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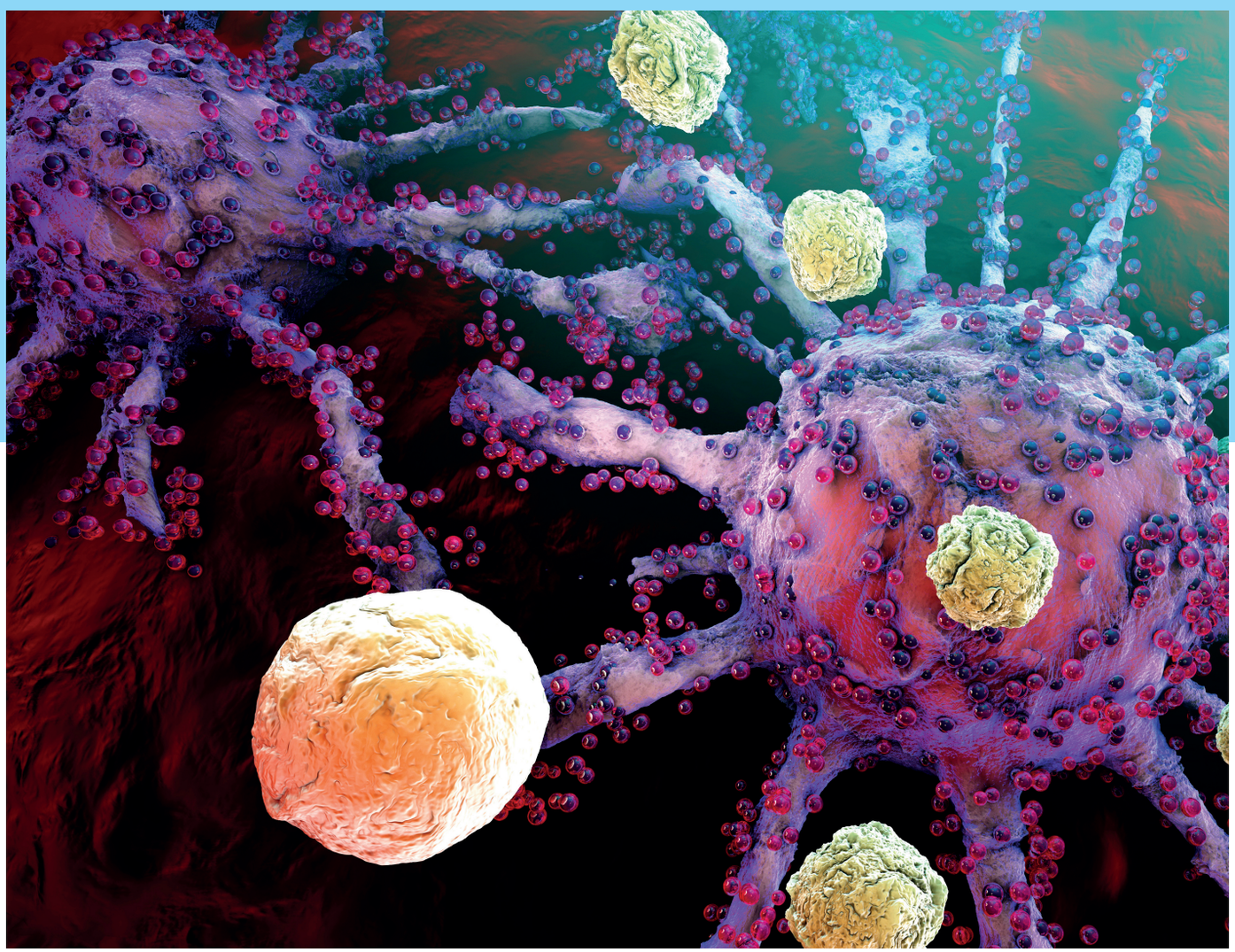

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#Based on analysis from R&D batches
IV: Intravenous, ADC: Antibody–Drug Conjugate, MoA: Mode of Action HER2+: Human Epidermal growth factor Receptor 2 positive, EBC: Early Breast Cancer, MBC: Metastatic Breast Cancer,
Reference: 1. Data on file.

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National Research Scholars' Meet 2021 Abstracts

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A001. Expression Pattern of CD244, a Novel SLAM Protein and Its Clinical Utility in the Diagnosis of Acute Leukemia

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Background: CD244, a member of the signaling lymphocyte-activation molecule (SLAM), is expressed on all Natural Killer cells, a subpopulation of T-cells, monocytes, and basophils, as well as leukemic stem cells. With the advent of immunotherapy, we aimed to assess the expression of CD244 in patients with acute leukemias.

Materials and Methods: Flow cytometric immunophenotyping was performed to study the expression pattern of CD244 on normal cells and leukemic blasts using 10- to 13-color panel. The study included 50-B-Acute Lymphocytic Leukemia, 50-TALL, 20-Myelodysplastic Syndromes Treatment, and 91-Acute Myeloid Leukemia cases, along with seven normal bone marrow controls. Analysis was done on Kaluza v-2.1 Software.

Results: NK-cells and T-cells were taken as positive controls with a median nMean Fluorescence Intensity of 8 (0.049–9.99) and 2 (0.32–7.75), respectively. Comparison of AML and MDS blasts versus granulocytes and monocytes revealed that CD244 can distinguish between AML blasts and normal granulocytes ($p < 0.0001$), monocytes ($p < 0.0001$), MDS blasts from granulocytes ($p < 0.0001$), and monocytes ($p < 0.0002$), respectively. NK cells express CD244 and can be distinguished from T-blasts which are negative for CD244 ($p < 0.0001$). Note the nMFI of hematogones is of normal bone marrow. The calculated median nMFI and range is given in **Table 1**.

Conclusion: This is the first study to demonstrate CD244-expression in acute leukemia and can be used as a potential marker for Minimal Residual Disease monitoring in AML, as it distinguishes MRD from granulocytes, monocytes and T-lymphoblasts from NK cells in T-ALL which may serve as a potential therapeutic target in patients with AML.

Table 1

CD244	AML blast	Granulocytes	Monocytes
Median nMFI	9	3	4
Range	0.23–48.42	0.0–9.46	0.23–10
CD244	**B-ALL blast**	**Hematogones control**	**B cells**
Median nMFI	0.026	0.158	0.138
Range	0.0–5.74	0.02–8.39	0.04–2.51
CD244	**T-ALL blast**	**T-cells**	**NK cells**
Median nMFI	0.41	2	8
Range	0.0–9.96	0.32–7.75	0.04–9.99
CD244	**MDS blast**	**Granulocytes**	**Monocytes**
Median nMFI	8	2	10
Range	1.35–9.71	0.27–6.24	3.69–10.55

Abbreviations: ALL, Acute Lymphocytic Leukemia; AML, Acute Myeloid Leukemia; MDS, Myelodysplastic Syndromes Treatment; MFI, Mean Fluorescence Intensity; NK, Natural Killer.

A002. An Efficient Diagnosis of Diffuse Parenchymal Lung Disease from Spirometry Exploring the Novel Role of FEF_{25-75}

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Background: The peripheral interstitial involvement in diffuse parenchymal lung disease (DPLD) can affect the small airways and, thus influence the FEF_{25-75} (forced expiratory flow), a marker of small airway function in spirometry.

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Here we evaluate the role of FEF_{25-75} for the diagnosis of DPLD that is otherwise diagnosed by high-resolution computerized tomography of chest.

Materials and Methods: FVC (forced vital capacity), FEF_{25-75}, FEV_1 (forced expiratory volume), and derived variables (FVC/FEF_{25-75}) are used to differentiate DPLD from normal, obstructive lung disease (OLD) populations. Using different combinations of variables, receiver operating characteristic curve and regression analysis are done to identify the best possible combination to diagnose DPLD from spirometry.

Results: We included 639 adult patients' (normal = 64, DPLD = 268, and OLD = 307 including asthma = 153 and COPD = 154) spirometry data. FEF_{25-75} alone could differentiate unmixed DPLD from OLD with sensitivity and specificity of 86.9 and 85.1%, respectively. The derived variables also showed promising diagnostic accuracies. A regression equation using age, FVC, FEV_1/FVC, and FVC/FEF_{25-75}(%) is found to have 79% diagnostic accuracy of DPLD on test population ($n = 639$). The same equation showed 90 and 87% diagnostic accuracy of DPLD in validation population of 427 mixed patients from same center and 100 mixed patients from different centers.

Conclusion: The inclusion of derived variables from spirometry and the regression equation using age, FVC, FEV_1/FVC, FVC/FEF_{25-75} (%) appears rewarding in the diagnostic exercise for DPLD. Hence, this spirometric approach needs further validation from multiple centers to be included in clinical practice.

A003. Identification of Profilin 2 in the Testis and Its Interaction with HDAC6

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Background: Previous reports have demonstrated the involvement of HDAC6 in sperm motility. Presence of HDAC6 has been reported in germ cells to get a snapshot on role the of HDAC6 at specific stage of spermatogenesis, GC-1spg cells were employed which represent cell type between type-B spermatogonia and preleptotene spermatocytes.

Materials and Methods: Mouse HDAC6 was transfected in GC-1spg cells, confirmed by real-time, western blot analysis, and indirect immunofluorescence (IIF), its effect on germ cell transcriptome was investigated by microarray gene expression array. IIF and coimmunoprecipitation experiments were performed for colocalization and protein interaction.

Results: Many transcripts that were differentially regulated, Profilin2 reported previously as a neuronal specific isoform was observed as one of the genes highly upregulated at the transcript level which was further confirmed by real-time PCR, protein confirmed by IIF. Profilin2 colocalized with HDAC6 both in GC-1 and sperm. On the sperm, presence of profilin2 was detected throughout the flagella, its colocalization with HDAC6 seen conspicuously in the mid piece region of the flagella. Coimmunoprecipitation confirmed Profilin2 interaction with HDAC6. Docking studies using Z dock suggested the interaction of eight residues of HDAC6 forming hydrogen bonds with 6 residues of Profilin2.

Conclusion: Present study identified Profilin2 as a novel interacting partner of HDAC6 and its presence in testis, GC-1cells, and sperm for the first time. Based on our observations and literature, we propose that their interaction may be integrated to regulate the actin cytoskeleton and possibly involved in migration during spermatogenesis.

A004. Modulation of Cellular Aging in the Drosophila Hematopoietic Organ Alters the Cell Lineage Trajectories and Blood Cell Homeostasis

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Background: Organismal aging is accompanied by a progressive decline in cellular and molecular functioning affecting organismal health and longevity. Stem cell aging is a hallmark of aging that results in disruption of normal functioning of organs. Our current study investigates the effect of aging on the hematopoietic system using *Drosophila*.

Materials and Methods: Using Gal4-UAS technology, we have overexpressed Atg8 or FOXO transgenes to stall/induce reverse aging in various cell populations of the Lymph Gland (LG), larval hematopoietic organ. These genetic models have been subjected to in vivo analysis using organ dissections and immunofluorescence analysis. Postacquisition analysis has been done using ImageJ.

Results: Stalling aging by spatially modulating expression of autophagy protein, Atg8, and transcription factor, FOXO in different cell compartments of LG has differential effects on the niche cells, hematopoietic progenitors, and the differentiated blood cells. Genetic manipulation of molecular circuitry of aging in the fat-body systemically alters dynamics of hematopoiesis in LG at many levels. Genetic modulation of aging alters cell lineage trajectories leading to either an increase or decrease in a given differentiated blood cell type.

Conclusion: Our data show that modulating cellular aging leads to changes in cellular differentiation potential thereby altering tissue homeostasis. Further studies will help in understanding the mechanistic framework and signaling that regulates cellular aging to maintain blood cell homeostasis.

A005. Intersubunit Cross-Talk via PDZ Synergistically Governs Allosteric Activation of Proapoptotic HtrA2

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Background: Mitochondrial serine protease, HtrA2, is associated with various diseases including neurodegenerative disorders and cancer. Despite availability of structural details, the reports on HtrA2's mechanistic regulation still remain nonconcordant. The purpose of this study is to identify the allosteric modes of communication adopted by HtrA2 under different activation scenarios.

Materials and Methods: We generated heterotrimeric HtrA2 variants differing in the number of protein-protein interaction domain, PDZ and/or active-site mutations via a protomer-mixing strategy. Various structure-guided biophysical studies such as enzyme kinetics, MD simulations,

single-molecule photobleaching, and X-ray diffraction studies were adopted to demonstrate differences in various conformational modes of these ensembles.

Results: Sequential deletion of PDZs from the trimeric ensemble significantly affected the total residual activity of each HtrA2 variant in a way that proffered a hypothesis advocating intermolecular allosteric crosstalk via PDZ domains in trimeric HtrA2. The single-molecule photobleaching studies established the existence of stepwise binding of substrate molecule to each subunit of the trimer, while enzymatic studies with active-site variants affirmed the role of transmediated allosteric communication within the pyramidal protease ensemble. Furthermore, structural and computational snapshots affirmed the role of PDZs in secondary structural element formation and coordinated reorganization of the N-terminal region and regulatory loops of the protease.

Conclusion: This study unequivocally describes different activation mechanisms of HtrA2 at the atomic level using a unique retrospective approach through dissecting and rebuilding protein structure. Apart from providing cues for devising structure-guided therapeutic strategies, it establishes a working model of complex allosteric regulation through a multifaceted transmediated cooperatively shared energy landscape.

A006. The Differential Interplay of the Notch-3/Jag-1 Axis Modulates Disease Progression in Epithelial Ovarian Cancer

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Background: A classical way of cell-to-cell communication is through receptor-ligand interaction, forming the basis of notch signaling. Epithelial Ovarian Cancer displays a heterotypic/homotypic-interplay between tumor-mesothelial/tumor-tumor cells, conniving its propensity of peritoneal metastasis. This warrants investigating differential notch-3 activation in the tumor through heterogenous mesothelial/cancer cell–mediated jagged-1 induction affecting cellular characteristics.

Materials and Methods: We developed a coculture-based model, NIH3T3 differentially overexpressing *jagged1* and SKOV3-expressing notch-3-sensor (10XCSL-driven firefly-luciferase; SCFT) and employed bioluminescence-imaging for monitoring notch-3 activity. We performed assays like CFSE, MTT, Matrigel invasion to study the cellular functions and transcriptional profiling of SCFT postdifferent cocultures by RT-PCR array to identify notch-3 effector candidates.

Results: We showed that incremental membranous-jagged-1 expression in NIH3T3 leads to proportional notch-3 activation in SCFT upon coculturing as reflected by the luciferase signal. We corroborated these observations in SCFT by cocultures with High Grade Serous Ovarian Cancer-patients' ascitic-mesothelial cell and jagged-1-expressing EOC cell lines (A2780, OVCAR-3, and OAW42). NIH3T3$^{J1\text{-highest}}$ and OVCAR3-cocultured SCFT showed proliferation index (48 h) 10.56 and 3.03 compared to SCFT. The resistance index (cisplatin) of SCFT cocultured with NIH3T3$^{J1\text{-highest}}$ and OVCAR-3 were 2.71 and 2.53, respectively. SCFT invaded two-fold higher after NIH3T3$^{J1\text{-highest}}$ coincubation. Five genes (CDKN1A, VEGFA, TNFSF10, SERPINA3, and FOXC1) showed significant fold-regulation across coculture conditions, and we assessed two genes with fold change^{max} (CDKN1A and VEGFA) for their roles.

Conclusion: We had developed a robust, sensitive, real-time coculture-based notch-3 reporter sensor. Further, we found that differential notch-3 activation affected several critical phenotypes like proliferation, invasiveness, and chemoresistance proportionately. Two putative critical regulators of notch-3-mediated effects, CDKN1A, and VEGFA, were identified, impacting the overall disease progression.

A007. Novel CDK-7 Inhibitor Suppresses Transcription of Oncogenes and Enhances Venetoclax-Mediated Apoptosis in Acute Myeloid Leukemia

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Background: Acute myeloid leukemia (AML) remains a highly fatal disease. Based on the available evidence of deregulated pathways of proliferation and apoptosis in AML, we hypothesize that pharmacological modulation of AML blasts by inhibitors of BCL-2 (Venetoclax) and novel CDK7 inhibitor (CRI-256) will be potently synergistic for the treatment of AML.

Materials and Methods: AML cell lines and primary blasts from 50 AML patients were used for antiproliferation assays. Cell proliferation and apoptosis was assessed using flow cytometry and western blotting. CDK7 CRISPR knockout was performed using pSp-Cas9-PURO-CDK7 vector. NOD/SCID mouse models of AML were used to evaluate antileukemic activity of CRI-256.

Results: Novel CDK7 inhibitor (CRI-256) downregulated proliferation in leukemic cell lines and in primary AML blast. CRI-256 inhibited the phosphorylation of RNA pol-II leading to decrease in transcription of oncogenes that plays major role in AML proliferation. RNA sequencing data provided further evidence that expression of genes involved in high cell proliferation was downregulated after treatment. Cell cycle arrest as well as induction of apoptosis was detected in dose dependent manner. Significant tumor reduction was observed in AML xenografts after 2 weeks of CRI-256. The antiproliferative data derived from AML cell lines treated with CRI-256 and Venetoclax has shown significant synergism.

Conclusion: The data indicated that targeted therapy by novel CDK7 inhibitor induce apoptosis, decrease cell proliferation, and has greater antileukemic activity compared to standard of care in AML.

A008. Multimodal Approach to Characterize Missense Mutation Identified in h-BRCA2

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Background: BRCA2 protein plays an essential role in homologous recombination. Germ-line mutations in BRCA2

confer an increased risk of breast cancer. Many missense mutations have been identified in BRCA2; however, most of them are classified as variants of "Uncertain Significance" due to a lack of structural, functional, and clinical studies.

Materials and Methods: Here, multidisciplinary in silico, in vitro and biophysical approaches have been explored to characterize an unclassified missense mutation, BRCA2 Arg2502Cys, identified from a case-control study. Wild-type and mutant proteins were overexpressed and purified using bacterial expression system. Comparative secondary and tertiary studies were performed using circular dichroism and fluorescence spectroscopy.

Results: Circular-dichroism and Fluorescence spectroscopy showed that the Arg2502Cys mutation in hBRCA2 (residues 2350-2545) decreases the α-helical/β-sheet propensity of the wild-type protein and perturb the tertiary structure conformation. In molecular dynamics simulation studies, the mutation perturbs the structural integrity and conformational dynamics by altering the intramolecular H-bonds, overall compactness and stability of the hydrophobic core. Principal component analysis indicated that Arg2502Cys mutant exhibits comparatively large conformational transitions and periodic fluctuation conformers throughout the 250ns trajectories.

Conclusion: Therefore, to our conclusion, BRCA2 Arg2502Cys mutation perturbed the structural integrity and conformational dynamics of BRCA2 which in turn may affect the function of the protein and could result in cancer predisposition.

A009. Localized Delivery of Gemcitabine Using Chitosan–Poly Vinyl Alcohol Film Inhibits Pancreatic Tumor Growth in Preclinical Settings

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Background: Gemcitabine (GEM) is used for treatment of pancreatic cancer (PC) patients. However, the limiting step in GEM dosage is its low plasma half-life, nonspecificity, and high cytotoxicity. To overcome these limitations, we have developed GEM loaded chitosan–poly vinyl alcohol (CP-GEM) based biodegradable film for localized and sustained release of GEM for PC management.

Materials and Methods: The CP-GEM was synthesized using solvent-casting method. The cell viability assays was carried in both 2D and 3D PC-cell culture with different dosages of GEM for 6 hours (mimicking the clinical scenario), 5 days, and equivalent amount CP-GEM for 5 days. Preclinical antitumor efficacy study was carried out in subcutaneous PC model.

Results: The GEM gets released in small dose over a period of 2 weeks. It was observed that CP-GEM has enhanced cell killing capability in both 2D- and 3D-cell cultures. Intriguingly, GEM resistant PC cells developed, on treatment of free GEM for 6 hours and 5 days, whereas no such development was observed on treatment with CP-GEM even after 5 days from end of the treatment. CP-GEM also inhibited cell proliferation and lead to DNA double strand break. Further, we observed that CP-GEM inhibited tumor growth in mice indicating the antitumor capability of the GEM when loaded in the film.

Conclusion: Thus, continuous and sustained release of GEM not only killed PC cells but also inhibited development of chemoresistance. The CP-GEM further inhibited tumor growth in mice. In future CP-GEM should be investigated for antitumor efficacy in orthotopic PC model.

A010. Reoxygenation Postacute Hypoxia Imparts Survival Advantage to Parental and Therapy-Resistant Breast Cancer Cells

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Background: In solid tumors, hypoxia due to insufficient blood supply associates with failure of anticancer treatment strategies. Intermittent reoxygenation of hypoxic cells is a clinically relevant condition potentially affecting biological outcomes. This study highlights the biological effect of hypoxia reoxygenation on parental and therapy-resistant breast cancer cells for better therapeutic targeting.

Materials and Methods: Parental and radioresistant breast cancer cells were exposed to hypoxia and/or reoxygenation for deciphering the cellular and molecular alteration compared to untreated cells. The comparative studies were carried out in cancer cells using the Raman spectroscopy, electron microscopy, western blotting, confocal microscopy, and other functional assays.

Results: In response to hypoxia and reoxygenation, the parental and radioresistant cells showed mitochondrial fusion, fission, and swelling with distinct spectral peaks of cytochrome-c at 750 and 1,585/cm compared to untreated cells. Incoherence, the increased levels of total and mitochondrial Reactive Oxygen Species with enhanced γH2AX foci formation and decreased pATR levels were also observed in hypoxia reoxygenation exposed parental and radioresistant cells compared to the untreated cell population. The mitochondrial structural and functional proteins also showed altered levels in parallel with changes in mitochondrial structural changes. These cells showed increased survival and migratory potential, possibly due to increased pAKT and pERK1/2 levels.

Conclusion: Differential mitochondrial alterations, ROS signaling, and increased levels of antioxidant enzymes play an essential role in cell proliferation and survival of parental and radioresistant cells under hypoxia and reoxygenation. Therefore, the targeted interruption of the mitochondria-to-cell redox pathway is potentially a promising target for future therapy.

A011. Withaferin-A Prevents the Onset of Acute Graft versus Host Disease by Inhibiting JAK2-STAT3 Signaling Pathway

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Background: Acute graft versus host disease (aGvHD) is a major complication of allogenic bone marrow transplantation (alloBMT). Withaferin-A (WA) as an anti-inflammatory and immunomodulatory agent may have clinical utility either as a single agent or in combination for the prevention of aGvHD.

Material and Methods: Recipient BALB/c (H-2K^d) mice were given 6.5 Gy of total body radiation. Irradiated BALB/c mice were transplanted with 5×10^6 bone marrow cells and 15×10^6 spleen cells from donor C57BL/6 (H-2K^b) mice. Further, efficacy of WA was evaluated and compared with standard prophylactic regimen of cyclosporine and methotrexate (CSA + MTX).

Results: Oral administration of WA significantly decreased the clinical severity score of aGvHD by 7.5 points with respect to control (9.5 vs. 2.0). Mortality associated with aGvHD was also significantly reduced compared to the control (HR = 0.07 (0.01–0.35); $p = 0.013$). Furthermore, WA arm had better overall survival compared with standard prophylactic regimen of CSA + MTX (HR = 0.19 [0.03–1.13], $p = 0.09$), although the effect was not statistically significant. In vitro WA treatment to splenic lymphocytes resulted in marked decrease in pJAK2 and pSTAT3 levels.

Conclusion: Oral administration of WA prevents aGvHD by inhibiting JAK2-STAT3 signaling. WA as a single agent demonstrated comparable efficacy as CSA + MTX. The efficacy of WA for the prophylaxis of aGvHD should be tested in prospective clinical trials either alone or in combination with standard regimen.

A012. Serum Raman Spectroscopy Study in DMBA-Induced Oral Carcinogenesis—Hamster Buccal Pouch Model

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Background: Oral cancer presents with poor 5-year survival rates owing to delayed clinical detection. Raman spectroscopy has demonstrated stratification of healthy, inflammatory, premalignant, and oral cancer conditions, proving potential in early diagnosis. This study aims to evaluate if the Serum Raman Spectroscopy (SRS) can probe the sequential biochemical changes occurring during oral carcinogenesis.

Materials and Methods: The serum collected across 14 weeks from DMBA-induced oral carcinogenesis in hamster buccal-pouch model was used to identify the sequential biochemical changes. Sequential spectral variations were observed in controls and carcinogen (DMBA) treated hamster sera. The Serum Raman spectra were subjected to PCA and PC-LDA analysis, both week and group wise.

