayanand Medical College and Hospital, Ludhiana. From 2016-18, I completed training on paediatric haematology and oncology at the Tata Medical Center, Kolkata and joined TTCRC as a clinical research fellow in January 2018. I was awarded a Wellcome trust-DBT Early career fellowship (clinical) in September 2019.

Aim: To identify strategies to decrease minimal residual disease (MRD) in acute lymphoblastic leukemia (ALL)

Hypotheses

Optimisation of the use of key biologic drug, Asparaginase, during treatment as well drug response profiling of patient-derived leukemic cells on in-vitro high throughput platform can detect sensitivity of available drugs that may otherwise not be given in induction treatment of ALL, can both act as strong strategies to decrease MRD (Figure 21, Top).

Acute lymphoblastic leukemia is the most common childhood cancer, accounting for 80% of childhood cancers. In addition to genetic risk classification, molecular tests to detect post-treatment minimal residual disease (MRD) at sensitivities of 10-4-10-5 cells are now part of modern ALL management. Though high-risk cytogenetic subtypes have proportionately higher MRD levels at the end of induction, MRD remains an independent prognostic variable (1). Current therapeutic strategies for ALL is based on risk stratified therapy, based on pre-treatment genetic risk

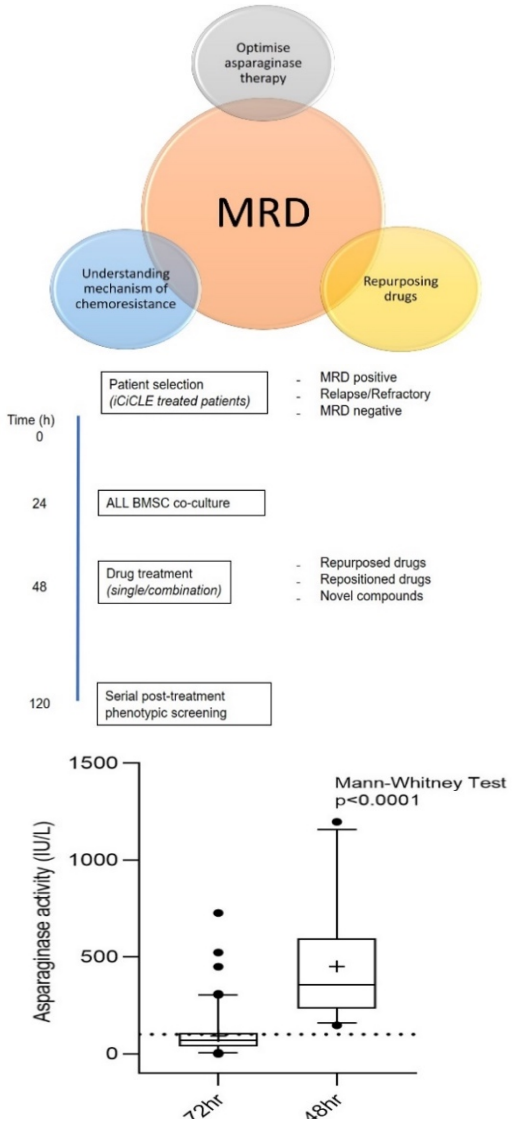

Figure 21.

Top. Components of proposed analyses to decrease the post-induction MRD Burden. **Middle**. Workflow for high-throughput phenotypic drug screening of patient-derived leukemic cells.

Bottom. Box and whisker plot of trough asparaginase activity during induction (IND) with every 72-hour administration and every 48-hour administration. Whiskers are at 5th and 95th centile, horizontal line indicates median and a plus sign within the box the mean. Adequate asparaginase activity (≥ 100 IU/L) was observed in 26% (29/110) of trough induction samples in 72-hr administration as compared to 100% (23/23) with 48hr administration.

stratification and post induction MRD assessment. Almost all protocols use the same 3-4 drugs in induction and an additional 6-8 are used to intensify post induction therapy to eradicate the MRD population. Current focus on newer therapies are directed at targeted small molecules and antibodies at a cost not affordable by our patients.

Progress so far

A key drug for induction is L-Asparaginase (ASNase), a bacterial enzyme whose activity varies according to source of the enzyme and manufacturing processes (2). I first proposed to standardise the dose and scheduling of a currently available generic native-ASNase to optimise its activity. Patients receive intramuscular asparaginase at a dose of 10000 U/m2 every 72 hours as per schedule of InPOG-ALL-1-ICiCLe. According to analysed pharmacokinetic data, in vivo enzyme activity of Leucoginase was adequate up to 48 hours' post-administration but showed inadequate levels at 72 hours (3). Based on the results, a pilot study has been initiated to administer the drug at 48 hours' interval, with prospective monitoring of trough activity and toxicity profile. Preliminary data shows optimal trough activity with 48hr schedule (Figure 21, bottom).

I am currently in the process of standardising the workflow for high-throughput phenotypic drug screening of patient-derived leukemic cells (seeding density of leukemic cell-stromal cell co-cultures, concentration range of drugs to be used, live cell image acquisition and analysis and quantification of drug response.

Future plan: This workflow will be used to screen the patient-derived leukemic cells against a range of repurposed drugs to identify sensitive agents and to identify survival pathways of residual resistant cells through transcriptomic analysis (Figure Top Left) (4).

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Amit Saxena
Head, TCS Genomics Lab

Anju Goel
Program Manager, TCS Life Sciences

Divya Narayan
Program Manager, TCS Life Sciences

TCS @ TTCRC

TCS is contributing to accelerate research initiatives by deploying platform and solutions for Translational Research. The Translational Research Platform allows for collecting and integrating clinical research data, molecular data, patient Electronic Medical Records (EMR) followed by cataloguing of data and creation of research database. The platform further has functionality to capture research hypothesis and generate hypothesis based patient cohorts. Specific concepts extracted from the research database can be used for exploratory analysis and visualisation or pattern mining and analytics for validating any scientific hypotheses.

Some of the enhancements planned for the platform include:

- Integration of imaging data e.g. cytometry
- Enhanced Project Management features and dashboards
- Enhanced functionlaties to the LIMS
- Enhanced analytics

Functionalities and solutions implemented as components of Translational Research Platform:

1.Data Ingestion System developed and deployed to extract data from electronic medical records in TMC's HMS system and upload into the research database as per the needs of clinicians / researchers.

2.HMS mirror setup for TTCRC to facilitate clinical data extraction from HMS

3.OMOP: Patient cohort creation by querying ontology based data elements from the HMS, is another functionality of the system. While the initial implementation of open source i2b2 for cohort creation based is operational, this is now being replaced with the OMOP model while is a more widely used open source model for healthcare data.

4.Functionalities of natural language processing using cTakes and visualizations and analytics using Kibana are in place, details of which can be viewed in previous annual report.

5.TCS also provides support to enable necessary IT infrastructure including server configuration, storage systems for genomic data processing, platform deployment and a 50Mbps ILL link to its Noida center.

6.Data Driven Discovery – One touch Analytics Platform within TRP**:** Module catering to data analytics needs of researchers integrated within TRP. Works in both Hypothesis driven as well as Data Driven modes.

7.LIMS: Lab Information Management System is an application developed for Equipment, Consumables and Project management. It helps lab user/admin to monitor equipment and consumables in an experiment and keep records for the same, such as Installation details, calibration details, vendor details, inventory etc. Major functionalities:

Equipment Inventory: user can view the whole list of available equipment. User can also add, bulk upload and update various details related to Equipment Inventory like Storage Location, SOP, vendor details, company details, AMC contracts, Engineer details, etc. This helps to track the Equipment management including usage, breakdown and repair.

Live status dashboard: There is also Live Status dashboard for alerts and

notifications. Lab user can directly keep track of Installation, Repair, Calibration and Maintenance status etc.

Project Management: In order to facilitate and streamline the activities of Research projects, the Project Management module has been developed and integrated with the Translational Research Platform.

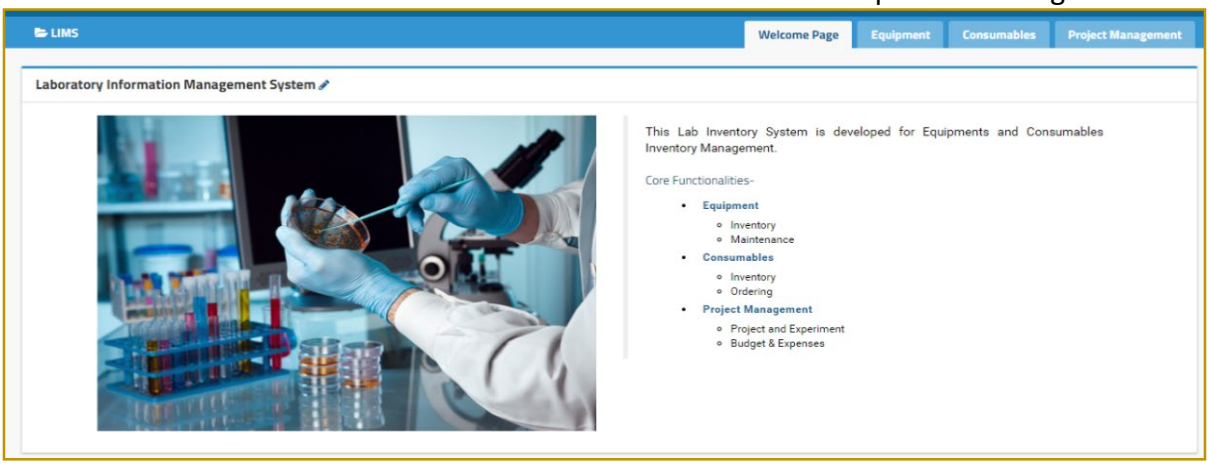


Figure 22. The Laboratory Information Management System

Consumable Management: LIMS also has feature to keep track of consumables in the laboratory. They can add, update and delete consumables from the list and check if the consumable is below certain threshold (minimum stock level). This would help in initiating the ordering process.

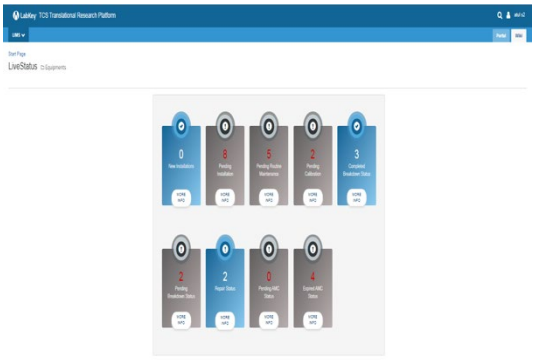


Figure 23. Equipment Status

Project consumable and minimum stock level report: LIMS also has facility to view Project Consumable report, where user can track all the consumables used in a particular project and associated experiments. Lab user can define new project and experiments, and which can be mapped to the consumables.

Project Management Module helps user create Research Projects and add subprojects- both Experiment and Analysis The module captures all the relevant data about the Research Project - Project Owner, Principal Investigators, Budget, Milestones, Deliverables etc. project, user can view the list of its sub-projects, i.e., Analysis Projects and Experiment Projects.

Sub Projects include details like, Principal Investigator, budget required, funding, project id etc.

Experiment Projects are linked to LIMS module and Analysis Projects are linked to Data Driven Discovery module to create hypothesis and then after analyse the results using various algorithms and tools.