Results: The PCA scatter plots show distinct clustering of spectra as the DMBA treatment progresses. In week-wise analysis, it is evident that serum spectra from the DMBA treated and physical injury group do not get misclassified as untreated group spectra. However, the serum spectra of untreated and physical injury controls showed misclassification with spectra from the DMBA treated group at later weeks. In group-wise analysis, the serum spectra from the untreated control, and DMBA-treated groups showed classification in their respective weeks, suggesting SRS can detect the age-related changes.

Conclusion: Detection of age-related changes demonstrates sensitivity of the technique. The misclassifications between the injury and DMBA group indicate mechanical irritations/injuries as an etiological factor. While no spectral classification of both above groups with untreated or vehicle controls, it suggests SRS as a rapid and objective screening adjunct in oral cancer diagnosis.

A013. Understanding the Histone Alteration during Drug Tolerance Leading to Survival of the Cancer Cells and Development of Drug Resistance

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Background: Recent studies have shown heterogeneity within cancer results in the generation of reversible early drug-tolerant persister (DTP) cells due to altered transcription. Further, these cells lead to stable drug-resistant cells (DRC). The present study focuses on differential histone alteration in DTP and DRC for their potential therapeutic importance.

Materials and Methods: The gastrointestinal cell lines treated with cisplatin were used to study sequential events in DTP and DRC. The characterization was performed based on phenotypic and molecular changes, such as cell cycle, cellular plasticity, apoptosis, and histone modifications in DTP, in comparison to parental cell lines of DRC.

Results: The sequential treatment of AGS and Hep3B cells with cisplatin leads to survival of 5 to 8% of the cell population in the early stage, defined as DTP cells. The long-term treatment leads to the development of DRC from the DTP population. The surviving DTP cells were in the quiescent state with significantly less cell death. Preliminary data have shown increased expression of stem-cell markers CD44 and CD133. Moreover, DTP cells showed an increased level of heterochromatin markers, H3K9me3 and H3K27me3, with increased incorporation of histone isoform, H3.2. The methylase, EHMT2, and demethylase, KDM3 of H3K9me3, showed increased and decreased expression respectively.

Conclusion: The G0/G1 arrest and increased heterochromatin marks in DTP cells provide an advantage for cell survival. Therefore, potentially targeting the altered histone modifiers required for histone modifications for the survival of DTP cells with specific inhibitors will help to prevent the development of drug-resistance cells.

A014. The Role of Lipocalin 2 (LCN2) in Regulating Ferroptosis and Therapy Resistance

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Background: Increased expression of LCN2 leads to chemoresistance and inhibiting LCN2 function results in chemosensitivity, due to LCN2-mediated inhibition of iron-dependent programmed cell death process called ferroptosis and induction of autophagy. We aim to assess how LCN2

stimulates ETS1 expression to inhibit ferroptosis and how LCN2 promotes autophagy leading to chemoresistance.

Materials and Methods: All the protocols have been described previously in Chaudhary et al. shRNA was cloned into pLKO.1-TRC cloning vector (a gift from David Root) to generate stable LCN2 knockdown in DLD1 cells to confirm the observation obtained with the LCN2 antibody (3D12B2).

Results: LCN2 overexpressing cells showed increased levels of ETS1, xCT, and GPX4, while LCN2 knockdown cells showed decreased levels, confirming the results obtained by treatment with LCN2 antibody, and consistent with the observation that LCN2 promotes resistance to 5-FU by inhibiting ferroptosis. Further, LCN2 overexpressing cells showed increased LC3II levels and increased LC3 foci, suggesting LCN2 promotes autophagy in response to therapy. Also, activated EGFR expression is decreased in LCN2 knockdown cells suggesting that the increase in ETS1 levels observed upon LCN2 expression is probably due to increased activity of EGFR.

Conclusion: LCN2 inhibits ferroptosis by increasing ETS1 expression and promotes autophagy. By inducing autophagy, it probably increases the recycling of EGFR, such that activation of EGFR signaling increases expression of ETS1 which inhibits ferroptosis to promote chemoresistance.

A015. Autophagy Inhibition Invoke Resistance to Arsenic Trioxide Induced Apoptosis in Acute Promyelocytic Leukemia

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Background: Despite significant advances in the management of acute promyelocytic leukemia (APL), 10 to 20% of de novo and 30 to 50% of relapsed patients show resistance toward arsenic trioxide (ATO) therapy. To investigate factors contributing ATO resistance using ATO sensitive and resistant APL cell model system, we examined the role of autophagy in ATO resistance.

Materials and Methods: PML-RARA positive ATO sensitive and resistant NB4 cell lines ($NB4^S$ and $NB4^R$) and ATO (INTAS pharmaceuticals). IC_{50} by CTG assay and synergy calculation using Calcusyn. Apoptosis assays by flow cytometry. The qRT-PCR and western blots for transcripts and protein levels were studied.

Results: The IC_{50} of ATO was found to be 3.8 times higher in $NB4^R$ compared to $NB4^S$ cells (0.72µMvs2.77µM). Flow cytometry data showed higher rate of apoptosis in sensitive than resistant cells (50 vs. 18% at 4µMATO). The protein levels of autophagy markers (p62, LC3-I/II) in $NB4^R$ remained unchanged at higher ATO concentrations. Levels of p62 were decreased 90% in dose dependent manner in $NB4^S$ cells. Conversion of LC3 I to II on ATO treatment was found abysmal in $NB4^R$ cells suggesting autophagy inhibition. Interestingly, 3µMATO eradicated PML-RARA protein in $NB4^S$ cells but failed to conduct in $NB4^R$ cells suggesting that degradation of PML-RARA in ATO sensitive cells is mediated through autophagy.

Conclusion: Preliminary data highlight the role of autophagy inhibition in resistance to ATO induced apoptosis leading to reduced degradation of PML-RARA.

A016. Structural and Molecular Dynamics Studies of Mutations Identified in Functional Domains of Ribosomal Protein S6 Kinase A1

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Background: Ribosomal protein S6 kinase (RSK) is one of the effector kinases of RAS-MAPK pathway. Cancer associated mutations identified in the different domains of RSK1 have not been well studied for their structure and dynamics. Current work focuses on structure and functional studies of the mutations in RSK1-CTKD.

Methods: Mutations were retrieved from cBioPortal and characterized for pathogenicity by in-silico prediction tools (PROVEAN, PhD-SNP, pMut, and PANTHER). Evolutionary conservation was assessed by the ConSurf. Effect of mutation on protein stability was then determined with iStable tool. Molecular dynamics simulation was used to investigate the structural changes induced by the mutations.

Results: A total of 140 retrieved nsSNPs were screened for pathogenicity and 10 nsSNPs were identified in highly conserved regions and were categorized as deleterious. These mutations were located at functionally important region and ERK1/2 interaction interface. The molecular dynamics simulation analysis revealed major structural alterations in 6 nsSNPs (R434P, T701M, A704T, S720C, R725W, and S732F). Principal component analysis uncovered few eigenvectors deciphered to be principal components and that can be corelated with their collective motion. The Gibbs free energy landscape of PC1 and PC2 of R725W, S732F, and S720C were observed to have higher conformational stability than wild type.

Conclusion: This study unveils that highly pathogenic mutation affects the stability and dynamics of RSK1-CTKD.

A017. Multiplexed STAT3-PhosphoBRET Sensor for Ready Distinction between Canonical versus Noncanonical PTM Mediated Pathway Activation in Cancer Cell

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Background: STAT3 is an intracellular signaling protein which gets activated via phosphor-PTMs marks at Y705 (canonical) and S727 (noncanonical activation). Considering the complexity of STAT3 activation and signaling in various cancer contexts, we aim to develop a multiplexed, color-coded STAT3-phosphoBRET sensor to detect canonical or noncanonical PTM within a cancer cell population in live culture.

Methods: Fusion constructs of Nluc-STAT3, Turbo-STAT3, and mOrange-STAT3 were synthesized in N-terminus of STAT3 by PCR based cloning method. Mutant STAT3 (Y705F and S727A) constructs were developed using SDM. Plasmid DNAs were cotransfected in HT1080 and MCF7 cell lines using Lipofectamine 2000 protocol. Protein expression was measured by western blot and immunofluorescence. BRET imaging was done using IVIS spectrum.

Results: Expression of Nluc-STAT3, Turbo-STAT3, and mOr-STAT3 was detected in transiently transfected cells. STAT3 activation and dimerization was observed with an increase in BRET signal using multicolor based STAT3-BRET pairs, Nluc-STAT3-Turbo-STAT3, and Nluc-STAT3-mOr-STAT3 with different WT and mutant constructs post-EGF stimulation and drug treatment, including static and niclosamide. Time-dependent STAT3 phosphorylation with EGF or drug treatment was also confirmed with western blotting and survival assays.

Conclusion: While BRET has proved to be a useful live-cell assay for measuring protein dimerization, this study, for the first time, shows spectral multiplexing-based distinction and screening possibility of STAT3 activation in live-cell formats. Notably, this multiplexed STAT3 sensor can be used to screen potential drug molecules against complex STAT3 signaling.

A018. Noninvasive Imaging-Guided In Vivo Estimation of Photothermal Therapy Using Cancer-Targeted Gold-Solid-Lipid Nanomaterial

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Background: Photothermal therapy (PTT) is a promising cancer treatment modality which employs near-infrared laser on gold-nanomaterials for localized heat-mediated tumor ablation. We estimate PTT effect of a novel CSC-targeted photothermal agent, that is, hyaluronic acid (HA)-tagged gold-solid-lipid nanoparticle (Au-SLN) on breast cancer (BC) cells and xenograft tumors.

Methods: Au-SLN was surface functionalized with HA and characterized. In vitro uptake and biocompatibility of HA-Au-SLN over Au-SLN were done. To evaluate PTT efficacy in vivo, 4T1-FL2 BC orthotopic NOD-SCID mice were given PTT (750 nm NIR-laser, 650 mW, and 4 minutes) after intratumoral Au-SLN injection. Tumor regression was quantitated by 2D-BLI and DLIT of IVIS-spectrum.

Results: HA-Au-SLN showed no shift in NIR absorbance efficiency. HA coating was confirmed by FTIR and TEM. In vitro studies showed significant cell death (>75%, $p < 0.0001$) within 2 minutes of PTT across BC cell lines. Enhanced uptake of HA-Au-SLN over Au-SLN was observed in MDA-MB-231 cell line. HA-Au-SLN showed higher biocompatibility over Au-SLN at concentrations beyond 100 µg/mL in L929 cell line. In concordance to planar imaging estimation, volumetric assessment by DLIT simulation signified a 10^4-fold dip in source signal upon photothermal insult, leading to complete regression in primary tumor.

Conclusion: This is the first report of an actively targeted gold nanomaterial for PTT showing excellent antitumor efficacy bringing this therapy closer for clinical application.

A019. BEND4, a Novel Prognostic Marker for Adverse AML

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Background: Cytogenetic and molecular abnormalities–based prognostication in acute myeloid leukemia (AML) is useful for managing the heterogeneous disease. Most of these abnormalities are either mutation or gene fusion based. Unfortunately, gene expression–based biomarkers, which are easy to assess in clinical settings, are understudied due to lack of availability of paired normal tissue.

Materials and Methods: Raw transcriptome data of $n = 1{,}775$ patient's (six independent datasets) available in public domain were processed and independently analyzed for *Z*-score–based statistic, differential gene expression, bivariate, multivariate, and survival analyses using R software. The qRT-PCR and IHC-IF were used to validate the identified gene in two independent cohorts of patient samples.

Results: In an independent analysis of each transcriptome dataset, BEND4 was identified as the only common gene with high expression in adverse cytogenetic and in relapsed AML patients, leading to poor survival. These findings were further validated in our sample cohort of 31 de novo adult AML samples and 6 paired AML relapse patients. Bivariate analysis shows association of BEND4 expression with known AML cytogenetic risk factors like blast count, FLT3-ITD, and NPM1 mutation. Importantly, multivariable analyses, adjusted for those cytogenetic risk factors, as a time-dependent variable, confirmed the independent association of high BEND4 expression with a higher hazard ratio.

Conclusion: We identified significant association of BEND4 high expression in AML patients of adverse prognosis with 81% sensitivity and 91% specificity. Thus, we demonstrate that BEND4 can be a novel poor prognostic marker for AML and provide rationale for further investigation of its biological function.

#These authors contributed equally to this work

A020. SFRP1 in Regulation of Skin Cancer and Cancer Stem Cells Associated through Noncanonical WNT Signaling

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Background: Wnt-signaling involved in regulation of different cancer and cancer stem cells (CSCs). SFRP1, a Wnt-inhibitor, is downregulated in various human cancers; earlier study observed inverse correlation between SFRP1 and SOX2 in human oral and breast cancers. However, role of SFRP1 and molecular mechanism involved in skin tumor and CSC regulation remains unexplored.

Methods: We used the cutaneous skin squamous cell carcinoma cell line (A-3886). Flow cytometry was performed to isolate CSCs by using $CD133^+$, while $CD133^-$ as non-CSCs control. Expression profiles was performed using microarray.

Further, the differentially expressed genes were validated by RT-PCR and protein expression was checked by western blot.

Results: We observed 6 to 8% of CSCs population from A-3886 cells. CSCs population was checked by IFA using the CD133 marker, sphere formation and in vivo tumorigenesis assay. Expression profiling of CSCs showed upregulation of genes involved in EMT (Vimentin, TWIST1), stemness marker SOX2, and noncanonical Wnt signaling that suggest an enhanced tumorigenic potential of CSCs. We observed the upregulation in protein expression of noncanonical Wnt signaling cascade (Rac1-Dvl2-JNK-c-JUN-SOX2). We observed the interaction of c-JUN and SOX2 by coimmunoprecipitation and ChIP-qPCR in the absence of SFRP1. Further, we found that knockdown of JNK1 decreases the SOX2 levels and tumorigenic potential.

Conclusion: Our data revealed the role of SFRP1 and molecular mechanism behind the skin CSCs regulation through non canonical Wnt signaling cascade (Rac1-Dvl2-JNK), and interaction between c-JUN and SOX2, thereby resulting in an increased tumorigenic potential of CSCs.

A021. Investigating the Molecular Association Between p53 and HER2 Expression in Gastric Cancer

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Background: Gastric cancer (GC), the fifth most common cancer worldwide shows frequent p53 mutations and around 20% cases exhibit HER2 overexpression. In multiple cancers, mutant p53 regulates HER2 signaling. We aim to investigate the association and molecular relation between p53 and HER2 in GC cell lines and Indian patients.

Materials and Methods: Immunohistochemistry is performed for p53 and HER2 in paired (chemonaïve and NACT) patient tumor blocks. Role of wild type p53 in regulating HER2 expression was studied by ChiP assay in AGS cells. Isogenic cellular model systems expressing mutant and wild-type p53 in p53 null KATO-III cell line are under construction.

Results: In a small cohort of GC patients ($n = 19$), 8 among 19 patients possess mutant p53 and the rest harbor wild type p53. Intriguingly, 3 of 19 cases showed intense HER2 membrane staining and all of them harbor mutant p53. In AGS cell line, platinum treatment led to enhanced p53 and decreased HER2 expression at protein, as well as mRNA level. Using JASPAR software, three p53 binding sites were identified on HER2 promoter (score >80%). ChiP assay revealed increased p53 binding to at sites 2 and 3 on the HER2 promoter upon platinum treatment in AGS cell line.

Conclusion: This preliminary study suggests that wild type/mutant p53 might transcriptionally regulate HER2 expression in GC cell lines and tumor tissues. Detail molecular mechanism and identification of specific p53 mutations are currently under investigation.

A022. Mitochondrial Targeted Curcumin Inhibits Glutathione Reductase and Modulates Mitochondrial Redox: A Potential Novel Strategy for the Treatment of Resistant NSCLC

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Background: The dedicated thioredoxin and glutathione redox systems are the central antioxidant defense mechanisms by which mitochondria neutralize the excess ROS. In cancer, these antioxidant systems get upregulated to cope with oxidative stress insult caused due to dysfunctional mitochondria. These upregulated antioxidant systems lead to drug resistance in lung cancer.

Materials and Methods: The cytotoxicity of mitocurcuminin A549 cells at different time points and its IC_{50} was calculated by MTT assay. Cell-free and cell-based glutathione reductase (GR) inhibition was studied by DTNB reduction assay. Autodockvina and LigPlot tools were used for molecular docking and ligand interaction studies.

Results: Mitocurcumin exhibited cytotoxicity on A549 cells with IC_{50} of 7.91, 5.37, and 3.37 µM at 24, 48, and 72 hours, respectively. It inhibited recombinant GR in cell-free system with IC_{50} of 1.27 µM and mitochondrial GR in A549 cells. GR inhibition was independent of NADPH which serves as the cofactor in enzyme catalysis and showed mixed-II-type inhibition where Km and Vmax both decreased. The inhibition of GR-affected mitochondrial and cellular GSH pool by increasing both mitochondrial and cellular ROS in a dose- and time-dependent manner. In silico-docking studies revealed that mitocurcumin binds to the site other than active site of GR.

Conclusion: Mitocurcumin affects proliferation of A549 cells in dose- and time-dependent manner. It inhibits GR in cell-free system and in A549 cells which in turn modulates mitochondrial redox leading to ROS dependent apoptosis in A549 cells.

A023. Isolation and Characterization of Circulating PLAP$^+$ Serum Exosomes Across Pregnancy

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Background: Exosomes are cellular vesicles (30–100 nm) of endosomal origin released in extracellular space and body fluids. During pregnancy, exosomes are produced by the syncytiotrophoblast and released into the maternal blood circulation. The characterization of these exosomes in maternal blood during pregnancy may represent a clinically useful, noninvasive test for placental physiology.

Materials and Methods: The isolated exosomes were characterized in terms of their shape using TEM, exosomal size using DLS method, and expression of placental enriched markers, PLAP, and Cullin-7 by western blot. The DNA methylation status of LINE-1 promoter by pyrosequencing and miRNA expression including C19MC cluster were assessed.

Results: The ratio of PLAP-specific exosomes to serum exosomes in pregnant women (third trimester) was 74% (±8.37) and 39% (±26.84) in nonpregnant women. The C19 miRNA cluster miRNAs (miR 515-5p-519e and miR-520f) along with miR-133-3p, miR210-3p and miR-223-3p were found to be present in PLAP-specific exosomes. LINE-1 promoter methylation pattern in DNA derived from PLAP-specific exosomes corroborated with previous studies on LINE-1

methylation in placental tissue. Exosomal marker CD63, and placental markers PLAP and Cullin-7 were present in the total protein lysate of PLAP-specific exosomes.

Conclusion: These findings suggest that the circulating $PLAP^+$ exosomes may be a noninvasive tool to determine placental health during pregnancy.

A024. Role of GATA3-Expression in the T-Cell Lineage Assignment for the Diagnosis and Classification of Acute Leukemia

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Background: GATA-binding protein 3 (GATA3) is the transcription factor that regulates early T-cell differentiation. Other markers of T-cells including CD7, CD5, and CD2 are frequently aberrant and not lineage specific. Hence, we evaluated the utility of GATA3 expression and studied its utility as a T-lineage marker in the lineage-assignment of acute leukemia.

Materials and Methods: Anti-GATA3 (PECF-594 and clone-L50-823) antibody staining was standardized and studied in the leukemic blasts along with CD7, CD5, sCD3, CD34, CD45, and TdT using MFC. We also evaluated four permeabilization reagents: FACS Lyse (BD Biosciences), Fix-&-Perm (Invitrogen), Foxp3-fixation-kit (eBiosciences), and true-nuclear transcription-factor staining buffer set (Biolegend).

Results: GATA3 expression was studied in 60 T-ALL, 11 AML, and 7 MPAL with T/myeloid patients. B-cells and normal T-cells were taken as negative and positive controls with a median nMFI of 0.13 (0.0–5.44) and 0.80 (0.0–25.0), respectively. Median nMFI of GATA3 in T-ALL, AML, and MPAL patients were 2.57 (0.0–8.04), 0.17 (0.00–5.23), and 2.02 (0.00–6.92), respectively. Taking a cut-off of 8.33, 71.66, 71.42, and 36.66% of T-ALL, MPAL, and AML, respectively, were positive for GATA3. The sensitivity and specificity of GATA3 for T-cell lineage is 71.67 and 72.73%, respectively.

Conclusion: This is a first-time standardized flow cytometric assessment of GATA3 expression in the clinical setting. FACS Lyse (BD Biosciences) was the best permeabilization reagent. Flow cytometric GATA3 expression can give confidence in assignment of T-lineage in the presence of dim cCD3 in difficult cases of AL.

Table 1

GATA3	B-cells negative control	Normal T-cells positive control	T ALL blast	AML blast	MPAL blast (T/myeloid)
Median nMFI	0.13	0.80	2.57	0.17	2.02
Range	0.0–5.44	0.0–25.0	0.0–8.04	0.0–5.23	0.0–6.92

Abbreviations: AML, Acute Myeloid Leukemia; GATA3, GATA binding factor 3; MFI, Mean Fluorescence Intensity; MPAL, Mixed Phenotype Acute Leukemia.

A025. Genetic Modulation of Molecular Circuitry Governing Cellular Ageing in the Drosophila Intestinal Stem Cells Alters Tissue Homeostasis

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Background: Ageing is the progressive physiological decline of cellular and molecular functions. On the cellular level, ageing drives genomic instability, chronic inflammation, altered protein turnover, and mitochondrial dysfunction. These result in the accumulation of derogatory effects, causing age-related disorders. Here, we have investigated the effects of ageing on intestinal tissue homeostasis.

Materials and Methods: To elucidate the cellular ageing phenomenon and to understand their effects on tissue homeostasis using thorough in vivo analysis, we genetically either accelerated or stalled ageing in the *Drosophila* larval midgut using the Gal4 -UAS–based technology. The guts were dissected, immunostained, and analyzed using confocal microscopy and Image J Software.

Results: Acceleration of ageing specifically in the intestinal stem cells using inflammageing approach or via production of reactive oxygen species results in an increased DNA damage, apoptosis, and differential cellular proliferation. On the contrary, when ageing is stalled by overexpression of a key autophagy regulator, Atg8 in the intestinal stem cells, there is decrease in DNA damage, cell differentiation, and shows increased intestinal stem cell proliferation.

Conclusion: Given, the considerable conservation between *Drosophila* and mammalian intestinal pathophysiology and signaling pathways, we aim to gain mechanistic insights into the cellular and molecular circuitry that governs cellular ageing. Here we have shown that modulating ageing in either direction will have differential impact on various cellular parameters and organismal function.

A026. Utility of Flow Cytometry in Cytokine Profiling from Human Plasma Samples

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Background: Cytokines, biological molecules pivotal in induction and regulation of immune responses and emerged as focal point of biomarker research. Detection of cytokines by flowcytometric multiplex bead–based assays increases the clinical utility. In this study, we established the normal range, evaluated changes in cytokine levels under different pathological conditions in cancer setting.

Materials and Methods: Whole blood collected in K_2-EDTA vacutainer tubes. The tubes centrifuged at 1,000 × g for 10 minutes for plasma separation. BD CBA-FLEX set assays were procured for human selected cytokines. Cytokine assay was performed using 50 µL plasma as per manufacturer's protocol. Samples were acquired on BD LSRFortessa Data analysis was performed on FCAPArray v3.0.1.

Results: Normal range of cytokines was evaluated in 33 healthy samples. Cytokine profiling was performed in 323 plasma samples including, but not limited to, suspected GVHD, sepsis, HLH diagnosis, and immunotherapy monitoring. Lower limit of detection for IL-6 was 0.49, IL-8 1.04, IL-10 0.57, IFN-γ 0.79, MIP-1α 0.11, and GM-CSF 0.08 pg/mL. We noticed elevation in levels of inflammatory cytokines under the conditions evaluated ($p < 0.05$). Our results indicate a stark increase in IL-8 during sepsis ($p < 0.0001$). Concordant with literature, we observe a significant increase in IL-8 in HLH ($p = 0.02$). Additionally, we have used these assays monitoring response to novel-therapeutic agents like blinatumomab.

Conclusion: Our study indicates that cytokines are increased in disease-specific manner and can be used as diagnostic/prognostic markers. Although our cohort included only plasma samples, similar large-scale studies on other clinical specimens might prove useful.

A027. Gcn5 Regulates Blood Cell Lineage Differentiation and Maintains Tissue Homeostasis in the Drosophila Hematopoietic Organ, the Lymph Gland

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Background: General-controlled nonrepressed 5 (GCN5) is the histone acetyl transferase (HAT) involved in leukemic stem cell maintenance and overexpressed in acute myeloid leukemia (AML). Here, we are employing the *Drosophila* larval lymph gland (LG) to understand the physiological function and regulation of Gcn5 during developmental hematopoiesis which remains unexplored.