User also has the ability to view Project Dashboard where details of all Research Projects, Experiment and Analysis Projects and related are displayed.

Clinical Trial Management:

TCS has deployed its IDM 3.0 platform for ICiCLE trial. The platform is operational at 5 centres with ~ 1700 patients screened and ~1500 patients enrolled. Some of the

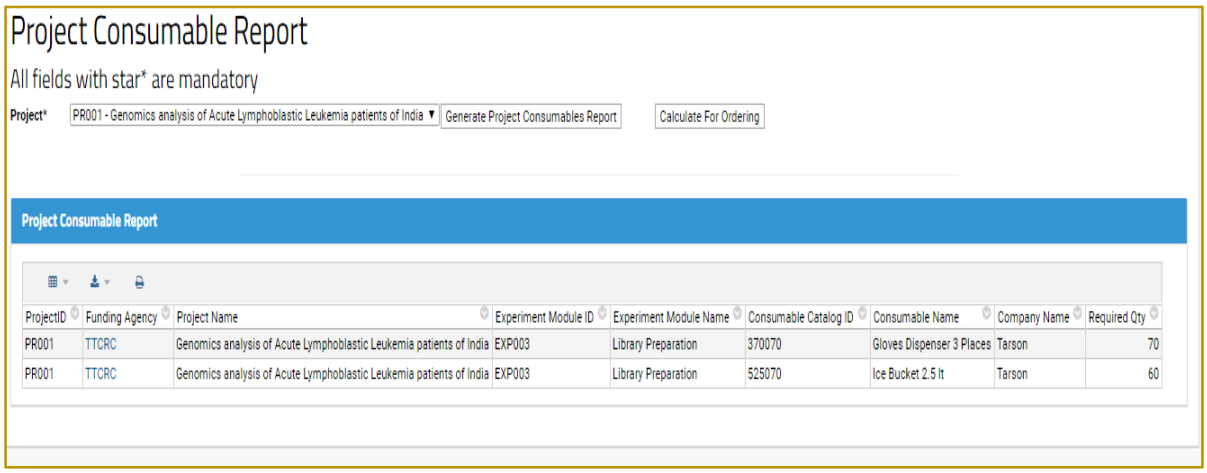
major enhancements done to the EDC include

New form for collection of cytogenetic data

Introduced Page Lock feature for critical CRFs

Creation of standard listings (for CRFs available in study)

Creation of study specific listing (Dataset creation)



ProjectID	Funding Agency	Project Name	Experiment Module ID	Experiment Module Name	Consumable Catalog ID	Consumable Name	Company Name	Required Qty
PR001	TTCRC	Genomics analysis of Acute Lymphoblastic Leukemia patients of India	EXP003	Library Preparation	370070	Gloves Dispenser 3 Places	Tarson	70
PR001	TTCRC	Genomics analysis of Acute Lymphoblastic Leukemia patients of India	EXP003	Library Preparation	525070	Ice Bucket 2.5 lt	Tarson	60

Figure 23. Project Management

TCS also provide data management activities such as data compilation for DSMC submission and preliminary presentation that included CONSORT, Patient and Response Characteristics, Cytogenetics, Events Classification; site queries for discrepant and missing data; responses to queries from sites related to specific issues and content compilation for Data Management Training.

TCS also provides maintenance support for User Access Management, deletion of users, unlock requests and resolving issues of all site through JIRA tool.

ALL R1

ALL R1 is a risk-adapted treatment protocol, to standardise management of children with untreated first relapse of Acute Lymphoblastic Leukaemia (ALL) at Tata Medical Centre. The protocol is a modification of the international ALLR3 protocol (ISRCTN45724312) and uses a combination of patient clinical information to identify and treat patients with relapsed ALL. This protocol has been introduced as a multicentre Indian Paediatric Oncology Group clinical study (InPOG-ALL-19-02; CTRI/2019/10/021758) coordinated by the Clinical Trial Unit.

For ALL R1, advice on the support, design and customization of database is done by

TCS. Data Management related activities like collecting, recording, managing and extracting of trial data is carried out using our EDC system - Integrated Data Management- IDM (v 4·0), developed by TCS. Similar to the ICiCle trial, TCS will also plays an important role in establishing and maintaining Quality Control (QC) methods and process like creation of data listings, review of listing to identify ambiguous, incorrect, missing data and following up with sites to verify and rectify the same to ensure that trial related data meet overall quality goals. Additionally, steps for Quality Assurance will be ensured through creation of documents like Data Management Plan, CRF completion guidelines and its adherence. The database support activities like User and Access Management for all the participating centres will also be managed by TCS. Ongoing pertinent and feasible modification to the database due protocol

amendments is another task that will be supported by TCS

Extracting cumulative billing details of patients from HMS and then the data by creating boxplots (T-test method) using R.

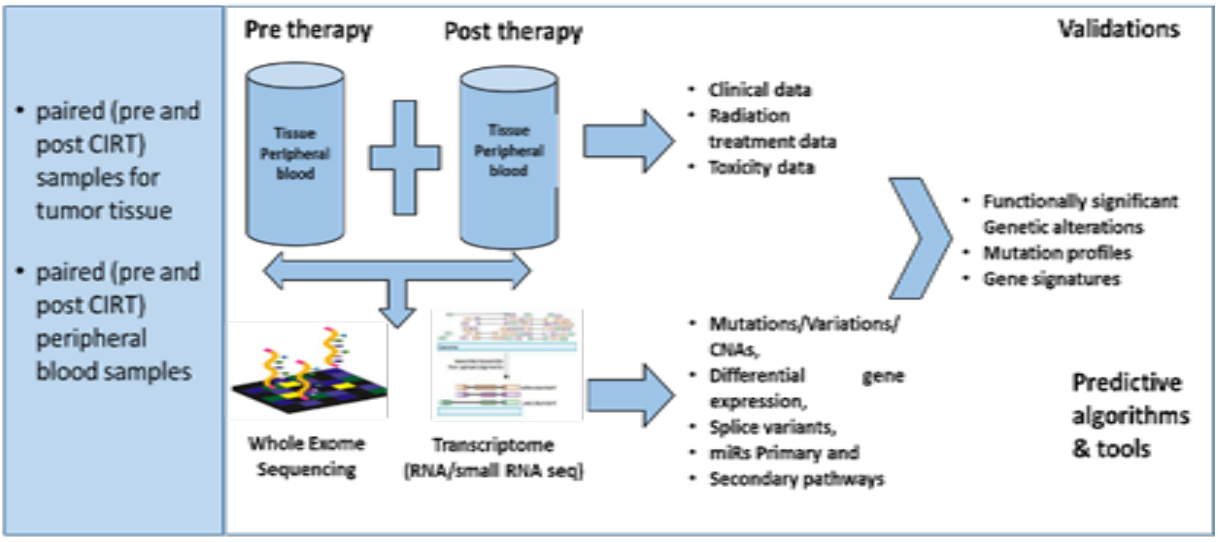


Figure 24. Breast Tumour Project

Ongoing Projects

Creating the latest dataset for DSMC report by combining data related to Registration, Risk Stratification, Study Consent forms along with details of End of Induction as well as Death, Relapse and Trial Withdrawal forms.

Creating the stored procedure based on the above forms which is then deployed in CTMS production environment and the complex report is thus created in CTMS IDM platform which can be used for real time data analysis.

Extracting data based on the paediatric patients and which departments did they visit and what type of diagnosis is done for all these patients and also this data is analysed using Kibana.

Analysis of Gall Bladder cancer data using Kibana which is based on various categories of Gall bladder cancer as per morphological classification.

Analysis of Gall Bladder cancer data using Kibana which is based on various categories of Gall bladder cancer as per morphological classification.

Extracting data based on no of males as well as females who remains as Standard risk patients and those patients who

moved from Standard Risk to High Risk.

Some of the future projects planned:

Integration of Gall Bladder Cancer project related data from both Redcap as well as HMS into a single database platform.

Creating various graphs and figures based on the pre-trial dataset.

Translational Study in Breast Cancer: Markers and tools for the prediction of response to radiation therapy /or sensitivity: Dr Binuja Verma

Collaborators: Dr. Sanjoy Chatterjee, Dr. Rosina Ahmed

The ongoing project is a part of HYPORT Trial which aims to develop markers and tools for the prediction of response to radiation therapy /or sensitivity in breast cancer patients undergoing Hypo-Fractionated Radiotherapy Schedule of 35GY in 10 Fractions in advanced incurable Breast Cancer

Radiation genomics has merged as an important translational research domain, investigating the association between patient genomic data and the response to radiation therapy. Several mechanisms have been contributing to radioresistance which include micro-environmental hypoxia, abnormal intrinsic DNA damage response (DDR) activity, deregulated survival pathway engagement through

constitutive activation of growth factor receptors, and/or mutations of oncogenes or tumour suppressors (Begg et al, 2011). Besides, a proportion of patients have a greater predilection to develop late radiation toxicity.

Although a large number of genetic association studies in breast cancer and other solid tumors are reported in last 10 years, most studies have involved only small patient numbers and replications carried out failed to validate previous findings. The lack of independent validation for any of the SNPs studied makes it impossible to say with certainty whether any predispose a patient to suffer toxicity and these studies have lacked the statistical robustness required for changing patient management. So also, the predictive power of gene expression based radiation sensitivity signatures (RSS) have to be validated in cancer patients as most of correlations are based on post-radiation clonogenic survival data in the cell lines.

A major challenge for radiogenomic studies is to obtain cohorts of patients with comprehensive toxicity data along with other data on possible non-genetic risk factors. As radiation therapy is a key modality in the treatment of cancer, it is of tremendous importance to increase our understanding of the molecular pathogenesis of radiotherapy toxicity. It is critical to develop tools for early prediction of treatment response and patient outcome as it offers a better management of treatment regime thereby making decisions to continue or to change treatment options. This is imperative to maximize patient survival while minimizing adverse effects and maintaining patients' quality of life.

We have initiated the genome sequencing project with the objective to analyze and identify the genetic variations associated with clinical toxicity and treatment response. The study will allow us to evaluate the role of genetic variations in well-characterized and uniformly treated breast cancer patients. This will be followed by functionally characterizing these genetic alterations with transcriptomics. The role of microRNA in radiation toxicity is would also be explored. In spite of the immense potential clinical utility of miRs, very few studies have developed strategies to analyse the significant miRs in patients receiving radiotherapy.

Cohort of 30 breast cancer patients treated with hypofractionated radiotherapy schedule of 35GY in 10 fractions. The clinical data for radiation induced toxicity measured by using CTCAE version 4 and LENT SOMA toxicity criteria (analyzed physiological methods and validated questionnaires). The clinical assessment and outcomes will be evaluated for acute and late radiotoxicity endpoints (breast pain, ulcer, arm oedema, tumour size etc.) at prescribed intervals. Radiological assessment using PET-Scan and response will be assessed using PERCIST 1.0 criteria. Assessment of quality of life also will be carried out and patients will be followed up for weekly during radiotherapy, then monthly for 1st three months, then 3 monthly for two years, then 6 monthly for

subsequent 3 years (Ref: TMC-HYPORT Protocol)

Pilot Genomics study

In this phase we attempted to standardize and establish the experiment and analysis pipeline for exome sequencing using DNA extracted from a subset of FFPE study samples (N=12/fine needle biopsy samples) using International guidelines (TCGA and Genomics england https://www.genomicsengland.co.uk/about-genomics-england/the-100000-genomes-project/information-for-gmc-staff/sample-handling-guidance/).