Materials and Methods: A multipronged genetic approach consisting of whole animal *gcn5* mutants, modulation of expression using knockdown or overexpression of Gcn5 in various cell populations of the LG will be used. The genotypes are then subjected to in-vivo analysis using organ microdissections and immunofluorescence analysis using confocal microscopy.

Results: Our results indicate that abrogation of Gcn5 expression by knockdown or overexpression in niche cells, stem cells, or differentiated blood cells leads to deregulation of hematopoiesis resulting in a leukemia-like scenario. Specifically, there is an alteration in the number of niche cells and the number of differentiated blood cell types.

Conclusion: Gcn5 regulates blood cell homeostasis in the *Drosophila* LG. Our study will help in understanding the mechanistic role of Gcn5 during hematopoiesis thereby gaining insights into the biology of AML.

Table : Median and range (minimum–maximum) of cytokines evaluated in healthy and disease conditions

Sample	IL-6 (pg/mL)	IL-8 (pg/mL)	IL-10 (pg/mL)	IFN-γ (pg/mL)	MIP-1α (pg/mL)	GM-CSF (pg/mL)
Normal	3.13 (1.24–5.70)	3.91 (3.13–5.67)	2.33 (1.36–4.09)	5.59 (1.63–7.84)	1.26 (0.36–2.36)	1.33 (0.77–2.37)
Sepsis	83.85 (16.28–1,322.74)	254.50 (16.72–2,355.95)	19.56 (5.38–162.36)	10.08 (0.00–13.53)	10.05 (1.75–54.31)	2.51 (0.00–12.22)
High-grade fever	50.84 (0–856.51)	84.17 (3.57–5,681.29)	12.08 (0.82–34.13)	4.41 (0–97.34)	7.61 (0–38.27)	2.53 (0–26.12)
HLH	54.49 (53.14–55.83)	251.4 (214.44–288.36)	12.62 (6.75–18.49)	2.71 (2.33–3.09)	2.43 (0.11–4.85)	2.28 (2–2.56)
Autoinflammatory syndrome	65.43 (25.61–105.25)	48.66 (7.65–89.66)	14.69 (4.62–24.77)	92.29 (16.20–168.37)	14.60 (4.96–24.21)	4.81 (3.06–6.57)
Multiple myeloma	45.21 (11.85–856.51)	19.88 (16.72–263.04)	15.64 (3.13–34.80)	14.43 (4.12–26.32)	12.96 (9.92–54.31)	4.30 (2.10–6.74)
GVHD	345.40 (38.04–2085.48)	63.77 (23.45–176.12)	16.11 (6.77–163.20)	13.17 (2.1–71.97)	6.89 (2.36–13.42)	1.24 (0–6.10)
CMV infection	18.60 (7.53–29.68)	41.94 (6.12–77.76)	23.76 (13.39–34.13)	2.50 (2.40–2.60)	10.32 (6.82–13.82)	2.16 (2.13-2.20)

Abbreviations: CMV, Cytomegalovirus; GM-CSF, Granulocyte Monocyte Colony Stimulating Factor; GVHD, Graft-versus-host disease; HLH, Hemophagocytic Lymphohistiocystosis; $IFN_γ$, $Interferon_γ$; IL-10, Interleukin-10; IL-6, Interleukin-6; IL-8, Interleukin-8; MIP1-α, Macrophage Inflammatory Protein 1-alpha.

A028. AMPing up the Search: A Structural and Functional Repository of Antimicrobial Peptides (AMPs) for Biofilm Studies (Biofilm-AMP), and a Case Study of its Application to Corynebacterium striatum, an Emerging Pathogen

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Background: Antimicrobial peptides (AMPs) have been recognized for their ability to target crucial biofilm formation processes. Identifying potential AMP candidates remains a significant challenge, necessitating preliminary in silico studies prior to in vitro and in vivo studies.

Materials and Methods: We have developed biofilm-AMP (B-AMP), a curated 3D structural repository of AMPs, focusing on AMPs relevant to biofilm studies. To demonstrate the user applicability of B-AMP, we use the sortase C biofilm target of the emerging pathogen *Corynebacterium striatum* as a case study.

Results: B-AMP contains predicted 3D structural models of 5544 AMPs (from the DRAMP database) generated with a suite of molecular modelling tools, with user friendly, multioption search capabilities. AMPs are annotated to existing biofilm literature, consisting of over 10,000 publications, enhancing the functional capabilities of B-AMP. For the case study, 100 structural AMP models from B-AMP were subjected to in silico protein-peptide molecular docking against the catalytic site residues of the *C. striatum* sortase C protein. We propose a preference scale based on docking scores and interacting residues to select candidate AMPs for further evaluation.

Conclusion: B-AMP (*https://b-amp.karishmakaushiklab.com/*) is a comprehensive structural and functional repository of AMPs, serving as a starting point for future studies on AMPs in biofilms. B-AMP will be regularly updated with AMP structures, interaction models with potential biofilm targets, and annotations to biofilm literature.

A029. Study of FGFR-3 by Flow Cytometry is Highly Sensitive and Specific For Prediction of t(4;14) Translocation In Multiple Myeloma.

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Background: Multiple myeloma (MM) is a genetically heterogenous plasma cell neoplasm comprising of varied translocations. Translocation t(4;14) involving IgH-FGFR3 fusion is reported in 6% cases and is associated with poor outcome. Flow cytometry is a sensitive and reliable method to detect FGFR3 expression, hence predicting presence of t (4;14) and helpful in risk stratification.

Methods: MM cases with more than 10% plasma cells (PC) on bone marrow aspirate were included. FGFR3 expression was studied by FC using 13-color antibody panel. Data were acquired on CytoFlex (Beckman Coulter) and analyzed on Kaluza version 2.1 software. Cytogenetic analysis by FISH was done to identify t(4;14) in all cases.

Results: Our cohort comprised of 140 cases of myeloma. Median age of patients was 57 years (range: 17–79). Cut-off of $\geq$15.8% FGFR3-positive plasma cells using ROC curve analysis was used to define FGFR3 positivity. In our study, FGFR3 was positive in 23 of 135 (17.03%). Cytogenetic results were available in 115 and t(4;14) was positive in (10%) patients. The sensitivity and specificity of FGFR3 expression by FC for the prediction of t(4; 14) in MM by FISH was 90.9 and 85.6%, respectively.

Conclusion: We standardized and studied FGFR3 expression in multiple myeloma by FC. It is highly sensitive and specific for detection of cases of MM with t(4;14) and therefore useful in clinical staging. Hence, it should be used as a reliable and alternate screening method in routine practice.

A030. A Biomimetic, 4D Preclinical Platform to Evaluate Novel Wound Biofilm Treatments

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Background: Evaluation of novel antibiofilm agents against wound infections has relied on in vitro models which fail to recapitulate the infection state, or scientifically and ethically questionable animal models. Biomimetic preclinical platforms that represent the wound infection state and enable the evaluation of novel and combination antibiofilm therapeutics are urgently needed.

Materials and Methods: We built a 3D host-cell scaffold of fixed dermal fibroblasts and epidermal keratinocytes, on which biofilms of *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA) were grown in an in vitro wound milieu[2] (host matrix and biochemical factors). Biofilms were characterized using confocal microscopy and the XTT assay for antibiotic susceptibility.

Results: The recapitulated 4D platform supports the development of mono- and mixed-species PA and SA biofilms over 24 hours, with characteristic features of biofilm structure and interspecies interactions. Notably, the thickness and distribution of PA and SA biofilm aggregates are influenced by microenvironmental factors such as host cells and the IVWM. Further, antibiotic tolerance of PA biofilms (to tobramycin) is higher in the composite 4D platform compared to standard laboratory media; SA biofilms (with vancomycin) did not show a difference.

Conclusion: We have developed a 4D biomimetic platform that recapitulates key features of wound biofilm structure and antibiotic tolerance. It can serve as a high throughput, selective, and precise approach to characterize

novel and combination antibiofilm approaches, and thereby fill a gap in the development of biofilm treatments.

A031. "Wound Infection on a Chip": A Macrofluidic Platform to Recapitulate the Dynamic Wound Infection Microenvironment

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Background: The wound infection state is a dynamic microenvironment with intricate interplay across host elements, biofilms, chemical cues, and biophysical factors. Biofluidic forces influence range of wound healing outcomes including cell proliferation, migration, and communication. However, the effects of biophysical forces in context of the composite microenvironment, remains to be explored.

Materials and Methods: Using 3D printing, we have developed a palm-sized "macrofluidic device" made of biocompatible polymer, polylactic acid, consisting of a transparent centrally placed chamber, with inlet and outlet channels connected to an in-house developed peristaltic pump. Human epidermal keratinocytes and dermal fibroblasts are seeded from the top portion of the chamber.

Results: The central chamber of the reconstituted "wound infection on a chip" supports the proliferation of HaCaT and HDFa cells, alone and in coculture. Using the scratch assay, to mimic wound injury, the cocultured "wound bed" demonstrates robust host cell migration and wound closure. These effects are currently being evaluated in presence of flow and shear stress applied at various time intervals.

Conclusion: The next steps include leveraging the "wound infection on a chip" system to study the effects of biophysical forces on the formation and development biofilms grown on host cells and dissect host-microbe interactions, with the inclusion of a relevant wound chemical milieu.

A032. An In Vitro Breast Cancer Cellular Dormancy Model

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Background: Cancer recurrence at distant sites is common among breast cancer patients. Dormancy plays a pivotal role in mediating these late recurrences. The processes involved in the regulation of quiescence in disseminated tumor cells (DTCs) persist predominantly undiscovered. Isolation and analysis of dormant DTCs from clinically disease-free patients is exceptionally complicated.

Materials and Methods: We developed an in vitro model that replicates dormant estrogen-positive (ER+) breast cancer cells in the bone marrow (BM), as they are likely to remain dormant for an extended period. The BM comprises basic fibroblast growth factor (FGF-2) and fibronectin, providing a favorable microenvironment for the DTCs to enter dormancy.

Results: MCF-7 cells were incubated at clonogenic densities in the presence of FGF-2, on fibronectin-coated dishes. FGF-2 treatment was given for 7 days, resulting in the formation of dormant clones on fibronectin-coated dishes. These treated cells were nonproliferating, had a large cytoplasm to nucleus ratio, and a distinctly flat appearance. In addition, a high p38/ ERK ratio and a negative senescence staining demonstrated that they were at a quiescent stage. Conversely, MCF-7 cells incubated without FGF-2 form much bigger colonies on fibronectin-coated and noncoated dishes.

Conclusion: Both FGF-2 and fibronectin have to be present in the microenvironment of DTCs for them to enter a state of dormancy. This assay functions as a tool to glean information about the molecular mechanisms necessary for the establishment and survival of dormant tumor cells.

A033. Prevalence and Susceptibility of Carbapenem Resistance Gram-Negative Bacteria in an ICU Setup

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Background: With the increasing use of carbapenems in clinical practice has led to emergence of carbapenem-resistant Gram-negative bacilli which now poses a great threat to human health. Carbapenem resistant Gram-negative bacilli are priority pathogens with limited options available for their treatment.

Materials and Methods: This is a prospective study over a period of 5 months during which, of the samples received from ICU, 59 samples grew Gram-negative bacilli for which identification and susceptibility was performed by Vitek-2 method. Carbapenem resistant Gram-negative bacilli (CRGNB) were considered in the study. Intrinsic carbapenem resistant bacilli, non-ICU samples were excluded from the study.

Results: Of the 60 carbapenem resistant isolates, 23.7% belonged to 41 to 50 years of age group followed closely by 51 to 60 (22.03%) and 61 to 70 (18.6%) years of age groups; males outnumbering the females. Most of the isolates were cultured from Respiratory samples (sputum, tracheal aspirate, and BAL). *Klebsiella pneumoniae* constituted the highest proportion (36.7%) of CRGNB followed by *Acinetobacter spp.* (22%). Tigecycline, colistin, and aminoglycosides were the most useful antibiotics for CRGNB. *Klebsiella spp*. had the highest resistance (75%) to tigecycline while *Acinetobacter spp*. had the highest resistance to colistin.

Conclusion: CRGNB is on the rise in ICU set-up, *Klebsiella spp*. dominating the scenario. The available options for these bacilli are limited to tigecycline, colistin, and aminoglycosides. Tigecycline is the most effective antibiotic for *E. coli* and *Acinetobacter spp*. but colistin should be preferred for *Klebsiella spp*.

A034. Scaffold Based Gene Delivery for Immunotherapeutic Applications

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Background: Gene delivery is one of the major achievements in the field of immunotherapy, but the cost and complexity of manufacturing large numbers of

genetically programmed cells remain a major hurdle. We thereby illustrate a method to deliver genes to cells in native microenvironment thus overcoming existing limitations.

Materials and Methods: Polyethylene glycol–based scaffold implant was fabricated and carbamide chemistry was used to conjugate poly-L-lysine and immobilize gene carrying lentiviruses on the scaffold. This implant was characterized for physical and biological properties. Further, GFP and luciferase expressing lentiviruses were loaded in the scaffold and efficiency of gene delivery was characterized.

Results: Characterization of scaffold showed a highly interconnected macroporous structure in SEM, thereby revealing a higher surface area for immobilization of lentiviruses. The scaffold was also biocompatible and hemocompatible. Further, conjugation of poly-L-lysine significantly improved the immobilization of lentiviruses on the scaffold. In vitro studies show efficient delivery of GFP gene into HEK293T cells via the scaffold loaded with GFP expressing lentiviruses. Mice implanted with luciferase expressing lentivirus loaded scaffold showed higher transduction efficiency when compared to bolus lentivirus delivery.

Conclusion: Here, we demonstrate a practical, low-cost, broadly applicable gene delivery strategy that can genetically program cells without the requirement for ex vivo manipulation of patient cells. Further, engineering techniques can be used to attract and modify specific cells for various disease settings.

A035. Prevalence of ESBL-Positive Gram-Negative Bacteria in Community-Acquired UTI in a Tertiary Care Centre in the Eastern Part of India

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Background: Urinary tract infections (UTIs) are one of the most common infections in humans. The aim of the study was to find the incidence of Gram-negative bacteria along with its antibiotic sensitivity pattern in community-acquired UTI and to determine the prevalence of extended spectrum beta lactamase (ESBL) positivity among them.

Materials and Methods: A total of 515 urine samples were tested and the causative bacteria were identified. ESBL test was performed to identify the prevalence of ESBL-producing isolates. Antibiotic susceptibility test was performed for all the isolated bacteria.

Results: From a total of 515 samples, 65 (12.65%) were positive for UTI. The rate of infection was higher in females than males. *Eschereria coli* was the most frequently isolated pathogen followed by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Out of total positive, 43.08% were ESBL-producing strains. The antibiotic susceptibility pattern revealed that the isolates showed resistance to ciprofloxacin and cefixime and sensitivity to gentamicin, ertapenem, and nitrofurantoin.

Conclusion: Females have higher prevalence of UTI than males. Mostly, females suffer from UTI in their reproductive years, whereas males suffer in their old ages. UTI treatments should be done properly, and antibiotic resistant and sensitivity patterns should be considered to prevent overuse and misuse of antibiotics.

A036. Investigating the Molecular Basis of c-FLIP/Calmodulin Interaction for Modulating Apoptosis

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Background: Overexpression of antiapoptotic protein c-FLIP in cancer and its interference with DISC leads to apoptosis evasion. Preventing c-FLIP recruitment to DISC is difficult due to its structural similarity with procaspase 8. The c-FLIP needs to bind to calmodulin to access the DISC. Thus, targeting c-FLIP/CaM interaction can be a good strategy.

Materials and Methods: The genes were cloned in expression vectors. Proteins were purified using Ni-NTA and amylose resins followed by circular dichroism and fluorescence spectroscopy. Interactions were checked using pull-down assays. Schrodinger software was used for in silico experiments. Calmodulin Sepharose resin was used to check interactions with mutant and wildtype c-FLIP.

Results: We confirmed the interaction between the two proteins and also the critical amino acid residues of the binding interface of CaM/c-FLIP using in silico methods, protein engineering, and biochemical tools. The structural conformation and thermal stability of the purified mutant and wild type proteins was determined, and they were found to be stable. Furthermore, using this information, we have designed peptides that interfere with this binding. Crystal trials for the c-FLIP DED domains showed microcrystal formation in few buffer systems.

Conclusion: This array of information will be crucial toward devising small molecules for therapeutic benefit after appropriate testing and validation.

A037. Identification and Characterization of FDA-Approved Drugs as Novel Binders of CLIC1

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Background: CLIC1, a metamorphic ion channel, can exist as a soluble monomer and on sensing oxidative stress can dimerize and further oligomerize to form a functional ion channel on the membrane. CLIC1 overexpression is associated with poor prognosis and metastasis in GBM, colorectal cancer, HNSCC, and HCC. Ion channels are promising therapeutic targets, but most of them have a normal physiological function and targeting them have adverse side effects. Since CLIC1 is known to preferentially localize on the membrane of cancer cells, taking advantage of the existing CLIC1 structure to repurpose FDA-approved drugs reduces the risk of adverse side effects.

Materials and Methods: Potential inhibitors of CLIC1 were identified by docking of approximately 100 compounds using two different algorithms, Glide and AutoDock Vina.

Nano-differential scanning fluorimetry, limited proteolysis followed by SDS-PAGE, and Microscale thermophoresis were used to test interaction of the best fit compounds to CLIC1 in vitro.

Results: All the drugs bound to CLIC1 but at varying affinities as observed by their ability to protect CLIC1 from proteolysis, MST, and nano-DSF. One of the compounds (Ash1) bound with low µM affinity and was used to set up cocrystals with CLIC1. Ash1 reduced ERK1/2 activation by phosphorylation, decreasing the migratory potential. These results provide an excellent opportunity to improvise the leads obtained to find an alternate inhibitor of CLIC1 which is already FDA approved.

Conclusion: Using a combination of in silico and in vitro methods, we have identified a promising first-generation inhibitor of CLIC1 which can be optimized into a potent inhibitor.

A038. Characterization of VRK2A-BclxL Interaction and Implication in Apoptosis

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Background: In intrinsic apoptotic pathway, there are many regulatory molecules that together form mitochondrial membrane protein complexes. Recently, VRK2A has been identified as one such regulator. It is a protein belonging to vaccinia-related kinase (VRK) family, a Ser-Thr kinase family that regulates several signal transduction pathways. Interestingly, VRK2A has been found to be a component of the well-established BAX-Bcl-xL complex; however, the exact role of the former is yet to be delineated. It has been hypothesized that at high VRK2A level; this ternary complex delays apoptosis induction possibly by stabilizing the ternary complex.

Materials and Methods: Cellular localization of VRK2A was analyzed through live cell imaging. Interaction between VRK2A and Bcl-xL has been validated using biochemical and cell biology probes. To identify the binding interface, as well as the critical residues involved, this interaction in silico studies, including molecular modeling and docking, were done.

Results: For better understanding of this complex formation and its significance in apoptotic pathway, characterization of these interactions become imperative. Therefore, we checked their expression levels in different cancer cell lines and mitochondrial localization. We identified crucial residues of both proteins involved in VRK2A and Bcl-xL interaction.

Conclusion: With these leads, variants of both the interacting partners are being generated to validate the interacting residues and determine their biophysical parameters. These studies would further extended toward studying the role of VRK2A in stabilization of Bcl-xL-Bax complex, so as to devise ways to modulate the interactions with desired characteristics for disease intervention.

A039. Unravelling the Epigenetics of Murine Testis–Specific Histone Variants, TH2A, and TH2B in the Mature Caudal Sperm

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Background: Sperm-retained histones/variants are one of the most significant epigenomic contribution of father to embryo. Two such histone variants, TH2A and TH2B escape spermiogenic nuclear remodeling and are retained in the mature sperm. Importance of TH2A/TH2B in histone eviction during spermiogenesis is acknowledged. However, their significance postspermatogenesis is unknown.

Materials and Methods: Chromatin Immunoprecipitation sequencing (ChIP-seq) analysis was done to understand the epigenomics of the retained TH2B and TH2A, using murine caudal sperm.

Results: Genomic distribution revealed 35% of TH2B peaks within ±5 kb of TSS whereas TH2A was mainly intergenic. TH2B was enriched at spindle assembly and meiosis-specific genes, and embryo development was the most significant term among TH2B-associated genes (TBAGs). This is important as TH2A/TH2B DKO mice have defective cohesin release. Also, TH2A was enriched with mitochondrial function genes. Presumably, TH2A is associated with mitochondrial DNA inserted in nuclear DNA. Also, there was 26% evolutionary conservation between human and murine TBAGs including genes crucial for embryogenesis. Most importantly, heterogeneity in the epigenetic landscape of TH2A and TH2B was seen.

Conclusion: The murine TH2A/TH2B ChIP-seq analysis has attributed novel functions to sperm retained TH2B regarding embryogenesis and spermatogenesis. Conservation of TH2B-DNA linkage across two mammalian species highlights the significance of programmed histone retention for embryogenesis. Also, TH2B/TH2A is relatively independent in their epigenetic landscape. The TH2A-MtDNA link needs to be explored.

A040. Expression Pattern of a New Marker, Gl7 in Different Stages of B-Cell Maturation and Its Utility in B-Lymphoblastic Leukemia/Lymphoma Measurable Residual Disease Assessment

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Background: Increasing use of targeted therapies such as anti-CD19 Blinatumomab/CAR-T-cells and anti-CD20 rituximab obviates the need for additional B-cell maturation–associated markers to evaluate deviation from normal in bone marrow (BM) in patients with B-lymphoblastic leukemia/lymphoma (B-ALL) by multicolor flow cytometry (MFC). GL7, a novel marker, has been studied for the same.

Materials and Methods: Expression of GL7 was studied on leukemic blasts in 65 B-ALL patients at baseline, 15 end of induction (EOI = D28-35) BMRD samples and on hematopoietic cells including B-cell precursors (BCP) from five uninvolved-staging BM samples using 16-color MFC. Expression pattern of GL7 was determined as MFI and CV-IF on Kaluza-v2.1.

Results: The proportion of various hematopoietic cells expressing GL7 (MFI and CV-IF) are provided in **Table 1**. GL7 appeared at the late BCP2 stage of BCP2 and in a subset of

Table 1

Population	Percentage of Expressing GL7 Median (range)	MFI of GL7+ proportion Median (range)	CV of GL7+ proportion Median (range)
B-cell precursor-1 (BCP1)	3.7 (1.1–3.8)	12.4 (9.5–13.1)	32.1.1 (11.7–41.4)
B-cell precursor-2 (BCP2)	51.6 (31.8–75.2)	24.6 (11.2–22.3)	51.6 (31.8–75.2)
Mature B	98.0 (95.1–99.3)	82.6 (61.6–84.3)	48.0 (41.1–54.4)
Plasma cells	47.1 (37.7–49.4)	16.1 (9.0–18.2)	68.2 (49.5–75.9)
T-cells	66.3 (39.3–87.1)	22.5 (15.3–43.8)	66.2 (64.6–77.9)
NK cells	19.7 (17.6–24.5)	13.7 (11.2–14.4)	51.6 (46.8–57.6)
Neutrophils	65.9 (64.4–92.0)	8.3 (7.4–8.3)	149.6 (141.7–176.7)
Plasmacytoid dendritic cells (PDC)	9.2 (7.7–15.2)	6.6 (4.2–7.1)	71.5 (22.5–97.7)
Leukemic B-blasts	58.7 (0.07–99.6)	14.1 (1.3–35.6)	127.7 (65.3–299.2)

Abbreviation: MFI, Mean Fluorescence Intensity.

BCP1. The brightest level of GL7 was observed in mature B-cells. Among non-B-cells, a distinct proportion of T-cells and neutrophils expressed GL7. Among B-ALL patients, a median of 58.7% (0.07–99.6%) of leukemic B-blasts expressed GL7; however, the expression level was dim to heterogeneous. Among 15 BMRD EOI samples, six patients had detectable MRD (median MRD = 0.024% [0.0006–9.7%]) and 8%(6.1–34.6%) of residual B-blasts expressed GL7. Asynchronous expression of GL7 was observed in two of six MRD+ patients.

Conclusion: GL7 appears in B-cell maturation in BM at late BCP2 stage and is expressed in a proportion of B-ALL patients. GL7 provides a highly useful additional marker for the assessment of B-ALL MRD.

A041. An Improved Bioavailability of Total Lutein Oxidized Products (LOPs) Extracted from Tagetes erecta Flower Petals in C57BL/6 Mice

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Background: The lutein extracted from shade dried *Tagetes erecta* flower petal powder by simultaneous solvent extraction and saponification, was exposed to sunlight ($31 \pm 2°C$) for 10 days. The resultant lutein oxidized products (LOP) exhibited significant inhibition of pancreatic lipase in in-vitro assay with IC_{50} of 1.5953 µg/mL.

Materials and Methods: Bioavailability and absorption kinetics of LOPs in comparison with lutein (parent molecule) were analyzed on C57BL/6J mice. Time course plasma kinetics was studied by collecting blood plasma, liver, intestine and eyes after first, second, third, sixth, and ninth hours of incubation.

Results: Postprandial LOPs concentration in plasma was maximum at second hour. The area under concentration (AUC) and area under moment concentration (AUMC) of LOPs were 1,139.418 and 17,750.69 $pg/h^2/mL$, respectively, with a half-life of 11.353 hours. The mean residence time (MRT) of LOPs was 15.573 hours with a volume of distribution (Vd) of 2.874 and clearance (CL) of 0.175. The eyes had 59.80 pg/mL at ninth hour after pooling due to less concentration of LOPs in single eye. Whereas, mean lutein concentration in plasma, liver, intestine, and eye was significantly less in lutein in comparison with LOPs.

Conclusion: Thus the above data suggest that LOPs reach systemic circulation and target tissue unaltered and enhance the absorption rate in comparison to the parental compound lutein and with further in vivo examinations, LOPs can be developed as a potent nutraceutical with antiobesity action.

A042. Pax5 Standardization for the B-Cell Lineage Assignment in Acute Leukemia by Flowcytometry.

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Background: PAX5 (B-cell specific nuclear protein) used in immunohistochemistry as B-lineage marker. In clinical settings, its expression by MFC has not been reported. In complicated cases, confident lineage assignment as per WHO criteria requires multiple markers. PAX5 expression was standardized by MFC, and its importance studied as a B-lineage marker in acute leukemia.

Materials and Methods: We standardized Anti-PAX5 (PE, clone-REA140) antibody staining and studied in the leukemic blasts using MFC. The permeabilization reagents used for evaluation were FACS Lyse (BD), Fix-&-Perm (Invitrogen), Foxp3-fixation-kit (eBiosciences), and true nuclear transcription-factor staining buffer-set (Biolegend).

Results: Best expression for anti-PAX5-antibody was obtained with Foxp3-fixation-kit (titer = 5 µL). Our cohort comprised of 21 Acute Myeloid Leukemia, 25 B-Acute Lymphocytic Leukemia, and 10 (Mixed Phenotype Acute Leukemia and B/myeloid) patients. Normal B-cells and T-cells served as positive and negative controls (**Table 1**). Median nMean Fluorescence Intensity of PAX5 in B-ALL and AML

Table 1 Median nMFI of PAX5 expression

PAX5	Negative control (T-cells)	Positive control (B-cells)	B-ALL blast	AML blast	MPAL blast (B/myeloid)
Median nMFI	1.84	5.7	14.7	−0.62	12.44
Range	0.18–1.4	3.19–17.3	3.18–33.1	(−2.4)–10.8	2.0–65.3

Abbreviations: ALL, Acute Lymphocytic Leukemia; AML, Acute Myeloid Leukemia; MFI, Mean Fluorescence Intensity; MPAL, Mixed Phenotype Acute Leukemia.

patients were calculated (**Table 1**). AML patients did not show PAX5 expression (nMFI < 3) except one t(8;21) translocated AML which showed aberrant CD19 expression. B-ALL cases showed strong PAX5 expression (nMFI > 7). PAX5 expression was homogenous in B-ALLs with median (SD): 58.9 (41.7) as compared to 101.5 (131.8) in AML. PAX expression was also noted in two CD10-negative pro-B-ALLs and four B/Myeloid MPAL patients.

Conclusion: We standardized and studied PAX5 expression by flow cytometry in clinical settings. Best permeabilization reagent was Foxp3-fixation-kit (eBioscience). PAX5 expression was highly specific for B-lineage and therefore could be used in challenging and difficult cases of acute leukemias for determination and confirmation of B-cell lineage.

A043. Analysis of Circulating Tumor DNA to Detect EGFR Mutations as a Diagnostic Tool for NSCLC Patients

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Background: Somatic DNA mutations are highly tumor specific which can be used as optimum diagnostic markers. Noninvasive mutation detection by assessing circulating tumor DNA (ctDNA) offers many advantages over tumor biopsy.

Materials and Methods: ctDNA from plasma of 96 nonsmall cells lung cancer (NSCLC) patients were analyzed for the presence of EGFR activating mutations using highly sensitive BEAMing polymerase chain reaction (PCR). We could identify mutations in ctDNA from 31 of 96 patients (32%). McNemar's test was done to determine the concordance between BEAMing and qPCR done on original tumor tissues.

Results: Our results showed high concordance with *p*-value of 1.00 for all the exons (19, 20, and 21) between BEAMing and qPCR-EMR. Similarly, BEAMing data when compared with qPCR-EasyLine data showed *p*-value of 1.00 for exon 21 and $p = 0.625$ for exon 19. Exon 20 BEAMing versus qPCR-EMR/qPCR-EasyLine were not estimable as all cases were negative in one of the comparative methods. Since the McNemar's test, *p*-value is larger than the significance level ($\alpha = 0.05$), here the null hypothesis could not be rejected which signifies our data were highly concordant between BEAMing versus qPCR-EMR and BEAMing versus qPCR-EasyLine kit.

Conclusion: EGFR mutation detection by BEAM*ing* PCR showed high overall agreement and good correlation with that in tumor biopsy with a detection sensitivity of 0.1%. Thus, feasibility and practicality of ctDNA analysis is a reliable technique and may translate into an alternative tool for anti-EGFR treatment selection.

A044. Detrimental Impacts of Amygdalin Compound against Honeybees Present in Pollen and Nectar of (clove) Syzygium aromaticum (L.) Merr. and L.M. Perry and Pliny the Elder

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Background: Some plants containing cyanogenic glucoside. Honeybees may be affected when they collect the nectar of CNglc-containing plants such as a clove. This study aimed to understand the response of honeybees against cyanogenic glucoside.

Materials and Methods: Almost 40 to 70 bees were introduced to hording cages. Cages were kept in laboratory in open conditions at temperature ranged from 17.5 to 25°C. Relative Humidity ranged from about 18 to 37%. Mortality rate of died bees was measured by counting on 1 to 8 days following initial dosing on day 0. Ad libitum was used for bees feeding. Data were analyzed to providing the estimation of the dose of CNglc. Analysis of controls to determine an LD50 provided a significant model but had 95% fiducial limits of one to infinity.

Results: **Table 1** shows the estimated duration of exposure. At each experimental dose of amygdalin required to kill 50% of the bees (LD 50) in days.

Conclusion: According to the results, it can be concluded that doses which are similar to those found in pollen and nectar of *S. aromaticum* are not having much toxic effects to kill honeybees, but extra doses of this toxic compounds may be harmful for honey bees.

Table 1

Dose of amygdalin (ppm)	Estimated time (days) for 50 % mortality	Lower (95)% fiducial limit	Upper (95)% fiducial limit
0[a]	16.1	14.2	19.4
2,250	4.6	3.0	6.3
4,500	2.7	1.7	3.6
9,000	2.3	2.1	2.4
18,000	1.9	1.7	2.0
36,000	1.8	1.5	2.0

A045. Bilateral Tubular Adenoma-Breast, A Case Report

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Background: Fibroadenoma is one of the most common benign neoplasm of breast in a young female; however, tubular adenoma is a much rare benign breast neoplasm occurring in young women. These are clinically indistinguishable from other benign breast neoplasms and can be diagnosed only on the basis of histopathological examination.

Materials and Methods (Clinical Presentation): A 20-years old female presented to our hospital with complaints of lump in both breasts. On clinical examination, lumps were felt in upper-outer quadrants of both breasts below the nipple and areola. USG findings were suggestive of bilateral fibroadenoma of the breast. Patient underwent lump excision under the same provisional diagnosis of bilateral fibroadenoma. Resected lumps were sent to our department for histopathological examination.

Results (Histopathological Evaluation): Grossly, the mass consisted of multiple irregular tissue bits which were pinkish white in color. Microscopic examination of H&E-stained sections revealed tightly packed proliferated tubules lined by cuboidal epithelium having normochromatic nuclei and scanty intervening stroma. Hence from the histopathological examination the final diagnosis was established as bilateral tubular adenoma-breast, in a 20-year-old female.

Conclusion (and Why this Case Was Chosen): Tubular adenoma is a rare benign epithelial neoplasm of breast seen in young females, and it cannot be diagnosed on the basis of clinical, cytological, or radiological examination and is often labelled as fibroadenoma. Histopathology remains the gold-standard method to diagnose this entity which is only seen in handful of cases and bilateral involvement being even rare.

A046. Clinical Utility Cytoplasmic TRBC1 Expression in the Diagnosis and Minimal Residual

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Background: TRBC1 is a novel flow cytometry-based T-cell colonialist marker. Recently, it is being studied in T-cell Non-Hodgkin lymphoma. However, studies evaluating its utility in the diagnosis, lineage-identification, and Minimal Residual Disease studies in T-cell acute lymphoblastic leukemia (T-ALL) are extremely rare.

Materials and Methods: Surface and cytoplasmic TRBC1 (Clone-Jovi1, PE) expression was studied 20 T-ALL patients at diagnosis and 4 T-ALL MRD samples, using 13c antibody panel on DxFLEX Beckman Coulter flow cytometry. Data analysis was carried out on Kaluza Analysis 2.0 software.

Results: We studied cytoplasmic TRBC1 expression in eight childhood T-ALL (median age = 6 years), 12 adult T-ALL (median age = 25 years), and 4 T-ALL MRD samples. There was clonal restriction of TRBC1 in the form of negative expression in T-ALL ($n = 7$) and TMRD ($n = 2$). There was clonal restriction of TRBC1 in the form of positive cytoplasmic expression in TALL (pediatric $n = 3$ and adult $n = 2$). Clonal TRBC1 expression was positive in >90% blast population.

Conclusion: Our study demonstrates that flow cytometric immunophenotyping of surface and cytoplasmic TRBC1 can aid in detection of abnormal blasts in T-ALL cases and has a high potential in T-ALL MRD monitoring.

A047. Synthesis of Zinc Oxide Nanoparticles using Aspergillus species Stain RR-1 and RR-2

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Background: Myconanotechnology is the interface between mycology and nanotechnology, new applied interdisciplinary science that may have considerable potential. Purpose of this study is to synthesize zinc oxide (ZnO) nanoparticles using *Aspergillus nidulans.*

Materials and Methods: Coriander seeds were taken (presterilized) and sampled on potato dextrose agar (PDA). The desired *A. nidulans* strains were isolated followed by lactophenol cotton blue (LCB) staining. Fungal strains were grown aerobically in potato dextrose broth (PDB) and after processing with $ZnSO_4$ salt, ZnO nanoparticles were synthesized.

Results: ZnO nanoparticles producing fungal strains RR-1 and RR-2 were isolated from the species. The fungal strain is grown in the PDB containing 1 mM of $ZnSO_4$ salt to determine their metal resistance. The ZnO nanoparticles were characterized by means of the UV-Vis spectroscopy and scanning electron microscopy. The synthesized ZnO nanoparticles showed an absorption, minimum and maximum at 340 and 520 nm, respectively.

Conclusion: ZnO nanoparticles which are synthesized using *A. nidulans* strains can be produced at large scale and used against many food-borne pathogens due to strong antibacterial activity. This work has future implication in the form of activity against cancer cells and gene induction to produce large quantity of nanoparticles.

A048. Investigating the Role of Herbal Compounds in Chemotherapy-Sensitive and -Resistant Gastric Cancer Cell Lines

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Background: Gastric Cancer is the third leading cause of cancer-related deaths worldwide. The treatment regimen consists of daily administration of capecitabine, increasing the likelihood of developing chemoresistance. However, the prognosis remains poor (5-year survival rate of <20%). So, there is an unmet need to develop alternative therapeutic strategies. This study aims to elucidate the role of herbal extracts of *Withania somnifera* and *Punica granatum* as potential therapeutics for their minimal side effects in chemotherapy-sensitive and -resistant GC.

Materials and Methods: We are developing 5-FU resistance model via pulse-treatment method in AGS cell line. *Punica* was dissolved in 30% DMSO. *Withania* was dissolved in 100% methanol. Cell viability assays and immunoblotting were performed to determine the IC50 values for 5-FU and herbal compounds to characterize autophagic flux and apoptosis in AGS.

Results: The IC50 values for *Withania*, *Punica*, and 5-FU are 2.5, 40 µg/mL, and 10 µM, respectively. Immunoblotting analysis revealed that both *Punica* and *Withania* showed a blockade in the autophagic flux in AGS-sensitive cells. *Punica* increased PARP cleavage in a concentration-dependent manner indicating induction of apoptosis. In the chemoresistance model which is in progress, cells after six and nine cycles of 5-FU treatment showed a two- and three-fold increase in their resistance indices, respectively.

Conclusion: We show that *Withania* and *Punica* impart cell death in chemotherapy-sensitive cells primarily via an apoptotic pathway. The mechanism of cell death in chemotherapy-resistant cells by these herbal compounds still remains elusive which will be explored further in the study.

A049. Investigating the Effect of Silencing of ATG5 Gene in Differentiation, Chemotherapy-Resistance and Stemness Properties of Cancer Stem Cells of Epithelial Ovarian Cancer

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Background: Cancer stem cells (CSCs) are implicated to be the prime cause for tumor relapse and chemotherapy resistance in ovarian cancer, as they are quiescent and intrinsically resistant to various chemotherapeutic drugs. Autophagy, a vesicular lysosome-mediated degradation mechanism maintains the homeostasis, stemness, and chemotherapy-resistance properties of CSCs. A key protein called ATG5 initiates the formation of the autophagosome membrane and is responsible for the fusion of autophagosomes with lysosome that marks the completion of autophagy flux.

Materials and Methods: In our study, we are knocking down ATG5 in an indigenously developed late stage chemotherapy-resistant A2780 cells. Two shRNA cassettes with one targeting 3′ UTR of *ATG5* gene and the other targeting both the 3′UTR and the coding region of the gene were designed.

Results: Two shRNA constructs against *ATG5* gene were successfully cloned into pLL3.7-EGFP plasmid. Positive clones were selected using the colony PCR technique and further validated by Sanger's sequencing. FACS-sorted GFP positive cells from transiently transfected HEK293FT cells showed a knock-down efficiency of 20 and 60% for shRNA1 and shRNA2, respectively. HEK293FT cells were transfected with plasmid carrying shRNA2, PΔ, and VSVG for production of lentiviral particles. Virus particles concentrated by ultracentrifugation will be transduced in $A2780^{LR}$ cells to generate stable ATG5 knockdown cells.

Conclusion: Successful cloning of shRNA1 and shRNA2 was achieved in pLL3.7 plasmid and 50 to 60% knock-down efficiency was achieved in HEK293FT cells. Virus particles have been constructed to transduce $A2780^{LR}$ cells to understand the consequences of autophagy blockade on the CSC phenotype with respect to stemness, proliferation, and chemotherapy resistance.

A050. An In Silico Analysis to Elucidate the Role of miRNAs in Hydroxyurea-Induced Fetal Hemoglobin in Sickle Cell Disease Patients

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Background: The most adaptive therapeutic form of sickle cell disease (SCD) treatment has been the induction of fetal hemoglobin (HbF) using hydroxyurea (HU). Since the role of miRNAs in HU-induced HbF production in SCD patients remains elusive, our study highlights the differentially expressed miRNAs and their regulated mechanisms through computational studies.

Materials and Methods: The miRNA expression profile data set GEOD32025 and GEOD11060 were obtained from the GEO database. The data were statistically analyzed with R software packages. The miRNA targets were identified, followed by the revelation of gene ontology and pathway enrichment analysis to highlight the involved cellular functions and pathways.

Results: Out of the 50 DE miRNAs filtered, 32 were downregulated, while the remaining ones were upregulated. A total of around 29,000 genes were identified as targets for the DE miRNAs. The gene set enrichment analysis was performed on the identified targets which revealed 125 enriched biological functions. Subsequently, pathway enrichment analysis divulged B-cell receptor signaling pathway, T-cell receptor signaling pathway, B-cell receptor signaling pathway, and MAPK signaling pathway. In GSE11060 dataset, four enriched pathways were upregulated notably, that is, axon guidance, renal cell carcinoma, pancreatic cancer, and endocytosis.

Conclusion: miRNAs might be plausibly involved in the HbF induction process following HU treatment. This information enlightens about the hydroxyurea efficacy mechanism while simultaneously pointing out novel targets for effective treatment of sickle cell anemia. The latter can be accomplished using recently developed genome editing and therapy tools.

A051. Application of Zinc Oxide Nanoparticles as a Catalyst in Dissipation Kinetics of Azoxystrobin 23 % SC in Different Soils under Photocatalytic

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Background: The dissipation kinetics of azoxystrobin 23 % SC under direct sunlight using zinc oxide nanoparticles as a catalyst. The nanoparticles are synthesized and characterized by SEM, UV, and FT-IR. The experiment was conducted by spiking into four different soils. The spiked sample was kept under sunlight. The sampling occasions were collected for four soils. The residues of azoxystrobin in different soils were dissipation to below the detectable level by 72 hours. DT50 values calculated using the following formula DT50 = ln 2 / (k).

Materials and methods: Reference standard from Sigma-Aldrich. The compound was purchased from local market. The UHPLC with PDA system used, the Xterra column was used and column temperature at 30°C. The injected volume was 10 µL. Mobile phase acetonitrile-to-water was 60:40 (%), and the flow rate was 1.0 mL/min and wavelength was 240 nm.

Results: The initial concentration of azoxystrobin 23 % SC in four soil samples were collected on 0th hours showed initial concentration 0.499 and 0.998, 0.324, 0.714, 0.265, 0.611, 0.156, 0.486, 0.056, and 0.186 µg/g, respectively and complete dissipation of residues of compound to below detectable limit was observed in 72nd hour.

Conclusion: The photocatalytic degradation of residues of azoxystrobin 23 % SC clearly indicates that the sunlight photolysis was influenced by the addition of zinc oxide nanoparticles as a catalyst. In the presence of catalyst, the compound persists very fast. The catalysis reaction proceeded rapidly degrading the molecules very fast in different soil samples.

Pediatric Acute Myeloid Leukemia in India: A Systematic Review

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Abstract

Background Lower-middle-income countries face unique problems in the management of pediatric acute myeloid leukemia (AML) due to which the outcomes have not kept pace with developed nations. In India, data on childhood AML is sparsely available, thus making a true assessment of disease trends difficult. The current systematic review was undertaken to assess the outcomes of childhood AML from published literature from India over a period of 10 years (2011–2021).

Materials and Methods A systematic search of MEDLINE, Google Scholar, and SCOPUS was performed as per preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement from January 1, 2011 to December 31, 2021. In addition, International Society of Pediatric Oncology (SIOP) conference abstracts were also screened for relevant studies on AML from India. This study was registered in PROSPERO (ID42021273218).

Results A total of 1,210 patients from 19 studies were included. Standard 3 + 7 and MRC AML based regimens were commonly adopted regimens for induction. Remission rates varied between 56 and 95%. Overall treatment-related mortality across studies was 23.2% (95% confidence interval [CI]: 10.3–35.9%). The mean incidence of treatment abandonment was 19.3% (95% CI: 10.9–27.5%). Event-free survival and overall survival were in the range of 28 to 55% and 15 to 66%, respectively. Hematopoietic stem cell transplantation was performed only on a small subset of patients.

Conclusion Outcomes of pediatric AML in India continue to be suboptimal with high treatment abandonment and toxic deaths. Ensuring uniform access to therapy and supportive care along with a robust social support system would improve outcomes of childhood AML in India.

Keywords

- acute myeloid leukemia
- India
- lower-middle-income countries
- abandonment

Introduction

Acute leukemia accounts for approximately one-third of all childhood malignancies, of which 15 to 20% cases comprise of acute myeloid leukemia (AML).[1] The outcomes of childhood AML in high-income countries (HICs) have currently surpassed 70% with an increased focus on targeted therapies to further these outcomes and also simultaneously reduce toxicity.[2,3] Lower-middle-income countries (LMICs) continue to have suboptimal outcomes due to various socioeconomic and disease-related factors.[3] There is limited data on childhood AML from India.[4] As a result, there is limited

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understanding of disease trends, which may ultimately compromise patient care. A previous systematic review from India, which included studies published between 1990 and 2010, highlighted several shortcomings of managing pediatric AML.[5] The current systematic review was undertaken to study the treatment strategies and outcomes of pediatric AML in India. The review included studies published between January 1, 2011 and December 31, 2021.

Materials and Methods

Protocol and Registration

This systematic review was registered on PROSPERO (ID42021273218).

Eligibility Criteria

Inclusion Criteria

1. Studies reporting on pediatric AML in India.
2. Studies written in English.
3. Prospective, retrospective, and ambispective studies.

Exclusion Criteria

1. Studies on pediatric AML not from India.
2. Case reports, reviews, and books.

Settings

There were no restrictions on the type of setting in which the studies were conducted.

Information Source

A systematic search of the MEDLINE, Google Scholar, and SCOPUS database for published studies on pediatric AML from India was conducted. In addition, SIOP conference abstracts were also screened. The reference lists of the included studies or relevant reviews were screened for other eligible studies.

Time

Search of database was from January 1, 2011 till January 31, 2021. SIOP conference abstracts were screened from year 2011 to 2020.

Literature Search

A comprehensive literature search was performed using text words "Acute myeloid leukemia," "AML," "child*," "India." Articles published in English alone were reviewed. Literature search was as per preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement. The full search strategy is shown in **supplementary material.**

Study Selection

Two review authors (S.S and V.R.M.G) independently screened the titles and abstracts yielded by the search against the inclusion and exclusion criteria. Full reports for all titles and abstracts were obtained if they appeared to meet the inclusion criteria and in case of any uncertainty. Review authors then screened the full text reports and decided whether the inclusion criteria were met. If necessary, additional information from study authors was sought to resolve questions about eligibility and disagreement was resolved through discussion. Reasons for excluding trials were also recorded. None of the review authors were blinded to the journal titles or to the study.

Data Collection Process

Data extraction from the included studies was performed using standardized data collection forms. Two reviewers (S.S and N.D) independently extracted the data to reduce the bias and errors in data extraction and the studies in question were jointly reviewed by the two investigators and the final determination was reached by consensus.

Data Items

The information that was extracted from each study included surname of the first author, year of study, median/mean age with range, number of patients, chemotherapy administered, induction mortality, complete remission (CR) rate, duration of follow-up, relapse, event-free survival (EFS), overall survival (OS), treatment-related mortality (TRM), treatment abandonment, prognostic factors, and use of hematopoietic stem cell transplant (HSCT).

Evaluation of Quality and Risk of Bias

Quality was assessed by two authors using the quality assessment tool for observational cohort and cross-sectional studies from the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health.[6]

Synthesis Method

All studies included were screened for the required data items and results were tabulated using Microsoft Word software. Categorical variables were expressed as the number of cases and percentages (%). Mean along with 95% confidence interval (CI) was calculated to report the incidence of TRM and abandonment rates. Statistical analyses were done using the R software version 4.0.2.

Results

Literature Search

A total of 1,057 studies and 15 SIOP conference abstracts were obtained after the initial search. Additionally, seven other studies were added after citation searching. After removing duplicates and screening the titles and abstracts of the publications, full text of 60 studies were assessed of which 19 were included for the systematic review. The PRISMA flowchart is shown in ►**Fig. 1**.