We have completed the standardization experiments and has generated the pilot set of whole exome data. The sequence data is being analysed for coverage and depth. As these are FFPE DNA, we will finalise the sequencing strategy by which would be feasible to identify both SNVs and CNVs.

Dr. Ruma Dey Ghosh
DHR Woman Scientist

Akash Bararia
Biobank Technician

Sudhritti Guha Majumdar
Research Intern

Head & Neck Cancer

Currently, we are working around the development of biomarkers mainly through non-coding RNA molecules for oral cancers that will make diagnosis more precise, offer tailored therapy, and reduce the burden of over treatment and improve outcome as well as to understand the reason for treatment failure in different stages of cancers. We are aimed to explore the biology of different non-coding RNA-mediated regulation of gene expression and its alteration during disease progression and to understand their role in differential prognostic outcome in patients.

The importance of non-invasive biomarkers in molecular diagnostics is undisputed. Accessing the molecular information through specific biomarkers from minimally invasive sampling methods is thus highly valued. The plasma-derived cell-free small non-coding RNAs (ncRNA) including miRNA could have diverse origin and function. Studies have demonstrated that the stability (the most clinically exploitable feature) of circulating cell-free miRNA. Recently, one of the major roles of these cell-free-miRNA is revealed in cell-to-cell communication as endocrine signals. It has also been found that the spectrum of these cell-free-miRNAs is altered under various patho-physiological conditions.

Therefore, in our study, preliminary we have used peripheral blood plasma collected from OSCC patients. To achieve our goal, extracellular cell-free microRNA profiling has been carried out through miRNA expression microarray (Agilent) for prognostic predictive biomarker development in patients with oral squamous cell carcinoma. In this study, the patients were subcategorized on the basis of nodal status, disease-risk prediction, and actual patient outcome (recurrent and non-recurrent) after follow up. In the present investigation, we have analyzed the cell-free miRNAs expression profile through miRNA expression microarrays for different groups of OSCC-patient's plasma samples. The results revealed differential expression of different miRNAs (p-value< 0.05) between the different groups of OSCC-patients from the Agilent miRNA expression microarray experiments. These differentially expressed down regulated or upregulated miRNAs are being evaluated through quantitative real time PCR (qRT-PCR) method using QuantStudio 7 Flex real-time PCR system.

In another investigation, we have also studied the potentiality of mature miR-100-5p whether it could be used as a predictive cell-free biomarker from OSCC-patient plasma samples for OSCC prognostication. Here, we want to correlate their functional relationship between the conventional histo-pathological predictions and the level of cell-free mature miR-100-5p expression in plasma to predict the disease prognosis in patients with OSCC. We have also studied that miR-100 can sensitise the cisplatin resistant OSCC cells (SCC25/R, SCC084/R and SCC131/R originated from their parental cell lines SCC25, SCC084 and SCC131) to cisplatin probably mediated through beta-catenin pathway.

Our goal is to identify specific miRNA expression signature in plasma associated with differential OSCC patient-outcome.

Gynaecological Oncology

Systems Medicine Cluster (SyMeC) is an ongoing project that is a proposal conceptualized by the National Institution of Biomedical and Genomics (NIBMG), Kalyani to conduct a multidimensional research project on integrating basic science, clinical and translational science in medicine using cancer as a prototype. Tata Medical Center is the clinical nodal center of this consortium with NIBMG, IICB, IISER, Bose Institute and ISI. This project is funded by Department of Biotechnology (DBT) from 2017 till 2021.

The SyMeC cervical cancer project team is involved in the prospective recruitment of 2500 women who undergo screening for cervical cancer and 300 women who are affected by cervical cancer for the study. The objective in cervical cancer screening study is to find possible biomarkers for HPV persistence in women which lead to cervical cancer and help triage them for colposcopy and treatment for precancerous lesions. The objective in cervical cancer patients is to find possible biomarkers for treatment failure to standard chemoradiation therapy and identify possible biomarkers and radiosensitizers that can reduce this failure and recurrence of cervical cancer.

TMC-K being the clinical nodal center provides the clinical data and biospecimen of screened women and women undergoing treatment for cervical cancer. The collected biospecimen in the form of tissue, vaginal fluid and blood are stored under required condition in the Institutional Biobank (TiMBER) as per protocol for the translational work on genomics, metabolomics, proteomics and immune studies are biobanked in the TTCRC repository and transferred to the respective institutions for various analysis as per protocol. A part of the cervical scrape is utilized for HPV detection via Qiagen HC2 Hybrid Capture II test. Genotyping of HPV is conducted by nested PCR. Identification of the various subtypes of HPV will be conducted via mRNA sequencing shortly.

We screened 1496 women this year (606 women within the hospital OPD and 890 from the community) thus completing our 2500 screening target. Primary HPV testing has been performed for all the women using HC2 technique. The targeted cohort of 2502 women will be followed till Aug 2021 to observe the outcome of the HPV positive women and correlate it with the viral and host factors that include genotyping, genomics, and microbiome environment. We have so far transferred 4000 biobanked samples that includes whole blood, plasma and cervical scrapes to NIBMG for genomic studies. 80 tissues and 1200 swabs from cancer patients have been transferred to IICB for flow cytometric and microbiome studies respectively. We have recruited 182 women with cervical cancer till date where the tumor tissue would be studied to help identify genomic and immune signatures/biomarkers that would be correlated to their outcomes to treatment in their follow-up. Primary cultures have been generated from 30 patients followed by characterization using epithelial and fibroblast/CAF markers. Functional imaging using of MRI images of these patients has been determined by analyzing the parameters such as texture and diffusion. Two other parameters viz. bold and perfusion are on the process of analysis. The identification of these biomarkers in cancer affected women would identify who would likely fail to respond to standard treatment and help clinicians tailor treatment with additional

modulating protocols that would improve their outcome to treatment.