Quality of Studies

The quality assessment tool for observational cohort and cross-sectional studies from the National heart, Lung, and Blood Institute of the National Institutes of Health was adapted to assess the quality of included studies (►**Supplementary Table S1**).[7] Overall, the quality of the study was poor in 1 (5%) study, fair in 8 (42%) studies, and good in

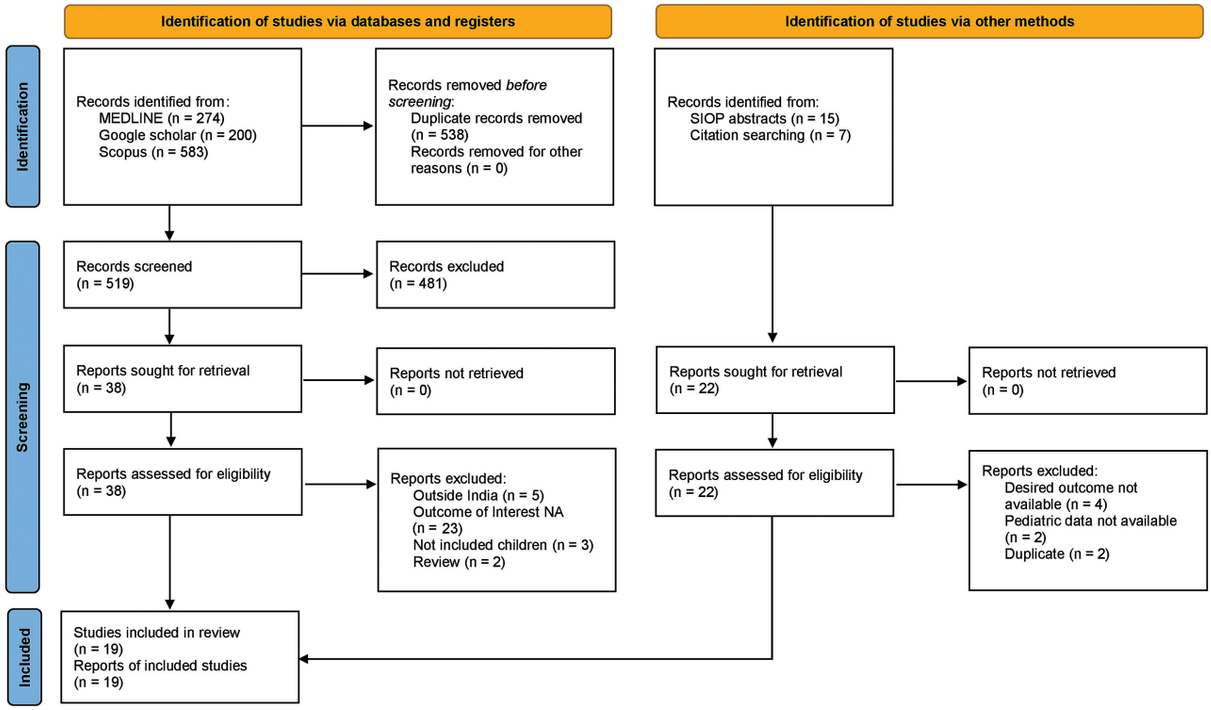

Fig. 1 Flow diagram of the systematic review according to preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines.

10 (53%) studies of the 19 studies included in the systematic review.

Characteristics of the Studies

A total of 1,210 patients were included from the 19 studies.[8–26] Three of the 19 studies also included patients with acute promyelocytic leukemia (APML).[19,21,22] Eight studies were published before 2015, while the remaining 11 studies were published in or after 2015. The various studies included patients between 1 and 19 years of age. There was slight predominance of males across majority of the studies. The salient features of the studies are summarized in ►**Table 1**.

Data regarding induction chemotherapy were available from 17 studies.[8,9,11–23,25,26] Anthracycline-based regimens were used for induction in all studies. 3 + 7 regimen consisting of daunorubicin (45–60 mg/m^2/day) and cytarabine (100–200 mg/m^2/day) was the standard regimen used in nine studies, while MRC AML based regimens that included an additional third agent (etoposide) were used in seven studies. In another study, patients were treated with AML-Berlin-Frankfurt-Munich (BFM) 98 protocol in which idarubicin was the anthracycline used. CR rate was available from 11 studies and varied between 56 and 95%, and overall there was no major difference in CR rates between 3 + 7 regimens (56–78%) and MRC-based regimens (64–95%)[7,9–11,13,14,16–18,20,22]. Six of the 17 studies used maintenance chemotherapy.[13,14,16,20,21,26] Six studies mentioned regarding HSCT (Reference: 8,9,16,17,22,25). A total of 20 patients underwent HSCT in these studies, 15 in CR1 and another 5 in CR2.[8,9,16,17,22,25] Overall induction mortality (10 studies) and TRM (9 studies) were 12% (95% CI: 6.4–17.8) and 23.2% (95% CI: 10.3–35.9), respectively.[7–9,12–20,22,24,25] Only three studies had a TRM of less than 10%, while four other studies had a high TRM over 20%.

Duration of follow-up was available from seven studies and the shortest and longest follow-up period was 7 and 31 months, respectively.[11,12,14,16,22,26,27] Data pertaining to event-free survival/disease-free survival (EFS/DFS) was available from 10 studies.[8,11,12,14,17–20,23,26] The overall EFS/DFS reported among these studies ranged between 28 and 52%. Data for OS was available from 16 studies.[8–11,13–21,23–25] Philip et al reported an OS of 70% with a follow-up of 7 months. Remaining 15 studies had OS ranging between 15 and 66%. In general, the OS of studies that used maintenance therapy (19–66%) was not different from those studies that did not offer maintenance (15–55%). Prognostic factors could be determined from four studies.[8,12,17,21] High-risk cytogenetics that included -5/del 5q, -7/del 7q, complex cytogenetics (defined as more than 3 structural and/or numerical abnormalities) were cited to have negative impact on CR and relapse rate. Sharawat et al highlighted the negative impact FMS-like tyrosine kinase 3 - internal tandem duplication (FLT3-ITD) mutations (DFS of 18% for FLT3-ITD-positive vs. 51% for FLT3-ITD-negative patients).[12] Kapoor and Yadav in their paper highlighted that the negative impact of adverse cytogenetic/molecular can be negated by HSCT in CR1.[22] The mean incidence of treatment abandonment that was available from 11 studies was 19.3% (95% CI: 10.9–27.5).[7–10,12,17,18,20–23] Six (55%) of these studies reported an abandonment rate over 20%.

Table 1 Characteristics of 19 studies included in the systematic review

Study	Type of study	Time period	Age (in years)	M:F ratio	Number of patients	Chemotherapy	Risk group included	Median follow-up	Abandonment (%)	CR (%)	Relapse/ refractory disease (%)	EFS/DFS	Induction mortality (%)	Overall TRM (%)	OS
Gupta et al 2011 [8]	Retrospective	2005–2009	Mean: 12.4 (1–18)	1.9:1	35	In-I: 3 + 7 (D45mg/m2 + Arac 100mg/m2) In-II: HAM Consol: HIDAC	All	NA	5.7%	77.1%	40%	2 years DFS: 40%	2.9%	5.7%	NA
Yadav et al 2011 [9]	Retrospective	2005–2010	NA	NA	51	UKAML12 protocol	All	NA	55%	NA	26%	NA	22%	48%	26%
Mohammed et al 2013 [10]	Retrospective	2006–2013	NA	NA	34	NA	All	NA	26%	56%	26%	NA	12%	NA	59%
Kota et al 2013 [11]	Retrospective	2007–2012	1–19	1:6:1	63	In: 3 + 7 Consol: NA	NA	11 months	21%	78%	NA	Median EFS: 11 months	NA	NA	3 year OS: 15%
Sharawat et al 2014 [12]	Retrospective	2008–2010	Median: 10(1–18)	3:1	64	In: 3 + 7 (60mg/m2 *3 days) Consol: HIDAC	All	18.3 months	NA	83%	NA	EFS: 30.2 ± 5.8% DFS: 43.03 ± 7.3%	NA	NA	37.1 ± 6.3%
Jain et al 2014 [13]	Retrospective	2000–2013	NA	NA	88	In: 3 + 7 & 5 + 2 Consol: HIDAC + M	NA	NA	34%	NA	NA	NA	NA	18%	NA
Jayabose et al 2014 [14]	Retrospective	2010–2014	NA	0.9:1	39	Modified MRC-10 protocol + M	All	29 months	NA	72%	21.4%	3 year EFS: 40%	NA	18%	3 year OS: 47.5%
Siddaiahgari et al 2014 [15]	Prospective + Retrospective	2009–2012		0.7:1	32	UK AML 15 protocol	All	NA	NA	94%	16%	NA	NA	6%	72%
Philip et al 2015 [16]	Retrospective	2012–2014	NA	NA	23	AML-BFM 98 protocol + M	NA	7 months	NA	NA	NA	NA	17%	NA	1 year OS: 70.4 ± 10.7%
Radhakrishnan et al 2015 [17]	Retrospective	2008–2013	Median: 9 (1–17)	2.25:1	72	In: DAE/DA Con: HIDAC	All	11.7 months	NA	72%	NA	EFS: 28%	5.5%	7%	OS: 36%
Ramamoorthy et al 2015 [18]	Retrospective	2004–2013	Mean: 7.3 ± 3.6	3.2:1	100	AML MRC 12 protocol	All	NA	3%	64%	25%	DFS: 34.7%	25%	48%	27.2%
Seth et al 2016 [19]	Retrospective	2011–2015	Median: 7.5 (1.5–13)	NA	71 (Included APML)	MRC-10 protocol	All	NA	25%	95% (excluding APML)	NA	3 year EFS: 43% (excluding APML)	5.4%	27%	3 year OS: 55% (excluding APML)
Narula et al 2017 [20]	Retrospective	2011	NA	NA	65	In: 3 + 7 Consol: HIDAC + M	All	NA	NA	NA	NA	3 year DFS: 66% 3 year EFS: 49%	<20%	NA	3 year OS:66%
Naseer et al 2017 [21]	Retrospective	2012–2017	NA	2:1	42 (included APML)	In: 7 + 3 and 5 + 2 Con: HIDAC + M	All	NA	9.4%	56%	25–28%	NA	18%	NA	19%
Kapoor et al 2018 [22]	Retrospective	2015–2018	NA	NA	24 (Included APML)	In: 3 + 7 Consol: HIDAC	All	31 months	4%	NA	29%	NA	NA	NA	67%
Peyam et al 2018 [23]	Retrospective	2011–2017	Mean: 6.96(1–12)	2.2:1	114	MRC 15 protocol	All	NA	8.8%	67.5%	22.8%	3 year EFS: 31.6%	NA	30.7%	NA
Sinha et al 2019 [24]	Retrospective	2014–2015	<15	1.7:1	65	NA	NA	NA	20%	NA	NA	NA	NA	NA	36.9% at 5 months after diagnosis
Uppuluri et al 2020 [25]	Retrospective	2002–2019	8	NA	48	MRC 15 protocol	All	NA	NA	NA	41%	NA	6.2%	NA	5 year OS:53%
Srinivasan et al 2020 [26]	Retrospective	2014–2017	9	NA	180	Upfront OMCT f/b 3 + 7 and HIDAC + M	All	25 months	NA	NA	NA	2 year EFS: 46–52	6.5%	NA	2 year OS: 47–53%

Abbreviations: APML, acute promyelocytic leukemia; AraC, cytarabine; consol, consolidation; CR, complete remission; DAE, Daunorubicin, Cytarabine, Etoposide; Dauno, daunorubicin; DFS, disease-free survival; EFS, event-free survival; f/b, followed-by; HAM, high-dose cytarabine, mitoxantrone; HIDAC, high-dose cytarabine; In, induction; M, maintenance; M:F, male:female; NA, not available; OMCT, oral metronomic chemotherapy; OS, overall survival; TRM, treatment related mortality.

Discussion

Treatment of AML in children continues to remain a challenge in LMICs. A previous systematic review published by Kulkarni and Marwaha in the year 2010 summarized two decades of experience of treating pediatric AML in India.[5] Their review included 322 children between the year 1990 and 2010, which is much smaller than our current review that included 1,200 children treated over a shorter duration of 10 years. Also, a recent systematic review on pediatric AML from LMICs acknowledged that maximum data was contributed from India.[3] This is a step in the right direction indicating that more Indian children with AML are being treated and reported. But, the true incidence of childhood AML in India is unknown. According to World Health Organization, the estimated number of new cases of leukemia from India, in the 0 to 14 age group, for the year 2020 was 11,850 and considering that approximately 15% of these patients have AML, the annual incidence of childhood AML should be approximately 1,750.[28] Thus, there continues to be underreporting and underdiagnosis of pediatric AML in India.

Treatment abandonment is a major hurdle and is one of the most common reasons for treatment failure in LMICs.[29] In fact, treatment abandonment is thought to contribute to at least a third of the survival difference between HICs and LMICs.[30] Though not systematically reported, the current review highlights an alarmingly high abandonment rates among children with AML, with no major improvements in comparison to previous reports.[5] Perceived prognosis of the disease, cost of treatment, and concerns of toxicity are few of the contributing factors to such high abandonment rates. Studies from India and other LMICs have highlighted that a comprehensive support group consisting of clinicians, as well as existing non-governmental organizations and governmental organizations can significantly reduce abandonment.[31–33]

TRM is the next biggest hurdle in the treatment of pediatric AML in LMICs. The standard of care for pediatric AML continues to be anthracycline-based induction followed by three to four cycles of consolidation. Most of the chemotherapy protocols for treating pediatric AML in India have been adopted from HICs, but the lack of essential supportive care and option of intensive care unit admission, which are considered to be indispensable during intensive AML treatment, have led to survival gap in comparison to HICs. For example, Yadav et al highlighted a very high TRM of 48% when treated with the UKAML12 protocol.[9] On the contrary, the original UKAML12 trial that used the same protocol had a TRM of only 10%.[34] Similar to previous studies from India, the current review estimated a high incidence of induction mortality (12%) and overall TRM (23%), which is much higher compared with 5 to 10% occurring in HICs.[4,5,35–37] High rates of infection with multidrug-resistant organisms, invasive fungal infections, and poor nutritional status have led to poor tolerance and subsequently a high TRM during intensive chemotherapy. Uppuluri et al highlighted that early intervention by the pediatric intensive care team and granulocyte transfusion positively impacts survival.[25] For patients in resource-limited settings with level two facilities, SIOP Pediatric Oncology in Developing Countries (PODC) guidelines recommend an alternative strategy, to begin treatment with inexpensive, low-intensity oral chemotherapy followed by low-dose or standard-dose induction to reduce TRM and abandonment.[38] For patients with baseline adverse host-related factors, use of upfront low-dose oral chemotherapy as a bridge to intensive chemotherapy has been shown to be safe and reduce TRMs with comparable outcomes to those who directly receive intensive chemotherapy.[26] Another modifiable factor that contributes to TRM is malnutrition. The reported incidence of malnutrition among Indian children with leukemia is approximately 50%.[27,39,40] Promoting routine nutritional assessment and ensuring availability of nutritional supplements that are affordable and culturally appropriate, such as ready-to-use therapeutic foods, must be incorporated into the care of childhood leukemia in India.

Lack of uniform access and high cost contribute to low rates of HSCT in India. In the current review, HSCT rate was less than 2%. This remains a significant concern for a disease like pediatric AML in which a third of the patients are thought to be high risk and thus would qualify for HSCT in CR1. Also, patients from LMICs do not have access to many of the newer therapies such as antibody drug conjugates and small molecule inhibitors, which are currently available for AML. Incorporating a simple risk stratification, which will otherwise identify the favorable risk group who can be cured by chemotherapy alone, will be helpful, especially in the setting of limited access to HSCT. Identifying certain high-risk mutations such as FLT3 can be of therapeutic benefit in light of access to targeted therapies such as tyrosine kinase inhibitors.

The survival of pediatric AML in HICs has reached 70% and is mostly attributed to advanced diagnostic techniques, better supportive care, and improved salvage options including HSCT. This has not been the scenario in India and other LMICs where the OS ranges between 10 and 50%.[3] Compared with previous studies published in India, our current review shows no major improvement in survival trends in the past 30 years.[5] The lower survival rate in India can be attributed to high TRMs, high treatment abandonments, and low salvage rate after relapse. While the HICs continue to improve upon the benchmark survival of 70% through refinement of molecular risk stratification and increased efforts toward personalized targeted therapy approaches, the immediate steps in LMICs must address both socioeconomic and disease-related challenges as discussed.

The current systematic review has certain limitations. Of the 19 studies included, 9 studies were published only in abstract format and 3 studies included APML patients that are often analyzed as a separate subset. Certain characteristics including baseline comorbidities, risk stratification, and delay in diagnosis were not captured. The median follow-up time was not mentioned in majority of the studies and among those studies which mentioned it, three had a follow-up duration of less than 1 year.

Conclusion

In conclusion, the treatment outcomes of pediatric AML in India are substantially inferior compared with HICs. Lowering TRM and abandonments is of utmost importance. A holistic approach of including a social support team, intensified patient counselling, ensuring uniform access to cancer therapy and supportive care will go a long way in improving the outcomes of pediatric AML in India. Collaboration and prospective multicenter studies may not only ensure standard of care treatment but also reduce abandonment rates. The Indian Pediatric Oncology group (InPOG) initiative is a step in that direction.[41]

Other Information

Protocol registration: PROSPERO (ID42021273218).

Competing Interests

The authors do not have any competing interests to declare.

All data that have been collected for the purpose of this systematic review have been from published literature and are available in public domains.

Authors' Contributions

Shyam Srinivasan was involved in conceptualization, designing, definition of intellectual content, literature search, clinical studies, experimental studies, data acquisition, data analysis, manuscript preparation, manuscript editing, and manuscript review.

Venkata Rama Mohan Gollamudi contributed to literature search and manuscript preparation.

Nidhi Dhariwal did literature search, data acquisition, and data analysis.

Conflict of Interest

None declared.

References

1 Ward E, DeSantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics, 2014. CA Cancer J Clin 2014;64(02): 83–103

2 Chen J, Glasser CL. New and emerging targeted therapies for pediatric acute myeloid leukemia (AML). Children (Basel) 2020; 7(02):1–15

3 Van Weelderen RE, Klein K, Natawidjaja MD, De Vries R, Kaspers GJ. Outcome of pediatric acute myeloid leukemia (AML) in low- and middle-income countries: a systematic review of the literature. Expert Rev Anticancer Ther 2021;21(07):765–780[Internet]

4 Arora RS, Arora B. Acute leukemia in children: a review of the current Indian data. South Asian J Cancer 2016;5(03):155–160

5 Kulkarni KP, Marwaha RK. Childhood acute myeloid leukemia: an Indian perspective. Pediatr Hematol Oncol 2011;28(04):257–268

6 Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies. [Internet]. [cited 2022 May 14]. Accessed July 12, 2022 from: https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools.

7 National Heart, Lung, and Blood Institute. Quality assessment tool for observational cohort and cross-sectional studies [cited 2022 January 5]. Accessed July 12, 2022 from: https://www.nhlbi.nih.gov/healthtopics/study-quality-assessment-tools.

8 Gupta N, Seth T, Mishra P, et al. Treatment of acute myeloid leukemia in children: experience from a tertiary care hematology centre in India. Indian J Pediatr 2011;78(10):1211–1215

9 Yadav SP, Ramzan M, Lall M, Sachdeva A. Pediatric acute myeloid leukemia: final frontier for pediatric oncologists in developing world. Pediatr Hematol Oncol 2011;28(08):647–648

10 Mohammed R, Yadav SP, Meena L, Verma IC, Sachdeva A. PUB-0194, cytogenetic findings in acute myeloid leukemia: a developing country experience. Abstracts of the 45th Congress of the International Society of Paediatric Oncology (SIOP) 2013. Hong Kong, China. September 25–28, 2013. Pediatr Blood Cancer 2013; 60(Suppl 3):228

11 Kota R, Linga VG, Gullipalli M, et al. PUB-0228, outcome of childhood acute myeloid leukaemia-Institutional experience from Indian subcontinent. Abstracts of the 45th Congress of the International Society of Paediatric Oncology (SIOP) 2013. Hong Kong, China. September 25–28, 2013. Pediatr Blood Cancer 2013; 60(Suppl 3):236

12 Sharawat SK, Bakhshi R, Vishnubhatla S, Gupta R, Bakhshi S. FLT3-ITD mutation in relation to FLT3 expression in pediatric AML: a prospective study from India. Pediatr Hematol Oncol 2014;31 (02):131–137

13 Jain K, Udgire S, Mudaliar S, Swami A, Shah N. EP-316, maintenance therapy in acute myeloid leukemia: experience from a developing country. 46(th) Congress of The International Society of Paediatric Oncology (SIOP) 2014 Toronto, Canada, 22(nd) -25 (th) October, 2014 SIOP Abstracts. Pediatr Blood Cancer 2014;61 (Suppl 2):324

14 Jayabose S, Kasi VT, Vignesh SR, Priya R, Rathnam K. EP-317, Use of codified MRC-10 protocol for acute myeloblastic leukemia in Indian children. 46(th) Congress of The International Society of Paediatric Oncology (SIOP) 2014 Toronto, Canada, 22(nd) -25(th) October, 2014 SIOP Abstracts. Pediatr Blood Cancer 2014;61 (Suppl 2):324

15 Siddaiahgari S, Jillella B, Manikyam A. EP-327, Improving survival rates of acute myeloid leukemia in developing countries using AML_15 protocol. 46(th) Congress of The International Society of Paediatric Oncology (SIOP) 2014 Toronto, Canada, 22(nd) -25(th) October, 2014 SIOP Abstracts. Pediatr Blood Cancer 2014;61 (Suppl 2):326

16 Philip C, George B, Ganapule A, et al. Acute myeloid leukaemia: challenges and real world data from India. Br J Haematol 2015;170 (01):110–117

17 Radhakrishnan V, Thampy C, Ganesan P, et al. Acute myeloid leukemia in children: experience from tertiary cancer centre in India. Indian J Hematol Blood Transfus 2016;32(03):257–261

18 Ramamoorthy J, Trehan A, Bansal D, Varma N, Jain R. PD-053, acute myeloid leukemia: treatment related mortality is abane in a developing country. 47th Congress of the International Society of Paediatric Oncology (SIOP) Cape Town, South Africa, October 8–11, 2015. Pediatr Blood Cancer 2015;62(Suppl 4):223

19 Seth R, Pathak N, Singh A, Chopra A, Kumar R, Kalaivani M. Pediatric acute myeloid leukemia: improved survival rates in India. Indian J Pediatr 2017;84(02):166–167[Internet]

20 Narula G, Prasad M, Jatia S, et al. Clinicoepidemiological profiles, clinical practices, and the impact of holistic care interventions on outcomes of pediatric hematolymphoid malignancies - a 7-year audit of the pediatric hematolymphoid disease management group at Tata Memorial Hospital. Indian J Cancer 2017;54(04):609–615

21 Naseer M, Ankit P, Purva K, Shraddha C. VP K. PO-012, acute myeloid leukemia: correlation of cytogenetics and outcome in a tertiary care pediatric centre. Abstracts From the 49th Congress of the International Society of Paediatric Oncology (SIOP) Washington, DC, USA October 12–15, 2017. Pediatr Blood Cancer. 2017; 64(Suppl 3):S442

22 Kapoor R, Yadav SP. Genetics-based risk stratification of pediatric acute myeloid leukemia in India. Indian Pediatr 2018;55(11): 1006–1007

23 Peyam S, Trehan A, Jain R, Bansal D, Varma N. PO-205, acute myeloid leukemia: incremental improvement in outcome in a developing country. Abstracts from the 50th Congress of the International Society of Paediatric Oncology (SIOP) Kyoto, Japan November 16–19, 2018. Pediatr Blood Cancer. 2018(Suppl 2): S186

24 Sinha S, Brattström G, Palat G, et al. Treatment adherence and abandonment in acute myeloid leukemia in pediatric patients at a low-resource cancer center in India. Indian J Med Paediatr Oncol 2019;40(04):501–506

25 Uppuluri R, Swaminathan V, Ravichandran N, et al. Chemotherapy for childhood acute myeloid leukemia and associated infections over two decades in India: timeline and impact on outcome. Indian J Med Paediatr Oncol 2020;41(06):869–873

26 Srinivasan S, Dhamne C, Moulik N, et al. 0037 / #975. A host-factor based approach impacts survival of children with acute myeloid leukemia (AML) at high-risk for induction mortality and/or early treatment abandonment. Abstracts from the 52th Congress of the International Society of Paediatric O. Pediatr Blood Cancer. 2020; 67(Suppl 4):S22

27 Radhakrishnan V, Ganesan P, Rajendranath R, Ganesan TS, Sagar TG. Nutritional profile of pediatric cancer patients at Cancer Institute, Chennai. Indian J Cancer 2015;52(02):207–209

28 WHO estimates of acute leukemia.. Data [Internet][Cited on 2022 Jan 5]. Accessed July 12, 2022 from: https://gco.iarc.fr/today/online-analysis-table?v=2020&mode=cancer&mode_population=continents&population=900&populations=356&key=asr&sex=0&cancer=39&type=0&statistic=5&

29 Arora RS, Pizer B, Eden T. Understanding refusal and abandonment in the treatment of childhood cancer. Indian Pediatr 2010; 47(12):1005–1010

30 Gupta S, Yeh S, Martiniuk A, et al. The magnitude and predictors of abandonment of therapy in paediatric acute leukaemia in middle-income countries: a systematic review and meta-analysis. Eur J Cancer 2013;49(11):2555–2564[Internet]

31 Atun R, Bhakta N, Denburg A, et al. Sustainable care for children with cancer: a Lancet Oncology Commission. Lancet Oncol 2020; 21(04):e185–e224

32 Jatia S, Narula G, Sankaran H, et al. Holistic support coupled with prospective tracking reduces abandonment in childhood cancers: a report from India. Pediatr Blood Cancer 2018;2019:1–8

33 Howard SC, Zaidi A, Cao X, et al. The My Child Matters programme: effect of public-private partnerships on paediatric cancer care in low-income and middle-income countries. Lancet Oncol 2018;19(05):e252–e266[Internet]

34 Gibson BES, Webb DKH, Howman AJ, De Graaf SS, Harrison CJ, Wheatley KUnited Kingdom Childhood Leukaemia Working Group and the Dutch Childhood Oncology Group. Results of a randomized trial in children with acute myeloid leukaemia: medical research council AML12 trial. Br J Haematol 2011;155 (03):366–376

35 Creutzig U, Zimmermann M, Reinhardt D, Michael D, Stary J, Lehrnbecher T. Early deaths and treatment-related mortality in children undergoing therapy for acute myeloid leukemia: analysis of the multicenter clinical early deaths and treatment-related mortality in children undergoing therapy for acute myeloid leukemia: analysis. J Clin Oncol 2004;2004(22):4384–4393

36 Rubnitz JE, Kaspers GJL. How I treat pediatric acute myeloid leukemia. Blood 2021;138(12):1009–1018

37 Klein K, Van Litsenburg RRL, de Haas V, et al. Causes of early death and treatment-related death in newly diagnosed pediatric acute myeloid leukemia: recent experiences of the Dutch Childhood Oncology Group. Pediatr Blood Cancer 2019;2020:1–10

38 Bansal D, Davidson A, Supriyadi E, Njuguna F, Ribeiro RC, Kaspers GJL. SIOP PODC adapted risk stratification and treatment guidelines: recommendations for acute myeloid leukemia in resource-limited settings. Pediatr Blood Cancer 2019(October):e28087

39 Sonowal R, Gupta V. Nutritional status in children with acute lymphoblastic leukemia, and its correlation with severe infection. Indian J Cancer 2021;58(02):190–194

40 Tandon S, Moulik NR, Kumar A, Mahdi AA, Kumar A. Effect of pre-treatment nutritional status, folate and vitamin B12 levels on induction chemotherapy in children with acute lymphoblastic leukemia. Indian Pediatr 2015;52(05):385–389

41 Singh R, Bakhshi S. (InPOG) e collaborative research in India comes of age. Pediatr Hematol Oncol J 2016;1(01):13–17

A Retrospective Observational Study of Dicentric (9;12): A Unique, Nonrandom Translocation Defining a Cytogenetic Subgroup with Favorable Outcome in Acute Lymphoblastic Leukemia

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Abstract

Introduction Cytogenetic abnormalities are integral to the risk stratification of acute lymphoblastic leukemia (ALL).