CLINICAL HEMATOLOGY & HCT

Dr. Vivek S Radhakrisnan
Senior Consultant

Our key areas of clinical and lab research interest are focused on mainly the following categories i) Investigator Initiated Registry Studies, ii) Translational Projects (Investigator initiated) and iii) Investigator Initiated Prospective Clinical Research Protocols, iv) Pharma Sponsored Registry Studies and v) Pharma Sponsored Clinical Trials.

Investigator Initiated Registry Studies

a)Lymphoma Registry Project- Database formation of details of lymphoma patients in ONCOCOLLECT software.

b)CML Registry Project- Database formation of details of CML patients in ONCOCOLLECT software

c)Cost-Analysis and Outcomes project in Acute Myeloid Leukemia and MDS, includes the formation of a database of the entire treatment expenses of retrospective and prospective AML and MDS patients taking treatment from Tata Medical Center, in REDCAP software.

d)T-cell Project 2.0- This ongoing project includes making of a detailed database of response and outcome T-cell lymphoma patients taking treatment in Tata Medical Center.

e)CRIMSON Project- Database formation of details of cancer patients receiving immunotherapy and precision medicine in Tata Medical Center.

f)CIBMTR Registry / Database for Hematopoietic Cell Transplantation and Cellular Therapies- CIBMTR (Center for International Blood & Marrow Transplant Research).

g)IMAGE Study- Creating a database of Multiple Myeloma Patients in the software called Care 4 Myeloma (Dr. Jeevan Kumar).

Translational Projects (Investigator initiated)

a)ALTITUDE Study- Acute Myeloid Leukemia: exploring the feasibility of multimodal-omics based genomic characterization, MRD evaluation and Computational Drug Modeling to inform disease management

Aim and Objectives:

To establish a Precision oncology work platform at Tata Medical Center in patients with Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome with excess blasts (MDS-EB).

Multimodal-omics based comprehensive genomic Characterization of a uniformly treated cohort of AML patients, accompanied by computational modeling POP model and MRD assessments.

b)Immuno-Oncology: GIFT Study- Genomic Immune proFile of Tumour- Excavating the relationship between genomic alterations and tumor immune microenvironment in oral squamous cell carcinoma – gingivo buccal (OSCC-GB) to inform immunotherapy

Aim and Objectives:

To excavate the relationship between the burden and nature of genomic/epigenomic alterations (single nucleotide variants, copy number variants, structural variants, methylation status) in tumour and the immunological profile (cell types, immune repertoire etc.) of the tumour micro environment (TME) and blood in a prospective cohort of locally advanced OSCC-GB patients, and to correlate with pathological features and treatment outcomes.

Investigator Initiated Prospective Clinical Research Protocols

a)R-BED Study- Phase II study of Bortezomib, Etoposide, Dexamethasone combination therapy, with or without

Rituximab, in Adult Relapsed or Refractory, B-cell Acute Lymphoblastic Leukemia who are transplant ineligible.

Aim and Objectives:

To determine the safety and activity of a combination therapy containing Bortezomib, Etoposide and Dexamethasone, with or without Rituximab, in relapsed refractory adult patients with B-ALL.

b)PRIME STUDY: Effect of Pomalidomide-Bortezomib-Dexamethasone induction on MRD status in patients with newly diagnosed Multiple Myeloma

Aim and Objectives:

To determine the activity of a combination of Pomalidomide-bortezomib-dexamethasone as initial therapy in NDMM, by assessing response using MRD assessment.

c)Immuno-Oncology, Cellular Immunotherapy: CLARION- CAR-T cell therapy in Lymphomas- Phase I Clinical Trial of CD19 Chimeric Antigen Receptor (CAR) T Cells Children and Adults with Relapsed or Refractory Indication i.e. CD19 Positive Acute Lymphoblastic Leukemia or Lymphoma.

This is an upcoming study, provisionally funded

Aim and Objectives:

To examine the feasibility of manufacture of autologous CD19 CAR T-cells at a minimum target dose using the Miltenyi CliniMACS Prodigy® automated system, to determine the safety of infusion with chimeric antigen receptor T cells targeting CD19 and to find the recommended phase II dose (RP2D) for recurrent/refractory ALL or lymphoma.

Pharma Sponsored Registry Studies

a)Lymphoma: RITUXIMAB generic (Reditux) Promise Registry to compare Effectiveness, Safety, and Resource Utilization of Reditux (Rituximab) vs. the reference Medicinal product to treat Diffuse Large B –Cell lymphoma and Chronic Lymphatic Leukemia in Routine Clinical Practice

b)Lymphoma: RITUXIMAB generic (Mabtas) A multi-center, observational, data collection registry study to monitor the routine clinical use of MABTAS in Indian patients". The enrollment of patients for this study is ongoing.

Pharma Sponsored Clinical Trials: Ongoing

a)A Phase 1 Study to Determine Safety, Tolerability, Pharmacokinetics, and Activity of K0706, a Novel Tyrosine Kinase Inhibitor (TKI), in Subjects with Chronic Myeloid Leukemia (CML) or Philadelphia Chromosome Positive Acute Lymphoblastic Leukemia (Ph+ALL) Protocol No.: CLR_15_03 (Dr. Vivek S Radhakrishnan)

b)A phase 3 randomized, controlled, open-label study of selinexor, bortezomib, and dexamethasone (svd) versus bortezomib and dexamethasone (vd) in patients with relapsed or refractory multiple myeloma (rrmm) karyopharm. Protocol No.: KCP-330-023 (Dr. Jeevan Kumar)

c)Safety and efficacy study of Azadine® (Azacitidine) in treatment of myelodysplatic syndrome in indian patients. Protocol No.: 484-14. (Dr. Vivek Radhakrishnan)

d)Clinical Outcomes of CLL and MCL patients treated with Ibrutinib: An Observational retrospective medical chart review from India that may require exchange of certain information that is confidential and proprietary in nature. (Dr. Vivek S Radhakrishnan)

Upcoming, Confirmed:

a)A prospective, multicenter, open label single arm Phase IV clinical trial to assess the safety of Imbruvica TM (Ibrutinib capsules 140 mg) in India patients with chronic lymphocytic leukemia or mantle cell lymphoma who have received at least one prior therapy or chronic lymphocytic

leukemia with 17p deletion. Protocol No: 54179060LYM4005. (Dr. Vivek S Radhakrishnan)

b)A Prospective, Single-Arm, Multicenter, Pragmatic Phase-IV Trial Investigating Safety and Effectiveness of DARZALEX (Daratumumab)In Indian Subjects With Relapsed and Refractory Multiple Myeloma, Whose Prior Therapy Included a Proteasome Inhibitor and an Immunomodulatory Agent.

c)A randomized, double-blind, placebo-controlled phase III multi-center study of azacitidine with or without MBG453 for the treatment of patients with intermediate, high or very high risk myelodysplastic syndrome (MDS) as per IPSS-R, or Chronic Myelomonocytic Leukemia-2 (CMML-2).

d)Phase III Multicenter Open-Label Randomized Trial to Evaluate Efficacy and Safety of CPI-613® (devimistat) in Combination with High Dose Cytarabine and Mitoxantrone (CHAM) Compared to High Dose Cytarabine and Mitoxantrone (HAM) in Older Patients (≥50 years) with Relapsed/Refractory Acute Myeloid Leukemia (AML).

Closed

a)A Phase 2, Open-Label Randomized Trial Evaluating the Efficacy and Safety of Two Dosages of Once Daily Oral CA-170 in Patients with Selected Relapsed Advanced Tumors (ASIAD). Protocol No.: CA-170-201. (Dr. Vivek S Radhakrishnan)

b)A Randomized, Double-blind, Multi-center, Multi-national Trial to Evaluate the Efficacy, Safety, and Immunogenicity of SAIT101 Versus Rituximab as a First-line Immunotherapy Treatment in Patients with Low Tumor Burden Follicular Lymphoma Protocol No.: AGB002. (Dr. Vivek S Radhakrishnan)

c)A Phase 2b Open-Label Study of Selinexor (KPT-330) in Patients with Relapsed/Refractory Diffuse Large B-Cell Lymphoma. Protocol No: KCP-330-009. (Dr. Vivek S Radhakrishnan).

Dr. Susri Ray Chaudhuri
Administrator Operations

Administration

This team coordinates HR, Finance and Estates activity at TTCRC. We also organise all the meetings that are held including external speakers.

Dr Soumita Das
Research Support Officer

Number of interns for the year (2018-19) are as follows:
Ms Nidhi Satishkumar ; Ms Sangita Pachal ; Ms Sanjali Mitra ; Ms Sohini Samaddar; Ms Sudhritti Guha Majumdar; Mr Sourav Roy; Dr Shatarupa Sinha

Sukanya Guha
Administrative Assistant

We have welcomed the following staff in TTCRC in the year 2019
Ms Dipshikha Chatterjee ; Dr Soumita Das ; Dr Nandana Das ; Mr Parag Das ; Ms Uzma Zaheer; Ms Parna Choudhury ; Mr Arko Bhaowal ; Ms Ankita Dutta ; Ms Kankana Das ; Dr Tushar Mungle

Dipshikha Chatterjee
Secretary

We convey our best wishes for our ex colleagues for the year 2019:
Dr Avishek Banerjee; Dr Satyam Banerjee; Priyanka Bose ; Soumasree Tapadar

Arbind Kr Mahato
IT Support Engineer

And last but not least we thank our housekeeping staff who are essential to the day to day functioning of the centre, Housekeeping staff: Sukumar Polye; Raju Bala; Supriti Sahoo; Debabrata Chowdhury; Debashis Biswas and Kaushik Chakraborty.

Seminars and Workshops

The internal seminar series called Radium which take place on every Wednesday at 8.30am is a bridge between the TTCRC researchers, clinicians and national and international researchers working in the field of cancer biology. We have enjoyed the scientific interaction throughout the year and with an excellent set of internationally renowned speakers visiting the Institute. Following are the details of Invited speakers in the year (2019).

1.Dr. Barun Thakur (Flame University, Pune)
"Arsenicosis, Health Damages & Cost of Treatment: Evidence from Arsenic affected areas of Bihar"
30th Jan 2019

2.Dr Uri Tabori (Institute of Medical Science, University of Toronto)
"Pediatric train tumours: from the lab to clinical trials".
13th Mar 2019

3.Dr Suman Mukhopadhyay (National Cancer Institute, USA)
"Exploiting cancer cell signalling & metabolism: Implications for therapeutic approach".
22nd May 2019

4.Prof Bhaswati Ganguli (Dept. of Statistics, University of Calcutta)
"Determination of the functional form of the log based ratio for a Cox model".
29th May 2019

5.Prof Tannistha Reya (Pharmacology and Medicine, University of California, San Diego, USA)
"Leukemia and Pancreatic Cancer".
19th June 2019

6.Dr Stephanie Sohier (ICG, Roussy, France)
Caspases:from fundamental studies to cancer fighting strategies."
10th July 2019