Objectives The present study aimed to highlight a rare, yet nonrandom cytogenetic abnormality notably dicentric (9;12), which was observed in ALL patients who presented to our institute. The study analyzed the frequency, clinicohematological features, and treatment response of these patients.

Materials and Methods A single-group observational study was conducted from April 2014 to April 2020. Cytogenetic analysis was done on bone marrow aspirate samples of the patients referred to the cytogenetics laboratory with clinical diagnosis of acute leukemia. Cytogenetic, clinical, and hematological data were collected from respective departmental records, case files, and patients.

Results Dic(9;12) was identified in 1.2% of ALL (19 out of 1,544 patients). They showed striking preponderance in teen and young adult males with characteristic precursor B cell immunophenotype. Majority of these patients displayed favorable risk profiles such as low total count, mild lymphadenopathy and splenomegaly, mild-to-moderate elevation of lactate dehydrogenase, and good response to first induction chemotherapy. Rare coexistence of dic (9;12) with well-established cytogenetic markers such as t(9;22) and t(1;19) was observed.

Conclusion Dic(9;12) is one of the most specific cytogenetic markers of precursor B cell (pre-B) ALL. It defines a subgroup with favorable clinical and biological profile. We

Keywords
- dicentric (9;12)
- precursor B cell acute lymphoblastic leukemia

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suggest inclusion of dic(9;12) in cytogenetic risk stratification of precursor B cell ALL. Long-term follow-up studies are recommended to establish the prognostic significance of this cytogenetic subgroup, which may benefit from less intensive chemotherapy.

Introduction

The World Health Organization (WHO) classification of B cell acute lymphoblastic leukemia (ALL)/lymphoma (2017) is refined by risk stratification model based on the recurrent chromosomal abnormalities. High hyperdiploidy, t(12;21), t (1;19), and t(9;22) are most commonly considered cytogenetic abnormalities in prognostication.[1]

Only 110 cases of dicentric (9;12) have been reported to date since its first discovery in 1985 by Faderl et al,[2] although it was Carroll et al in 1987, who recognized this abnormality as a recurrent finding in childhood ALL.[3] There has been no update until the last couple of years on this unique translocation, which is possibly associated with good prognosis. With this background, the present study aims to assess the overall frequency of dic(9;12) in ALL and comprehensively analyze the associated clinicohematological features.

Materials and Methods

This was a single-group retrospective observational study conducted from April 2014 to April 2020. Nineteen patients having dic(9;12) were retrieved from the cytogenetic records. Patients of ALL were identified based on the information obtained from cytogenetic registers (from 2014 to 2020) and departmental records of medical and pediatric oncology. Clinical and hematological parameters were obtained from the case files.

Cytogenetic Technique

Heparinized bone marrow aspirates of the patients were sent to the cytogenetics laboratory. Dual cultures of 24-hour and 48-hour incubation were set up in RPMI-1640 medium supplemented with 15% qualified, heat-inactivated fetal bovine serum (GIBCO, Invitrogen, United States). This was followed by mitotic arrest using Karyomax-Colcemid solution (0.10 µg/mL concentration) for 30 minutes. Subsequently, cells were subjected to hypotonic treatment in potassium chloride (0.075 M) at 37°C for 20 minutes and overnight fixation in Carnoy's fixative solution (3:1 concentration of methanol and glacial acetic acid). Giemsa-Trypsin-Giemsa banding was done on prepared slides, and chromosomal analysis was done in accordance with the International System for Chromosomal Nomenclature. Good quality metaphases were captured and analyzed using Olympus applied spectral imaging software version 8.1.0.47741.

Statistical Methods

Python 3.7 software was used for statistical analysis. Descriptive statistical analysis (frequencies, mean, median, and range) was done to analyze baseline parameters. Fisher's exact test was applied to find the association between complex karyotype and risk stratification. Survival probabilities were estimated by Kaplan–Meier method.

Ethics

The procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional) and with the Helsinki Declaration of 1964, as revised in 2013. Ethics Committee Waiver was obtained from Kidwai Memorial Institute of Oncology (dated 12–04–2020, KMIO/DPT/106/2021). The informed consent was waivered due to the retrospective nature of the study.

Results

Nineteen patients of dic(9;12) were identified among 1544 patients of ALL and 1214 patients of B cell ALL, accounting for frequencies of 1.2 and 1.6%, respectively.

The median age of patients was 14 years (range: 1–57 years). Majority of patients (12 out of 19 patients; 63%) were adolescents and young adults (10–23 years). Males were more frequently affected than females (M:F = 4:1). Clinical features and hematologic and biochemical parameters are summarized in ►**Tables 1** and **2** , respectively.

Bone marrow examination revealed blasts having L1/L2 morphology of French-American-British classification. Normal bone marrow elements were suppressed. Immunophenotypically, the blasts were positive for Tdt, CD34, CD10, CD19, cCD79a, and human leukocyte antigen-DR and negative for T cell and myeloid markers. CD20-positive expression was noted in only six cases. Features were consistent with the diagnosis of precursor B cell ALL.

Cytogenetic Studies

Hypodiploidy (modal number of chromosomes ranging from 44 to 45) was observed in 16 out of 19 patients (84%) (►**Table 3**). Patients with 45 chromosomes were considered

Table 1 Clinical features of patients with dic(9;12) at diagnosis

Signs and symptoms	Percentage of patients
Fever and fatigue	73–80
Others: cough, neck swelling, epistaxis	6
Moderate pallor	21
Severe pallor	79
Lymphadenopathy	87
Hepatomegaly	64
Splenomegaly	76

Abbreviation: dic(9;12), dicentric (9;12).

Table 2 Clinical, biochemical, and hematological parameters of patients with dic(9;12) at diagnosis

Parameters	Median	Range
Liver size on ultrasound	17 cm	13–20 cm
Spleen size on ultrasound	15.5 cm	10–20 cm
LDH	347	205–552
Hb (g/dl)	8	4–11
TC ($n \times 10^9$/L)	3	1–113
ANC ($n \times 10^9$/L)	0.45	0.02–15.36
Platelets ($n \times 10^9$/L)	25.5	2–71
PBL blast (%)	40	22–70
BM blast (%)	80	50–90

Abbreviations: ANC, absolute neutrophil count; BM, bone marrow; dic (9;12), dicentric (9;12); Hb, hemoglobin; LDH, lactate dehydrogenase; PBL, peripheral blood; TC, total count.

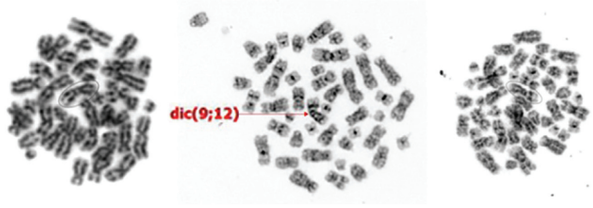

Fig. 1 Metaphases of dic(9;12) abnormality identified in different patients.

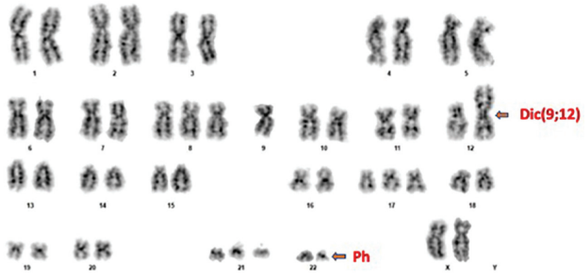

Fig. 2 Karyotype: 48,XX,+8,dic(9;12)(p13;p13),+17,+21,der(22)t (9;22) (q34;q11.2). dic(9;12); dicentric dic(9;12).

near-diploid since it occurred as a result of unbalanced translocation dic(9;12). ►**Fig. 1** represents the novel translocation dic(9;12). Complex karyotype is defined as metaphases having at least three chromosomal abnormalities, irrespective of their type. As per these criteria, 7 out of 19 patients (37%) in our study group had complex karyotype. The additional structural abnormalities observed in our study group included t(9;22), t(1;19), del(9p), del(7q), marker chromosomes, and numerical abnormalities. ►**Fig. 2** denotes coincidence of dic(9;12) and Philadelphia chromosome along with numerical abnormalities in a single metaphase. Although derivative chromosome 9 appeared cryptic in the metaphase, fluorescent in situ hybridization confirmed *bcr-abl* translocation (►**Fig. 3**). Reverse transcriptase

Table 3 Cytogenetic findings of patients with dic(9;12) at diagnosis

Case number	Age (y)/gender	Karyotype	Complex karyotype
1	6/male	45,XY,dic(9;12)(p11;p11–12)[8]/46,XY[4]	No
2	10/female	48,XX,+8,dic(9;12)(p23;q13),del(9)(p21),+2mar	Yes
3	12/male	45,XY,dic(9;12)(p11;p12)	No
4	13/male	45,XY,del(7)(q31),dic(9;12)(p13;p13)	Yes
5	14/male	45,XY,dic(9;12)(p11–13;p11)	No
6	14/male	45,XY,dic(9;12)(p11;p11),t(1;19)(q23;p13)	Yes
7	15/male	45,XY,dic(9;12)(p13;p13)	No
8	15/female	44,XX,dic(9;12)(p13;p13),-17	No
9	17/male	45,XY,dic(9;12)(p13;p13)	No
10	21/male	45,XY,dic(9;12)(p13;p13)	No
11	23/male	45,XY,der(9)t(9;12)(p13;p13)t(9;22)(q34;q11.2)	Yes
12	20/male	44,X,-Y,dic(9;12)(p13;p13)	No
13	43/female	45,XX,dic(9;12)(p13;p13)	No
14	01/male	45,XY,dic(9;12)(p11–12;p13)	No
15	57/male	45,XY,-2,i(8)(q10),dic(9;12)(p11;p11),t(9;22)(q34;q11.2)	Yes
16	19/female	46,XX,dic(9;12)(p13;p13),-10,+2mar	Yes
17	11/female	46,XX,dic(9;12)(p13;q13),t(9;22)(q34;q11.2)	Yes
18	8/male	45,XY,dic(9;12)(p13;p13)	No
19	9/male	45,XY,dic(9;12)(p13;p13)	No

Abbreviation: dic(9;12), dicentric (9;12).

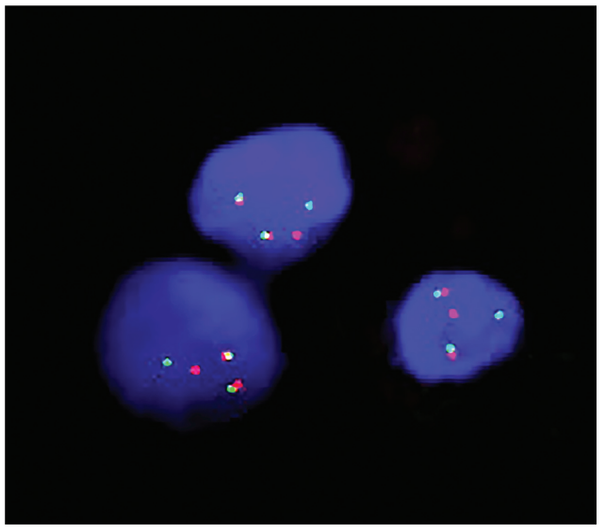

Fig. 3 Fluorescent in situ hybridization (dual color, dual fusion): Two red-green, one green, and one red signals confirm *bcr-abl* translocation.

polymerase chain reaction showed major *bcr-abl* transcripts in three patients harboring t(9;22).

Treatment and Follow-Up

Pediatric patients were treated according to the MCP-841 chemotherapeutic regimen until 2018 and Indian Childhood Collaborative Leukemia Group ALL-14 protocol since 2019. Adult patients were given chemotherapy according to BFM-95 ALL regimen. A 23-year-old male of Ph-positive ALL with dic(9;12) received R-Hyper-CVAD protocol along with imatinib. Majority of patients belonged to standard and intermediate-risk group (81%; 13 out of 16 patients) (►**Table 4**). All patients achieved complete hematologic and cytogenetic remission within 4 to 6 weeks of first induction chemotherapy. Minimal residual disease status was evaluated for six pediatric patients, who showed leukemic cell count <0.01% in the bone marrow.

Out of 19 patients, 10 were selected for survival analysis because other nine patients had still not completed treatment. The median duration of follow-up was 36 months (range: 7–51 months) (►**Table 4**). The overall survival was estimated to be 67% for 3 years. The survival curve is depicted in ►**Fig. 4**. A 15-year-old girl died of disease following discontinuation of treatment for 19 months. Deaths of a 43-year-old female and a 57-year-old male were attributed to their comorbid conditions.

Discussion

A dicentric chromosome is a single chromosome that has two centromeres, causing genomic instability and evolution of the disease in cancer.[4] There is considerable heterogeneity in breakpoints and the fusion gene partners in dicentric chromosomal rearrangements involving chromosome 9p.[5] Despite the

Table 4 Risk stratification, duration of follow-up, and survival status of patients with dic(9;12)

Case number	Risk stratification	Duration of follow-up in months	Survival status
1	Standard	36	Remission
2	Standard	51	Remission
3	Standard	7	On treatment
4	Standard	6	On treatment
5	Standard	37	Lost follow-up
6	Not available	36	Remission
7	Standard	40	Remission
8	High	19	Dead
9	Intermediate	12	On treatment
10	Intermediate	16	Lost follow-up
11	High	36	Remission
12	Intermediate	9	On treatment
13	Intermediate	7	Dead
14	Not available	9	On treatment
15	High	28	Dead
16	Intermediate	9	On treatment
17	Intermediate	8	On treatment
18	Not available	2	On treatment
19	Standard	2	On treatment

Abbreviation: dic(9;12), dicentric (9;12).

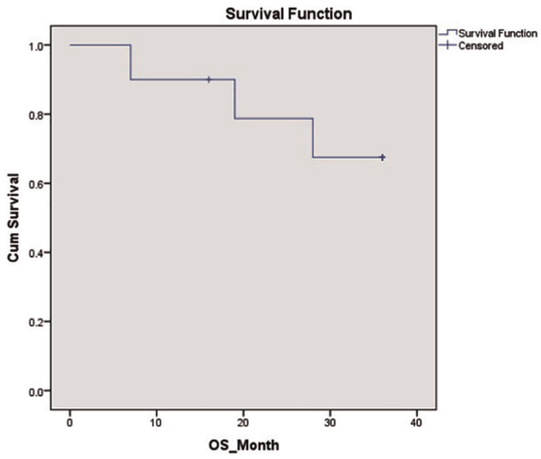

Fig. 4 Survival outcome analysis of patients with dic(9;12) by Kaplan–Meier method. dic(9;12); dicentric dic(9;12).

heterogeneity, molecular investigations have shown 9p rearrangements including deletion or gene fusions resulted in partial or complete deletion and under expression of *PAX5* gene.[5] The usual partner chromosomes are 7, 12, and 20.[6]

Dic(9;12)(p11–13;p11–13) is an unbalanced translocation with partial loss of short arms of chromosomes 9 and 12. This results in fusion of *PAX5* gene on short arm of chromosome 9 with *ETV6* gene on short arm of chromosome 12, resulting in *PAX5-ETV6* chimeric fusion protein.

Similar to earlier studies on dic(9;12),[7–9] the peak incidence was noted in teenage and young adult males in the present study. This translocation is rarely encountered in infants and the elderly. Only a single case of a 66-year-old woman with dic(9;12) in pre-B-ALL has been reported so far.[9] Two patients in our study group were older adults (aged 43 and 57 years).

Marked elevation of lactate dehydrogenase (LDH) serves as a surrogate for high leukemic blasts.[10] The present study showed marginal-to-moderate elevation of LDH and median blast of 40% (►**Table 2**). Dicentric translocations are seemingly most prevalent in leukemias of B cell lineage.[11] Dic(9;12) is predominantly a marker of precursor B cell ALL, though there are anecdotal reports of this abnormality in chronic myelogenous leukemia (CML)-blast crisis and T cell lymphoid malignancies.[3,8,9] All 19 patients in our study were diagnosed as precursor B cell ALL.

UKCCG 1992 reported better prognosis in patients with dic(9;12) than in patients with other translocations involving 9p and 12p. Patients with dic(9;12) possibly achieve complete remission with a 5-year survival rate of more than 95%, abrogating the need for bone marrow transplantation.[12,13]

Mahmoud et al identified 15 cases of dic(9;12)supposed to be dic(9;12)(p11;p12) in their study of 2,303 pediatric ALL patients. On median follow-up of 57 months, all were in remission.[8] Behrendt et al reported 31 cases of leukemia with dic(9;12)(p11–13;p11–12). Fourteen patients had pre-B-ALL with clinical and hematologic profile similar to our study group. Despite the presence of one or more adverse prognostic factors, 94% of these patients remained in remission on 5-year follow-up.[9] Illade et al reported a 14-year-old male with t(9;12)(p13;p13), who was in remission on 4-year follow-up.[14] In concordance with these studies, we observed overall survival of 67% for 3 years (►**Fig. 4**). Considering the death of two patients not directly related to the disease, the 3-year overall survival could be deduced as 90% in our study.

In general, low hypodiploidy having chromosomes <40 and complex karyotype is considered to be independent poor prognostic factors in ALL.[15,16] Majority of cases in our study were near-diploid or diploid, which is not considered as a poor prognostic factor. The relationship between presence or absence of complex karyotype and risk stratification was not statistically significant (Fisher's exact test, p = 0.504; ►**Table 5**). However, patients with complex karyotype also showed good initial treatment response.

Dic(9;12) and dic(7;9) frequently coexist with t(12;21) (*ETV6-RUNX1* fusion) and t(9;22)(*BCR-ABL1* fusion), respectively, which are considered to be important cooperating genetic events.[5] Bargetzi et al have reported dic(9;12) involving derivative chromosome 9 of t(9;22) in patients with CML-blast crisis.[17]

Conversely, three patients in our study showed dic(9;12) and t(9;22) as coexisting abnormalities (►**Fig. 3**) and all were diagnosed as de novo precursor B-ALL. One of these patients who aged 57 years and had comorbid conditions died at 28 months of treatment.

Table 5 Fisher's exact test to find the association between dic(9;12) with or without complex karyotype and risk stratification

Risk stratification of patients against presence and absence of complex karyotype	Standard	Intermediate	High	Total
Dic(9;12) with complex karyotype	2	2	2	6
Percentage within risk stratification	29	33	67	38
Dic(9;12) without complex karyotype	5	4	1	10
Percentage within risk stratification	71	67	33	63
Total	7	6	3	16
p = 0.504				

Abbreviation: dic(9;12), dicentric (9;12).

Conclusion

The current study showed that dic(9;12)(p13;p13) is a rare, yet recurrent chromosomal abnormality, with an overall frequency of 1.2% in ALL and 1.6% in B cell ALL. Based on the findings of this study and review of literature, we believe that patients with this abnormality show standard risk profile with initial good response to induction chemotherapy. Hence, we suggest incorporation of dic(9;12) in risk stratification of precursor B cell ALL as it represents a group with favorable risk profile and good initial response to treatment. We observed rare concurrence of two nonrandom translocations, dic(9;12) and t(9;22). Until the prognostic impact of dic(9;12) is well established by larger studies, it is better to consider t(9;22) over dic(9;12) in risk stratification of such cases. The current study is limited by small sample size and short duration of follow-up. Survival analysis could be confounded by different treatment protocols used among pediatric and adult patients. Additional studies are being sought to evaluate the prognostic value of this unique cytogenetic aberration, which may benefit from less intense chemotherapy.

Funding
None.

Conflict of Interest
None declared.

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References

1 Borowitz MJM J Borowitz. , Chan JKJ K Chan. , Downing JRJ R Downing. , Le Beau MMM M Le Beau. , Arber DAD A Arber. . B-lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. eds. WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues, Revised. 4th edition. Lyon: International Agency for Research on Cancer; 2017:200–209
2 Faderl S, Kantarjian HM, Talpaz M, Estrov Z. Clinical significance of cytogenetic abnormalities in adult acute lymphoblastic leukemia. Blood 1998;91(11):3995–4019
3 Carroll AJ, Raimondi SC, Williams DL, et al. tdic(9;12): a nonrandom chromosome abnormality in childhood B-cell precursor acute lymphoblastic leukemia: a Pediatric Oncology Group Study. Blood 1987;70(06):1962–1965
4 Mackinnon RN, Campbell LJ. The role of dicentric chromosome formation and secondary centromere deletion in the evolution of myeloid malignancy. Genet Res Int 2011:643628
5 An Q, Wright SL, Konn ZJ, et al. Variable breakpoints target PAX5 in patients with dicentric chromosomes: a model for the basis of unbalanced translocations in cancer. Proc Natl Acad Sci U S A 2008;105(44):17050–17054
6 Smith A, Das P, O'Reilly J, Patsouris C, Campbell LJ. Three adults with acute lymphoblastic leukemia and dic(7;9)(p11.2;p11. Cancer Genet Cytogenet 2006;166(01):86–88
7 United Kingdom Cancer Cytogenetics Group (UKCCG) Translocations involving 9p and/or 12p in acute lymphoblastic leukemia. Genes Chromosomes Cancer 1992;5(03):255–259
8 Mahmoud H, Carroll AJ, Behm F, et al. The non-random dic(9;12) translocation in acute lymphoblastic leukemia is associated with B-progenitor phenotype and an excellent prognosis. Leukemia 1992;6(07):703–707
9 Behrendt H, Charrin C, Gibbons B, et al. Dicentric (9;12) in acute lymphocytic leukemia and other hematological malignancies: report from a dic(9;12) study group. Leukemia 1995;9(01):102–106
10 Md Elbossaty WF. Lactate dehydrogenase (LDH) as prognostic marker in acute leukemia "quantitative method". J Blood Disord Transfus 2017;8:375
11 Raimondi SC, Privitera E, Williams DL, et al. New recurring chromosomal translocations in childhood acute lymphoblastic leukemia. Blood 1991;77(09):2016–2022
12 Huret JL, Heerema NA, Brizard A, et al. Two additional cases of t dic (9:12) in acute lymphocytic leukemia (ALL): prognosis in ALL with dic(9:12. Leukemia 1990;4(06):423–425
13 Strehl S, König M, Dworzak MN, Kalwak K, Haas OA. PAX5/ETV6 fusion defines cytogenetic entity dic(9;12)(p13;p13. Leukemia 2003;17(06):1121–1123
14 Illade L, Fioravantti V, Andion M, Hernandez-Marques C, Madero L, Lassaletta A. Dicentric translocation (9;12) in acute lymphoblastic leukemia: a chromosomal abnormality with an excellent prognosis? Tumori 2017;103(Suppl 1):e44–e46
15 Woo JS, Alberti MO, Tirado CA. Childhood B-acute lymphoblastic leukemia: a genetic update. Exp Hematol Oncol 2014;3:16
16 Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. Blood Cancer J 2017;7 (06):e577
17 Bargetzi MJ, Mühlematter D, Tichelli A, Jotterand M, Wernli M. Dicentric translocation (9;12) presenting as refractory Philadelphia chromosome-positive acute B-cell lymphoblastic leukemia. Cancer Genet Cytogenet 1999;113(01):90–92

Fear of Recurrence and Somatic Symptom Severity in Multiple Myeloma Patients: An Institution-Based Cross-Sectional Study

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Abstract

Introduction Psychosocial concerns especially fear of cancer recurrence (FCR) is less commonly addressed among patients with multiple myeloma in India. Myeloma being incurable, an understanding of this problem is essential for adequately addressing them.

Objectives To study the prevalence of FCR among patients with multiple myeloma and determine the prevalence of somatic symptoms among patients with multiple myeloma.

Materials and Methods A cross-sectional study was performed at our institution among patients with multiple myeloma who had been on treatment for 1 year or more. The study was conducted between July 01 and July 31, 2015. At least 49 patients were required to be recruited into this study to meet its first objective. Patients were administered fear of cancer recurrence inventory (FCRI) questionnaire and Physical Health Questionnaire-15 (PHQ-15) questionnaire.

Results Sixty-four patients participated in the study. The median age was 60 years (34–80 years) and majority were females ($N = 38$, 60%). ISS staging information was available in 53 (83%) patients. Of 53, 24 (45%) were ISS stage 3, 12 (23%) were ISS stage 2 and remaining stage 1. The mean total FCRI score in the study population was 27.95 (SD: 24.5). Moderate to high levels of FCR were seen in 40% ($N = 26$). Using PHQ-15, 54 (84%) patients had mild or lesser somatic symptom burden. Disease status of patients at the time of this study had a significant statistical association with PHQ-15 scores (mean score in partial response (PR) or more group 6.02 versus 8.00 in less than PR group, $p = 0.02$).

Conclusions Overall, FCR scores and somatic symptom severity were low among our patients with multiple myeloma. However, a significant proportion had moderate to high levels of FCR. Further studies involving larger numbers in a prospective manner required to confirm our findings of fear of cancer recurrence among patients with multiple myeloma.

Keywords
- fear of recurrence
- multiple myeloma
- PHQ-15
- FCRI

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Introduction

Fear of cancer recurrence/relapse (FCR) is one of the most distressing problems among cancer survivors.[1] FCR is defined as the fear or worry that the cancer will return or progress in the same organ or in another part of the body.[2,3] Higher FCR is associated with many problems such as higher physical symptom burden, higher emotional distress, and certain maladaptive behaviors such as alcohol use and lower physical activity.[4] Cancer patients with high FCR have higher unmet supportive care needs and in fact FCR has been one of the most important concerns among cancer patients and survivors.[5,6] FCR has been well studied among cancer survivors who had solid tumors and certain hematological cancers.[7–11]

Multiple myeloma survival has improved over time due to advances in diagnosis, risk stratification, and treatment.[12] However, myeloma is still incurable with patients experiencing multiple relapses and eventually becoming treatment refractory.[13] Myeloma is associated with significant symptom burden due to the nature of the illness and prolonged treatment required.[14] The presence of physical symptoms may be considered as disease not getting controlled or as a sign of reappearance of disease.[15]

There are limited studies exploring the fear of recurrence/relapse among myeloma patients.[13,16,17] With improvement in survival of myeloma, patients are in a state of persistent confrontation with likelihood of progression. In the studies by Hulin et al and Kelly et al, the issue of FCR was studied in a qualitative manner.[13,16] In the study by Xiaochun et al, it was observed that 56.4% of patients with myeloma had a high level of FCR using the Fear of Progression Questionnaire short form.[17] Persistent and high FCR are debilitating. Early recognition of FCR is essential as psychological interventions have shown to alleviate FCR-related symptoms.[18] Studies focusing on FCR in myeloma are lacking from India and hence we decided to undertake this study to assess the baseline prevalence of FCR among patients with multiple myeloma on treatment for 1 year or more and the prevalence of somatic symptoms among them. It is important to have a baseline assessment of the degree of FCR and somatic symptom burdens in our population. This will help us guide our intervention in the future to improve the patient well-being.

Materials and Methods

This cross-sectional study was conducted at the hematology outpatient department (OPD) at our center. All patients with multiple myeloma fulfilling the inclusion criteria mentioned below were recruited in to this study. The study was conducted between July 01 and July 31, 2015. Approval was obtained from the Institutional Review Board (IRB) before the start of the study. A single interviewer who was a qualified psychiatrist administered the fear of cancer recurrence inventory (FCRI) questionnaire and Patient Health Questionnaire-15 (PHQ-15) to all patients who satisfied inclusion criteria and came to OPD during the study period. The interview was conducted in the regional language, i.e., Malayalam. All patients were aware of the diagnosis. Patients were told about the prognosis by the treating doctor if they explicitly asked for such information. Responsible caregivers of patients were appraised about the prognosis. Socioeconomic status assessment was done using modified Kuppuswamy's scale.[19]

Inclusion Criteria

1. Patients with multiple myeloma on treatment for 1 year or more from the date of their diagnosis.
2. Patients who give informed consent to participate in the study.
3. Age 18 years and above.

Exclusion Criteria

1. Patients who were not willing to participate in the study or unwilling to provide informed consent.
2. Patients who were diagnosed with and on treatment for any psychiatric illness.

Fear of Cancer Recurrence Inventory

FCRI is a multidimensional questionnaire. Seven components of fear of cancer recurrence (FCR) along a continuum of severity are evaluated using this questionnaire. There are 42 items and key domains evaluated include triggers, severity, psychological distress, functioning impairments, insight, reassurance, and coping strategies. Each item is rated on a Likert scale ranging from 0 to 4. Score is obtained for each subscale and for the total scale by summing the items. A higher score indicates higher levels of FCR. Score ranges from 0 to 168.[2,6] If FCR mean scores are below the mid-points of these scales, it indicates low-to-moderate levels of FCR.[6] For the purpose of this study, patients were classified as having low FCR if they had FCR scores below the mean score, moderate if observed scores were between mean and 84 (midpoint of the total FCR score), and high if they had observed scores above 84.

PHQ-15

PHQ-15 is a somatic symptom subscale that was derived from the full patient health questionnaire (PHQ). It deals with the assessment of severity of 15 somatic symptoms commonly reported in outpatient settings. PHQ-15 score is a continuous measure. However, using cut offs of 5, 10, and 15, the PHQ-15 score can be demarcated into four classes. These are minimal, mild, moderate, and high. Scoring is done by asking patients how often they are bothered due to each symptom in the past 7 days. If a patient is not at all bothered due to the symptoms, it will be scored as "0," 1 if bothered a little; and 2 if bothered a lot. Range of PHQ-15 score is 0 to 30.[20]

Sample Size Calculation

Sample size was calculated using the formula of $4pq/d^2$ where p is the expected prevalence, q is 100-p, and d is the precision level. The prevalence of FCR was expected to be 67.[10] Assuming 20% as relative precision, sample size = 4

$\times 67 \times (100–67)/13.4 \times 13.4 = 49$. Thus, at least 49 patients were required for this study.

Statistical Analysis

The data were tabulated electronically in Microsoft Excel and analyzed using the software IBM SPSS 20.0 version (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). The demographic details of the participants are expressed in frequency and percentage. The associations between scores of FCR, PHQ, and other continuous demographic variables were examined by Pearson's correlation analysis if they were normally distributed, or Spearman's correlation if they were not. Independent samples *t*-test was used for comparison of means of FCR and PHQ with gender and response to treatment. For analysis in this study, response to treatment was divided into two groups, i.e., patients who had partial response or more and patients who had less than partial response (PR or more vs. less than PR). The response defined as one documented just before recruitment into this study. One-way analyses of variance (ANOVAs) were conducted to compare FCR and PHQ scores with socioeconomic class.

Results

A total of 64 patients participated in this study. The median age was 60 years (range: 34–80 years). More than half of patients were females ($N = 38$, 60%). The majority of participating patients were married ($N = 53$, 82%). Most of the study participants ($N = 57$, 89%) had education of primary school level or more. A significant number of participants were either unemployed (22, 34%) or were unskilled laborers (18, 28%). Forty-seven (73%) patients were from upper lower socioeconomic status. Other demographic details are mentioned in ►**Table 1**. Complete ISS staging information was available in 53 (83%). Of these 53 cases, 24 (45%) were ISS stage 3, 12 (23%) were ISS stage 2, and remaining stage 1. First-line treatment received by these patients is listed in ►**Table 1**. The majority received melphalan prednisolone thalidomide (MPT) (25, 39%) followed by lenalidomide dexamethasone (20, 31%). Three (5%) patients had undergone autologous stem cell transplant. After first-line treatment, responses observed were very good partial response (VGPR) in 44 (69%) patients, 16 (25%) had partial response (PR), and 4 (6%) had stable disease. At the time of this study, 15 (23%) had complete remission, 25 (39%) had VGPR, 11 (17%) had PR, 2 (3%) had stable disease, and 7 (11%) had progressive disease. Data were missing in 4 (6%). The mean duration from diagnosis to enrolment into this study was 28 months.

Table 1 Patient Characteristics

Parameter		*N* (%)
Median age (in years)		60 (range 34–80)
Gender	Male	26 (40)
	Female	38 (60)
Marital status	Married	53 (80)
	Single	11 (20)
Educational status	Illiterate	7 (11)
	Primary school	17 (27)
	Middle school	26 (41)
	High school	11 (17)
	Intermediate/ diploma	2 (3)
	Graduate	1 (1)
	Professional degree	0 (0)
Occupational status	Unemployed	22 (34)
	Unskilled	18 (28)
	Semiskilled	8 (12)
	Skilled	12 (19)
	Clerical/shop/farm	1 (2)
	Semi-profession	2 (3)
	Professional	1 (2)
Socioeconomic class	Lower	4 (6)
	Upper lower	47 (73)
	Lower middle	12 (19)
	Upper middle	0 (0)
	Upper class	1 (2)
ISS stage*	Stage 1	17 (32)
	Stage 2	12 (23)
	Stage 3	24 (45)
First-line treatment	MPT	25 (39)
	Lenalidomide-dexamethasone	20 (31)
	CyBorD	10 (16)
	Thalidomide dexamethasone	9 (14)
Treatment response to first line	VGPR or better	44 (69)
	Partial response	16 (25)
	Stable disease	4 (6)

Abbreviations: CyBorD, cyclophosphamide bortezomib dexamethasone; MPT, melphalan prednisolone thalidomide; VGPR, very good partial response.
*International Staging system (ISS) details were available only in 53 patients.

Fear of Cancer Recurrence

The mean total FCRI score in the study population was 27.95 (SD: 24.5). The mean score was numerically higher in females compared with males (29.42 vs. 25.81, $p = 0.56$). Moderate to high levels of FCR were seen in 40% ($N = 26$). One patient (2%) had FCR score more than the mid-point of this scale, indicating high levels of FCR. There was no correlation between total FCRI scores and age ($r = -0.192$, $p = 0.130$) or with duration of disease ($r = -0.072$, $p = 0.57$). There was no significant difference in the mean total FCRI score between patients who had partial response or more compared with those who had less than partial response at the time of this study (mean

Table 2 PHQ-15 Somatic Symptom Severity Scale

Levels of somatic symptom severity	Number (%)
Minimal (0–4)	25 (39)
Low (5–9)	29 (45)
Moderate (10–14)	10 (16)
High (15–30)	0 (0)

Abbreviation: PHQ-15, Patient Health Questionnaire-15.

score in PR or more group 29.37 vs. 22.67 in less than PR group, $p = 0.47$) or among socioeconomic groups ($p = 0.289$).

PHQ-15

Somatic symptom severity assessed by PHQ-15 revealed that the majority (84%, $N = 54$) had mild or lower somatic symptom burden. Frequency of patients in each class based on somatic symptom severity scale is listed in ►**Table 2**. There was a significant difference in mean PHQ-15 scores between patients who had partial response or more compared with those who had less than partial response at the time of this study (mean score in PR or more group 6.02 vs. 8.00 in less than PR group $p = 0.02$). There was no significant statistical difference in means of PHQ-15 between either gender ($p = 0.56$) or different socioeconomic groups ($p = 0.39$). There was no significant correlation of total PHQ-15 score with the duration of disease ($r = 0.05$, $p = 0.65$) or with FCRI total scores ($r = 0.140$, $p = 0.27$). Symptoms reported by patients to cause a little or a lot of bother in more than half of study subjects were feeling tired, nausea or indigestion, and pain in arms, legs, or joints.

Discussion

We studied FCR among myeloma patients on treatment for a year or more utilizing FCRI, which is a multidimensional tool with 42 items. We observed a mean FCRI score of 27.95 among our study participants. There are no studies assessing FCR among Indian patients with multiple myeloma using the FCRI tool. In our study, the mean total FCRI score observed was lower compared with other solid tumors in which the fear of recurrence has been studied using this tool.[2] The mean FCRI score has been reported as 39 among prostate cancer patients, 60 among breast cancer survivors, and 58 among colorectal cancer survivors and lung cancer survivors respectively.[2] Low FCRI scores may have been due to multiple factors. One reason could be that the majority of subjects in our study had disease under control and had lower symptom burden. Lower symptom burden and the thought that disease is under control would have led to lower FCR scores.[4,15] In India, patients may be aware of the diagnosis of cancer. However, they may not be fully aware of their prognosis.[21] In our society, a responsible family member, mostly a male is the decision maker on cancer management. Patients are kept in the dark especially about their prognosis. This is because relatives fear that a complete information regarding cancer to the patient may negatively impact a patient's physical and mental health.[21] Hence, the responsible caregiver comes to an understanding with treating oncologists and chooses in majority to conceal a lot of information regarding the patient's cancer.[21] Patient's lack of knowledge about prognosis of disease may be another reason for lower mean FCRI scores. A thorough knowledge can induce FCR.[22] However, we have not specifically looked into what extent patients themselves were aware of their disease prognosis. Across different cancer sites and with different assessment strategies, on an average, 49% of cancer survivors reported to have moderate to high levels of FCR.[6] In our study, we observed that 40% of patients had moderate to high levels of FCR and this is similar to what has been observed in lymphoma survivors.[9] However, tools used to assess FCR differ. It has been seen in some studies that patients are less apprehensive about recurrence or disease progression when they undergo frequent checkups including blood tests or when they are on some form of treatment.[23] Myeloma patients are continually on one or other forms of treatment and they undergo periodic blood testing as well. It is notable that the mean duration from diagnosis to enrollment into this study was around 28 months. Thus, patients would have developed a sense of understanding about the chronicity of their disease. This may also have contributed in some to the development of a sense of coherence and possibly altering their perception about the disease.[11,24] The majority of patients at the time of this study had disease in remission and this could be the reason for lower symptom burden. Most of the study subjects reported feeling tiredness, nausea, or indigestion and pain in arms, legs, or joints. These are common symptoms reported by myeloma patients on treatment.[14,25] These might be due to disease or due to drugs such as steroids used to treat these patients. Pain is one of the main somatic symptoms a myeloma patient would complain of because of involvement of the skeletal system by the disease process. There was a significant difference in the mean PHQ-15 scores between patients who had partial response or more compared with those who had less than partial response at the time of this study. Patients with progressive or non-responding disease have higher symptom burden.[26] A large majority of patients (84%) had mild or lower somatic symptom burden. This means that 16% had moderate-to-high symptom burden. This is lower than that reported in a population based data among transplant ineligible patients.[27] In this study from Canada, symptom burden was assessed among patients with myeloma within an year of their diagnosis. In our study, patients enrolled had been diagnosed with multiple myeloma for a year or more. The lower symptom burden seen in our study was possibly due to greater proportion of patients with partial response or better. In the study by Mian et al, the symptom burden was observed to steadily decrease over time.[27] Their study did not capture symptom burden in specific lines of treatment and other myeloma-related variables such as stage and response. Also, this study used a different tool—Edmonton symptom assessment scale (ESAS) to measure symptom burden among myeloma patients. ESAS is a validated tool for general cancer symptoms.[27] Females had a higher symptom burden in their study.[27] However, we

did not observe any difference in symptom burden based on gender.

There are a few strengths to our study. This is one of the few studies looking into fear of cancer recurrence among myeloma patients. In the study from the Chinese group, FCR was assessed among myeloma patients using the Fear of Progression Questionnaire short form which contains 12 items.[17] We have used the FCRI tool which contains 42 items. Longer multidimensional scales such as FCRI may be more useful where FCR is the primary outcome of interest.[28] Studies focusing on FCR in myeloma are lacking from India and this study provides initial reports regarding FCR among myeloma patients. There are no studies from India looking into symptom burden among myeloma patients on treatment. We provide here initial reports of symptom burden among myeloma patients who are on treatment for a year or more. However, this study is not without limitations. The FCRI tool has 42 items and is extensive. Hence, a shorter tool is recommended for administration on a day today basis in the outpatient department. The PHQ-15 tool has not been validated for use in cancers. Also, PHQ-15 tool does not capture all symptoms specifically related to multiple myeloma. It is important to have a baseline assessment of FCR among patients with myeloma. This will help us to guide psychosocial interventions to improve patient well-being.

Conclusions

This study has shown that patients with multiple myeloma have overall lower fear of cancer recurrence compared with solid tumors. One possible explanation for this may be related to our cultural practice of non-disclosure of prognosis to the patients. In our study, patients had lower physical symptom burden especially in patients who had partial response or better at the time of this study. However, a significant proportion had moderate to high levels of fear of cancer recurrence. Further studies involving larger numbers in a prospective manner are required to confirm our findings about fear of cancer recurrence among patients with multiple myeloma.

Ethics Approval and Consent to Participate

Approval was obtained from the Institutional Review Board before the start of the study.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Ethics

The procedures followed were in accordance with the ethical standards of the IEC and with the Helsinki Declaration of 1964, as revised in 2013. An approval was obtained from the Institutional Review Board (IRB) at Malabar Cancer Center, Thalassery before the start of the study. IRB number – 1616/IRB-SRC/13/MCC/04–07–15/4. Informed consent was obtained from all the patients prior to the study.

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None.

Conflict of Interest

None declared.

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References

1 Koch L, Jansen L, Brenner H, Arndt V. Fear of recurrence and disease progression in long-term (≥ 5 years) cancer survivors–a systematic review of quantitative studies. Psychooncology 2013; 22(01):1–11. Doi: 10.1002/pon.3022
2 Simard S, Savard J. Fear of Cancer Recurrence Inventory: development and initial validation of a multidimensional measure of fear of cancer recurrence. Support Care Cancer 2009;17(03):241–251. Doi: 10.1007/s00520-008-0444-y
3 Vickberg SMJ. The Concerns About Recurrence Scale (CARS): a systematic measure of women's fears about the possibility of breast cancer recurrence. Ann Behav Med 2003;25(01):16–24. Doi: 10.1207/S15324796ABM2501_03
4 Hall DL, Jimenez RB, Perez GK, et al. Fear of Cancer Recurrence: A Model Examination of Physical Symptoms, Emotional Distress, and Health Behavior Change. J Oncol Pract 2019;15(09):e787–e797. Doi: 10.1200/JOP.18.00787
5 Sarkar S, Sautier L, Schilling G, Bokemeyer C, Koch U, Mehnert A. Anxiety and fear of cancer recurrence and its association with supportive care needs and health-care service utilization in cancer patients. J Cancer Surviv 2015;9(04):567–575. Doi: 10.1007/s11764-015-0434-2
6 Simard S, Thewes B, Humphris G, et al. Fear of cancer recurrence in adult cancer survivors: a systematic review of quantitative studies. J Cancer Surviv 2013;7(03):300–322. Doi: 10.1007/s11764-013-0272-z
7 Fredette SL. Breast cancer survivors: concerns and coping. Cancer Nurs 1995;18(01):35–46
8 Lee-Jones C, Humphris G, Dixon R, Hatcher MB. Fear of cancer recurrence–a literature review and proposed cognitive formulation to explain exacerbation of recurrence fears. Psychooncology 1997;6(02):95–105. Doi: 10.1002/(SICI)1099-1611(199706)6:2<95:AID-PON250>3.0.CO;2-B
9 Walburg V, Rueter M, Lamy S, et al. Fear of cancer recurrence in non- and Hodgkin lymphoma survivors during their first three years of survivorship among French patients. Psychol Health Med 2019;24(07):781–787. Doi: 10.1080/13548506.2019.1574354
10 Mehnert A, Koch U, Sundermann C, Dinkel A. Predictors of fear of recurrence in patients one year after cancer rehabilitation: a prospective study. Acta Oncol 2013;52(06):1102–1109. Doi: 10.3109/0284186X.2013.765063
11 Black EK, White CA. Fear of recurrence, sense of coherence and posttraumatic stress disorder in haematological cancer survivors. Psychooncology 2005;14(06):510–515. Doi: 10.1002/pon.894

12 Kumar SK, Dispenzieri A, Lacy MQ, et al. Continued improvement in survival in multiple myeloma: changes in early mortality and outcomes in older patients. Leukemia 2014;28(05):1122–1128. Doi: 10.1038/leu.2013.313
13 Hulin C, Hansen T, Heron L, et al. Living with the burden of relapse in multiple myeloma from the patient and physician perspective. Leuk Res 2017;59:75–84. Doi: 10.1016/j.leukres.2017.05.019
14 Ramsenthaler C, Kane P, Gao W, et al. Prevalence of symptoms in patients with multiple myeloma: a systematic review and meta-analysis. Eur J Haematol 2016;97(05):416–429. Doi: 10.1111/ejh.12790
15 Hall DL, Lennes IT, Pirl WF, Friedman ER, Park ER. Fear of recurrence or progression as a link between somatic symptoms and perceived stress among cancer survivors. Support Care Cancer 2017;25(05):1401–1407. Doi: 10.1007/s00520-016-3533-3
16 Kelly M, Dowling M. Patients' lived experience of myeloma. Nurs Stand 2011;25(28):38–44
17 Hu X, Wang W, Wang Y, Liu K. Fear of cancer recurrence in patients with multiple myeloma: Prevalence and predictors based on a family model analysis. Psychooncology 2021;30(02):176–184. Doi: 10.1002/pon.5546
18 Tauber NM, O'Toole MS, Dinkel A, et al. Effect of psychological intervention on fear of cancer recurrence: a systematic review and meta-analysis. J Clin Oncol 2019;37(31):2899–2915. Doi: 10.1200/JCO.19.00572
19 Wani RT. Socioeconomic status scales-modified Kuppuswamy and Udai Pareekh's scale updated for 2019. J Family Med Prim Care 2019;8(06):1846–1849. Doi: 10.4103/jfmpc.jfmpc_288_19
20 Kroenke K, Spitzer RL, Williams JBW. The PHQ-15: validity of a new measure for evaluating the severity of somatic symptoms. Psychosom Med 2002;64(02):258–266
21 Ghoshal A, Salins N, Damani A, et al. To tell or not to tell: exploring the preferences and attitudes of patients and family caregivers on disclosure of a cancer-related diagnosis and prognosis. J Glob Oncol 2019;5:1–12. Doi: 10.1200/JGO.19.00132
22 Han PKJ, Gutheil C, Hutchinson RN, LaChance JA. Cause or effect? The role of prognostic uncertainty in the fear of cancer recurrence. Front Psychol 2021;11:626038. Doi: 10.3389/fpsyg.2020.626038
23 Raphael D, Frey R, Gott M. The nature and timing of distress among post-treatment haematological cancer survivors. Eur J Cancer Care (Engl) 2019;28(01):e12951. Doi: 10.1111/ecc.12951
24 Aderhold C, Morawa E, Paslakis G, Erim Y. Protective factors of depressive symptoms in adult cancer patients: the role of sense of coherence and posttraumatic growth in different time spans since diagnosis. J Psychosoc Oncol 2019;37(05):616–635. Doi: 10.1080/07347332.2019.1631931
25 Mols F, Oerlemans S, Vos AH, et al. Health-related quality of life and disease-specific complaints among multiple myeloma patients up to 10 yr after diagnosis: results from a population-based study using the PROFILES registry. Eur J Haematol 2012;89(04):311–319. Doi: 10.1111/j.1600-0609.2012.01831.x
26 Ramsenthaler C, Osborne TR, Gao W, et al. The impact of disease-related symptoms and palliative care concerns on health-related quality of life in multiple myeloma: a multi-centre study. BMC Cancer 2016;16:427. Doi: 10.1186/s12885-016-2410-2
27 Mian HS, Pond GR, Wildes TM, Sivapathasundaram B, Sussman J, Seow H. Symptom burden in transplant ineligible patients with newly diagnosed multiple myeloma: a population-based cohort study. Haematologica 2021;106(07):1991–1994. Doi: 10.3324/haematol.2020.267757
28 Cancer Australia. Recommendations for the identification and management of fear of cancer recurrence in adult cancer survivors. 2014:1–42

Comprehensive Germline Genomic Profiling of Patients with Ovarian Cancer: A Cross-Sectional Study

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Abstract

Introduction Ovarian cancer is the third most common cancer among Indian women. The data on the hereditary predisposition of these cancers and the clinical outcomes of those with pathogenic mutations is meager in India.