7.Dr. Ananthalakshmy Sunderaraman (School of Biochemistry, University of Bistol, UK)
"Targeting molecular switches regulating Anoikis resistance and Angiogenesis".
07th Aug 2019

8.Dr. Shatarupa Sinha (IIT, Mumbai)
"Mechanism of phosphor-alpha-tubulin driven motility in breast epithelial cells".
28th Aug 2019

9.Dr Somi Patranabis (Amity University, Kolkata)
"Non-coding RNAs: modulators of gene expression in all domains of life".
4th Sept 2019

10.Dr Sanhita SinhaRay (University of Texas MD Anderson Cancer Center, Texas, USA)
"Picturing the future of healthcare through molecular imaging".
16th Oct 2019

11.Dr Abhijit Chakraborty (Division of Vaccine Discovery La Jolla Institute for Allergy & Immunology)
"Organization and functional aspects of the human 3D genome".
23rd Oct 2019

12.Prof Amlam Chakraborty (Faculty of Engineering and Technology, University of Calcutta)
"Medical image Processing: Analysis & Visualization".
30th Oct 2019

13.Dr Siddharth De (CCBT, In Stem, Bangalore)

"To be or not to be: Pulsatile MAPK signalling modulates p53 activity to control cell fate decisions at the G2 checkpoint for DNA damage".
06th Nov 2018

14.Dr Tanja Gruber (St Jude Children's Research Hospital)
"Utilizing Next Generation Sequencing is a Discovery tool in Pediatric AML – Insights into novel subtypes, risk stratification, and biology".
27th Nov 2019.

Publications

1. **Efficacy and safety of a bortezomib and reduced-intensity cytarabine-based protocol, TMC ALLR1, for relapsed childhood ALL in India**. Roy P, Islam R, Saha D, Gogoi M, Kumar Mishra D, Arora N, Parihar M, Krishnan S, Saha V. *Br J Haematol*. 2019;186(6):861-5.

2. **Outcomes of patients with childhood B-cell precursor acute lymphoblastic leukaemia with late bone marrow relapses: long-term follow-up of the ALLR3 open-label randomised trial.** Parker C, Krishnan S, Hamadeh L, Irving JAE, Kuiper RP, Revesz T, Hoogerbrugge P, Hancock J, Sutton R, Moorman AV, Saha V. *Lancet Haematol*. 2019;6(4):e204-e16.

3. **Long-term follow up of pediatric Philadelphia positive acute lymphoblastic leukemia treated with the EsPhALL2004 study: high white blood cell count at diagnosis is the strongest prognostic factor.** Biondi A, Cario G, De Lorenzo P, Castor A, Conter V, Leoni V, Gandemer V, Pieters R, Stary J, Escherich G, Campbell M, Attarbaschi A, Li CK, Vora A, Bradtke J, Saha V, Valsecchi MG, Schrappe M. *Haematologica*. 2019;104(1):e13-e6.

4. **Impact of dose and duration of therapy on dexamethasone pharmacokinetics in childhood acute lymphoblastic leukaemia-a report from the UKALL 2011 trial.** Jackson RK, Liebich M, Berry P, Errington J, Liu J, Parker C, Moppett J, Samarasinghe S, Hough R, Rowntree C, Goulden NJ, Vora A, Kearns PR, Saha V, Hempel G, Irving JAE, Veal GJ. *Eur J Cancer*. 2019; 120:75-85.

5. **Ethics of cancer care: beyond biology and medicine.** Ghose S, Radhakrishnan V, Bhattacharya S. 2019. *Ecancermedicalscience*. Mar 28;13:911. doi: 10.3332/ecancer.2019.

6. **Spectrum and Immunophenotypic Profile of Acute Leukemia: A Tertiary Center Flow Cytometry Experience**. Gupta N, Pawar R, Banerjee S, Brahma S, Rath A, Shewale S, Parihar M, Singh M, Arun SR, Krishnan S, Bhatacharyya A, Das A, Kumar J, Bhave S, Radhakrishnan V, Nair R, Chandy M, Arora N, Mishra D. *Mediterr J Hematol Infect Dis*. 2019; 11(1):e.

7. **MYD88 and CXCR4 Mutation Profiling in Lymphoplasmacytic Lymphoma/Waldenstrom's Macroglobulinaemia.** Vinarkar S, Arora N, Chowdhury SS, Saha K, Pal B, Parihar M, Radhakrishnan VS, Chakrapani A, Bhartia S, Bhave S, Chandy M, Nair R, Mishra DK. *Indian J Hematol Blood Transfus*. 2019; 35(1):57-65.

8. **FxCycle™ Based Ploidy Correlates with Cytogenetic Ploidy in B-Cell Acute Lymphoblastic Leukemia and Is Able to Detect the Aneuploid Minimal Residual Disease Clone.** Gupta N, Parihar M, Banerjee S, Brahma S, Pawar R, Rath A, Shewale S, Singh M, Sasikumaran N, Remani A, Krishnan S, Bhatacharyya A, Das A, Kumar J, Bhave S, Radhakrishnan V, Nair R, Chandy M, Mishra D, Arora N. *Cytometry B Clin Cytom*. 2019; 96(5):359-367.

9. **Diagnosis of variant RARA translocation using standard dual-color dual-fusion PML/RARA FISH probes: An illustrative report**. Singh MK, Parihar M, Arora N, Mishra DK,

Bhave SJ, Chandy M. *Hematol Oncol Stem Cell Ther*. 2019; 12(1):50-53.

10. **Detection of BCR/PDGRFα Fusion Using Dual Colour Dual Fusion BCR/ABL1 Probe: An Illustrative Report.** Singh MK, Sasikumaran NRA, Bhave SJ, Mishra DK, Arora N, Parihar M. *Indian J Hematol Blood Transfus*. 2019; 35(3):570-574.

THANK YOU !