Objective The aim of the current study was to analyze the germline-genetic profile, clinicopathological characteristics, and outcomes of patients with ovarian cancer who were referred for genetic counseling at our Institute.

Materials and Methods It was a cross-sectional observational study. Patients with histological diagnosis of carcinoma ovary at our institute who were referred for genetic counseling from July 2017 to June 2020 were included in the study. All patients underwent pretest counseling. Most patients underwent multigene panel testing with reflex multiplication ligation-dependent probe amplification for large genomic rearrangements, while some received testing for *BRCA1* and *BRCA2* only. The variants were classified as pathogenic or benign based on American College of Medical Genetics (ACMG) guidelines. Data regarding the demographic profile, clinical characteristics, histopathological findings, family history, treatment received, and outcomes were extracted from the medical record system files.

Results One hundred and one patients were referred to the genetic clinic and underwent genetic counseling. All patients were advised for genetic testing; however, only 72 (71%) underwent testing. A multigene panel testing was done in 51 (70%)

Keywords
- germline
- ovarian cancer
- BRCA

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patients, and only *BRCA1* and *BRCA2* genes were tested in 21 (30%). Among the 72 patients who underwent a genetic test, the median age was 47 years (range, 28–82). The most common histopathology was serous (90%), while 85% were diagnosed having stage 3 and 4 ovarian cancer. A pathogenic/likely pathogenic (P/LP) BRCA or non-BRCA mutation was detected in 32 (44%) patients. Six patients (8%) had a variant of unknown significance (VUS). Among P/LP mutations, 85% were in the *BRCA* gene (75% in *BRCA1* and 10% in *BRCA2*), while 15% were in non-BRCA gene mutations (*RAD51, PALB2, MER11, HMMR*). Disease-free survival and overall survival were not different in mutation-positive and mutation-negative cohorts.

Conclusions We report 44% P/LP mutations in this selected cohort of patients with carcinoma ovaries. BRCA mutations constituted 85% of all the mutations, while 15% of mutations were in non-BRCA genes.

Introduction

Ovarian cancer (OC) is the eighth most common cancer in females (3.4% of all cancers) and leads to 4.4% of all cancer-related mortality.[1] Globally, there were 313,959 cases diagnosed with OC in 2020 and 207,252 deaths.[1] In India, the estimated incidence of OC in 2020 is 43,886 (6.7%) cases, and mortality is 32,077 (7.8%).[2] Though the prevalence is low, the survival of advanced OCs is dismal (20–40%).[3] The most important risk factor for OC is a genetic predisposition, that is, family history of breast or OC.[4] More than 20% of OCs are associated with a mutation in the tumor suppressor genes, most important being *BRCA1/2*.[5] The average cumulative frequency of OC at 70 years is estimated to be 35 to 46% for individuals with *BRCA1* mutations and 13 to 23% for *BRCA2* mutation.[6] Other genes implicated are *ATM*, *CHEK2, BRIP, RAD51C, RAD51D,* and *MUTYH* genes.[5]

Families with these mutations have a higher prevalence of multiple malignancies. Preventive strategies like salpingo-oophorectomy reduce the risk of OC by 96%.[7] Detecting mutation in the *BRCA* gene has therapeutic implications and is immediately applicable to patients in the first line as maintenance and in the recurrent setting where it has shown improvements in progression-free survival.[8,9] Identification of these pathogenic mutations is essential as this may help us plan screening and preventive strategies in families with identifiable mutations. The current literature regarding mutation-positive OC in India is meager and is often lumped with breast cancer data. The current study aimed to analyze the genetic and clinicopathological profile and outcomes of patients with OC from a tertiary care center in India.

Methodology

Materials and Methods

Patients

In this cross-sectional observational study, patients with histological diagnosis of carcinoma of the ovary, registered in our cancer center, who were referred for genetic counseling between Jan 2017 and Dec 2020 were included. A medical oncologist did pretest counseling. Patients who did not give informed consent were excluded. Most patients underwent multigene panel testing with reflex multiplication ligation-dependent probe amplification (MLPA) that detects gene specific single or multiple exon deletions and duplications, which may be missed by next-generation sequencing, while some received testing for *BRCA1* and *BRCA2* only. Those with a pathogenic/likely pathogenic (P/LP) mutation in any of the predisposing genes were discussed in a multidisciplinary tumor board for management and were offered post-test counseling. The variants were classified as pathogenic or benign based on American College of Medical Genetics (ACMG) guidelines.[10] Data regarding the demographic profile, clinical characteristics, histopathological findings, family history, treatment received, and outcomes were extracted from the medical record system files. The primary outcome was the frequency of PLA mutations identified in the sample. The secondary outcomes included the comparison of response rates, overall survival (OS), and disease-free survival between the groups with and without a P/LP mutation.

Sample Preparation and Multigene Panel Design

Blood samples were collected from each patient (10 mL), and germline DNA extraction was done by kits following the manufacturer's instructions. Genomic DNA was enzymatically fragmented. Regions of interest were selectively enriched using customed capture probes targeted against coding regions of the 104 genes (listed in supplementary material), including ten bp of flanking intronic sequences for the genes *BRCA1* and *BRCA2*. In 21 patients, only two genes (*BRCA1* and *BRCA2*) were studied. The libraries underwent next-generation sequencing to mean >80–100X coverage on the Illumina sequencing platform. The sequences were aligned to the human reference genome (GRCh37/hg19) build I.D. Gene bank NM_007300.3 and NM _000059.3 were used as reference transcript sequences for *BRCA1* and *BRCA2* genes, respectively. Gene annotation of the variants was performed using the variant effect predictor (VEP) program against Ensemble release 91 human gene model.

Mutations were annotated using databases: ClinVar, OMIM, GWAS, HGMD, and SwissVar. Variants were filtered based on allele frequency in 1000 Genome phase3, gnomAD, EVS, dbSNP, 1000 Japanese Genome, and Indian database.

MLPA Testing

Reflex MLPA testing was done for patients who tested negative on the multigene panel. Copy number change in 24 exons of *BRCA1* and 27 exons of *BRCA2* was identified by hybridizing with MLPA-based assay. Each MLPA probe consisted of two hemiprobes that were bound to an adjacent site of the target sequence. Upon ligation and subsequent PCR amplification, each probe generated an amplicon with a unique length. Copy number differences of various exons between test and control DNA samples were detected by analyzing the MLPA peak patterns. The classification of variants was done as deleterious (class 5—pathogenic or class 4—likely pathogenic), a variant of unknown clinical significance (VUS, class 3), likely benign (class 2), and benign (class 1) according to the ACMG guidelines.

Data regarding the demographic profile, clinical characteristics, histopathological findings, family history, treatment received, and outcomes were extracted from the medical record system files.

Statistical Analysis

Categorical data were summarized using percentages. Numerical data were summarized as the means and standard deviations or medians and ranges. Chi-squared tests and Fisher's exact tests were used to examine the relationships between qualitative variables. The survival analysis was performed using the Kaplan–Meier method. A log-rank test was used to compare the survival curves. All tests of hypotheses were conducted at an α level of 0.05, with a 95% confidence interval. The median duration of follow-up was estimated using the reverse Kaplan–Meier method. Disease-free survival) was defined as "the interval from histological diagnosis to the date of first progression or last follow-up." OS was defined as "the interval from histological diagnosis to the date of death or last follow-up." All analysis was done using STATA software (ver13, Texas, United States)

Ethics

The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1964, as revised in 2013. Ethics Committee Approval was obtained from the Institutional Ethics Committee vide letter no. IEC-511/05.06.2020, RP-50/2020 dated 30.9.2020 (see supplementary material).

Results

One hundred and one patients were referred to the genetic clinic and underwent genetic counseling. All patients were advised for genetic testing; however, only 72 of them (71%) underwent testing. The various reasons for not undergoing genetic testing were financial issues in 20 patients (20%), deferral of testing due to COVID pandemic in 5 of them (5%), and patient preference in 4 (5%). A multigene panel testing was done in 51 (70%) patients, and only *BRCA1* and *BRCA2* genes were tested in 21 (30%).

Table 1 Baseline characteristics of the patients included in the study

Baseline characteristics ($n = 72$)	Median/mean ± SD/ percentage
Age	45.5 years (IQR: 41.5–55) (Range, 28–82)
Ethnicity	
Hindu	65 (90%)
Muslim	04 (5%)
Christian	02 (3%)
Buddhism	01 (2%)
Histology	
Serous	68 (95%)
Mucinous	1 (1%)
Endometroid	2 (3%)
Clear cell	1 (1%)
Baseline stage	
I	7 (10%)
II	4 (6%)
III	48 (68%)
IV	13 (16%)
Newly diagnosed cases	5 (5%)
Relapsed cases	95 (95%)
Family history	
Yes	34 (34%)
No	38 (66%)
Family history of breast cancer	09 (26%)
Ovarian cancer	14 (41%)
Both breast and ovarian cancer	10 (29%)
Prostate cancer	01 (4%)

Abbreviations: IQR, interquartile range; SD, standard deviation.

Among the 72 patients who underwent a genetic test, the median age was 47 years (range 28–82) (►**Table 1**). Hinduism was the most common religion (89%), while four (5%) patients were Muslims and two (3%) were Christians. Seventy-three percent of these patients were literate. Seventy-four percent of these patients were from Delhi, Chandigarh, and Uttar Pradesh, while 18% were from eastern states (Bihar, Orissa, West Bengal). Six patients were from western and southern states (four from Rajasthan and two from Kerala).

Of all patients, 4 (5%) had recently been diagnosed with malignancy, whereas 68 (95%) were referred after progression on the first line of therapy. Sixty-four (89%) patients had OC, while 8 (11%) patients had both ovarian and breast cancer. The most common histopathology was serous (90%), while 85% were detected as stage 3 and 4 OCs.

A family history of breast/ovarian cancer syndrome was present in 34 (34%) cases (85% in first-degree relatives and 15% in second-degree relatives). Of these 34 patients, 9 of them (26%) had a family history of breast cancer, 14 (41%) patients had a history of OC, and 10 (29%) patients had a family history of both breast and OC.

A mutation was identified among the patients who underwent a genetic test in 38 (52%) patients. A P/LP BRCA or non-BRCA mutation was detected in 32 (44%) patients. Six patients (8%) had a VUS (►**Table 2**).

Mutations were more common in patients younger than 50 years (68%: $n = 22$). However, it was not significantly different from those older than 50 years ($p = 0.65$). Notably, among patients younger than 50 years and who had a positive family history, the detection rate of a mutation was 87%.

Among P/LP mutations, 85% were in the *BRCA* gene (75% in *BRCA1* and 10% in *BRCA2*), while 15% were non-BRCA gene mutations (*RAD51, PALB2, MER11, HMMR*). The mutations in *BRCA1* gene spanned over exon 2 to exon 24 region with maximum mutations present in exon 10 (►**Table 3**). The Ashkenazi Jewish founder mutation (c.68_69delAG) was present in two patients. The most common types of mutations in patients were nonsense mutation in 10 (42%), followed by frameshift mutations in 8 of them (33%), missense mutation in 3 (13%), splice-site mutation in 2 (8%), and a large genomic rearrangement in 1 patient (4%). In the *BRCA2* gene, all were frameshift mutations, while in non-BRCA genes, 80% were nonsense, and 20% were frameshift mutations.

VUS was present in 10 patients, among which four patients had both P/LP and VUS. Two patients had VUS in *BRCA2*, and three patients had in *ATM* gene. One patient each had VUS in *FANCI, MLH1, ERCC2, CHECK2,* and *FANCM* genes.

Table 2 Frequency distribution of mutations ($n = 72$)

Pathogenic/likely pathogenic (P/LP)	32 (44%)
Variant of unknown significance (VUS)	10 (14%)
Both P/LP and VUS	04 (6%)
Locus of P/LP mutations ($n = 32$)	*n* (percentage)
BRCA 1	24 (75%)
BRCA 2	03 (10%)
MER11	01 (3%)
PALB2	01 (3%)
RAD51	02 (6%)
HMMR	01 (3%)

Abbreviations: BRCA, breast cancer gene; HMMR, hyaluronan-mediated motility receptor; MER11, meiotic recombination 11 homolog; P/LP, pathogenic/likely pathogenic; PALB2, partner and localizer of BRCA2; RAD51, DNA repair protein RAD51; VUS, variant of uncertain significance.

Neoadjuvant chemotherapy was given in 50% of both mutation-positive and mutation- negative cohorts. Eighteen percent of mutation-positive patients achieved complete remission (CR), while 76% had a partial response (PR) Among mutation-negative, there were no CR, and 84% achieved PR (►**Table 4**). None of the included patients received maintenance PARP inhibitors during this period.

Table 3 Comparison of the clinical characteristics of patients with and without a P/LP mutation

Patients characteristics	Positive for P/LP mutation ($n = 32$)	Negative for P/LP mutation ($n = 40$)	*p*-Value
Age	47.5 ± 9	49.5 ± 12	0.77
Age group			
< 50 years	22 (68%)	24 (60%)	0.65
> 50 years	10 (32%)	16 (40%)	
Family history present (first or second-degree having breast/ovarian cancer)	25 (78%)	09 (22.5%)	0.001
First-degree relatives	24 (70%)	5 (15%)	
Second-degree relatives	5 (15%)	02 (6%)	
Personal history of breast cancer	05 (15%)	03 (8%)	0.31
NACT received	17 (48%)	19 (53%)	0.20
Response to NACT			
CR	03 (18%)	0	
PR	13 (76%)	16 (84%)	
SD	01 (06%)	02 (10%)	
PD	0	01 (05%)	
DFS (median)	20.2 months	21.4 months	0.26
OS (median)	91.4 months	71.5 months	0.38

Abbreviations: CR, complete response; DFS, disease-free survival; NACT, neoadjuvant Chemotherapy; OS, overall survival; P/LP, pathogenic/likely pathogenic; PD, progressive disease; PR, partial response; SD, stable disease.

Table 4 The pathogenic/likely pathogenic mutations, their description, location, and corresponding protein changes

Gene	Site	Variant	Protein change	Type of mutation
BRCA 1	EXON 10	c.3607C > T	p.Arg1203 Ter	Nonsense
BRCA 1	EXON 10	c.1450G > T	p.Gly484 Ter	Nonsense
BRCA 1	EXON 10	c.1008delA	p.Glu337LysfsTer4	Frameshift
BRCA 1	EXON 10	c.3147delC	p.Ser1050ValfsTer12	Frameshift
BRCA 1	EXON 10	c.2769del	p.Asn924IlefsTer76	Frameshift
BRCA 1	EXON 10	c.2338C > T	p.Gln780Ter	Nonsense
BRCA 1	EXON 10	c.2076_2080delTGACA	p.His692GlnfsTer18	Frameshift
BRCA 1	EXON 10	c.3607C > T	p.Arg1203 Ter	Nonsense
BRCA 1	EXON 10	c.1953_1956delGAAA	p.Lys653SerfsTer47	Frameshift
BRCA 1	EXON 11	c.4183 C > T	p.Gln1395Ter	Nonsense
BRCA 1	EXON 15	c.4738G > G/C	p.Glu1580 Gln	Missense
BRCA 1	EXON 15	c.4571 C > A	p.Ser1524Ter*	Nonsense
BRCA 1	EXON 15	c.4571 C > A	p.Ser1524Ter*	Nonsense
BRCA 1	EXON 15	c.4571 C > A	p.Ser1524Ter*	Nonsense
BRCA 1	EXON 15	c.4571 C > A	p.Ser1524Ter*	Nonsense
BRCA 1	EXON 16	c.4900_4901delinsGCC	p.Ser1634AlafsTer9	Frameshift
BRCA 1	EXON 2	c.68_69 delAG	p.Glu23ValfsTer17	Frameshift
BRCA 1	EXON 2	c.68_69 delAG	p.Glu23ValfsTer17	Frameshift
BRCA 1	EXON 7	c.470_471 delCT	p.Ser157Ter	Nonsense
BRCA 1	EXON 24	c.5572 T > C	p.Trp1858Arg	Missense
BRCA 1	EXON 24	c.5572 T > C	p.Trp1858Arg	Missense
BRCA 1	INTRON 17	c.5137 +1 G > A		Splice site
BRCA	INTRON	c.4547 + 1G > A		Splice site
1	14			
BRCA 1	LGR, EXON 1-20			
BRCA 2	EXON 11	c.3182del	p.Lys1061SerfsTer16	Frameshift
		c.4570_4573delTTTC	p.Phe1524IlefsTer18	Frameshift

(*Continued*)

Table 4 (*Continued*)

Gene	Site	Variant	Protein change	Type of mutation
BRCA 2	EXON 11			
BRCA 2	EXON 11	c.5967 _5968 del	p.Asp1990cysfsTer12	Frameshift
RAD51 D	EXON 5	c.423delA	pAla142GlnfsTer14	Frameshift
RAD51 D	EXON 5	c.423delA	pAla142GlnfsTer14	Frameshift
HMMR	EXON 12	c.1327C > T	p.Gln443Ter	Nonsense
MER 11	EXON 10	c.1086del	p.Val363TyrfsTer27	Frameshift
PALB2	Exon 5	c.2488delG	p.Glu830SerfsTer21	Frameshift

This mutation was found in a single family with 4 members affected with ovarian cancer.

Disease-free survival was not different in mutation-positive and mutation-negative arms. Although OS was numerically higher in the mutation-positive arm (91 vs. 71 months), it was not statistically significant (►**Fig. 1**).

Cascade testing was advised for the family members of patients who were positive for the mutation. However, only 13 members from 8 different families underwent testing. Four were *BRCA1* positive. Though risk reducing salpingo-oophorectomy was advised to the previvors, none of them had undergone the preventive surgery. All are under radiological surveillance.

Discussion

The current study is a cross-sectional analysis of selectively referred OC patients. We report 44% PLP mutations in this selected cohort of patients with carcinoma ovaries. BRCA mutations constituted 85% of all the mutations, while 15% of mutations were in non-BRCA genes. The prevalence of Ashkenazi Jewish founder mutation was very scarce (2.1%) in our cohort. The presence of BRCA mutation had no impact on survival outcomes. There was poor uptake of cascade testing.

In one retrospective analysis of OC ($n = 238$), where patients across India were selectively referred to the laboratory for multigene panel testing, 36% had pathogenic mutations (84.9% in *BRCA1/2* gene and 15.1% in non-BRCA gene).[11] In another study of selectively referred patients with OC by Mehta et al from north India ($n = 74$), where only BRCA mutation testing was done, 41.5% of patients carried a mutation.[12] Both the above-mentioned studies with selective populations showed comparable mutation rates to our study. However, Gupta et al, in a prospective study ($n = 239$)

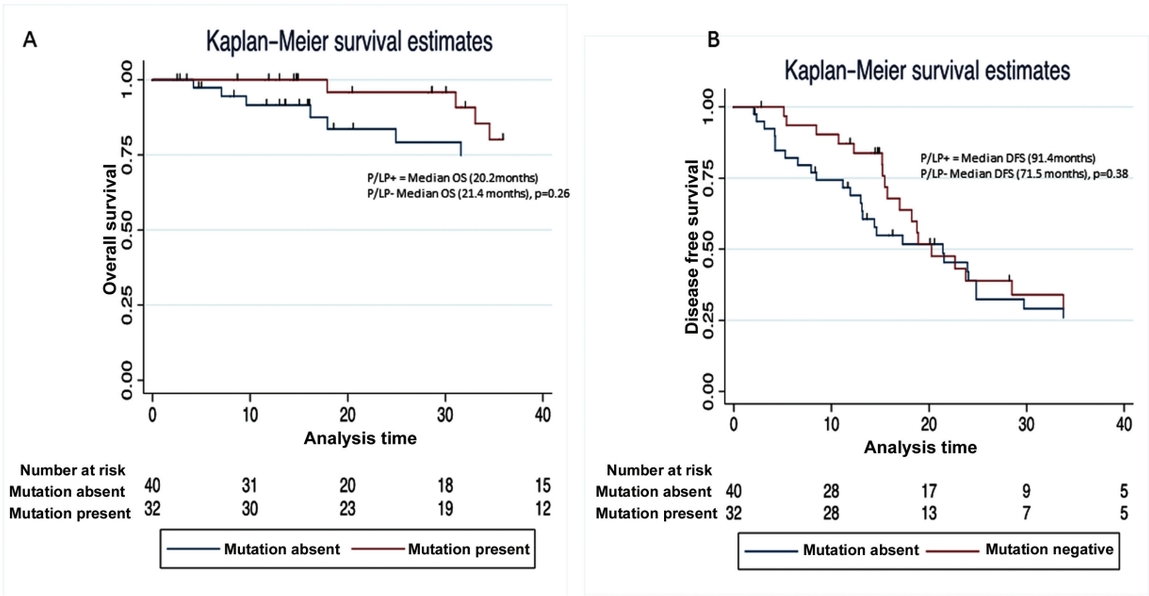

Fig. 1 Comparison of the (A) overall survival and (B) disease-free survival (DFS) of patients with and without a pathogenic/likely pathogenic (P/LP) mutation in any of the predisposing genes.

of unselected patients of carcinoma ovary from all across India, reported BRCA mutations in 25.5% of patients, which may be closer to true prevalence in the population.[13]

Reported literature from various ethnicity worldwide has shown a slightly lower prevalence of BRCA and non-BRCA mutations. In a prospective study of consecutive patients of carcinoma ovary done by Eoh et al in Korea ($n = 117$), 32.5% of patients with epithelial ovarian cancer had a pathogenic mutation in BRCA and non-BRCA genes (79.5% mutation in BRCA gene).[14] In the Japanese nationwide multicentric study ($n = 634$), where OC patients were recruited prospectively, 28.5% of high-grade serous ovarian cancers had germline BRCA mutations.[15] Similarly, a retrospective study by Ataseven et al in the German population ($n = 545$) showed 29.5% P/LP mutations in hereditary cancer-predisposing genes. Eighty-one percent of mutations were in the BRCA gene alone.[16] Walsh et al showed that in the American population of prospectively selected OC ($n = 360$), 24% had germline mutation,18% in *BRCA1/2*, and 6% in non-BRCA genes.[5] In the study done at Royal Marsden Hospital, London, where only *BRCA1* and *BRCA2* were tested, the yield was 16%.[17]

The Ashkenazi Jewish founder mutation was present in two patients in our study. In another study done in North India, this mutation was found in only one patient.[12] However, in the study by Singh et al, which had patients from across India, this mutation was repeated 34 times.[11] In another study done exclusively in the South Indian population, which included both breast and OC, the Ashkenazi Jewish founder mutation was present in 10 out of 44 patients.[18] The profile of BRCA1/2 mutation in South India appears to be different from North India.

Patients with pathogenic mutations had a better response to platinum-based therapy. Eighteen percent of patients with mutations achieved CR postneoadjuvant chemotherapy compared with none in patients with no detectable mutations. This may be explained due to increased platinum sensitivity in *BRCA* deficient tumors. In *BRCA* deficient tumors xenograft studies have shown differential gene expression and pathway modulation, which includes upregulation of RAD 52 and ERCC1/RRM1 downregulation that might be responsible for increased platinum sensitivity.[19] However, we did not find any difference in the PFS or OS. Some studies have reported longer PFS in BRCA mutated patients,[20] while others have shown no difference.[21] The heterogeneity in the outcome can be explained by other prognostic factors such as stage, optimal cytoreduction, and age.

Our study included multigene panel testing with reflex MLPA, and results were correlated with clinical details. This study reports the details exclusively from the North Indian population, chiefly Delhi and adjoining states, and the real-world challenges of access to testing, cascade testing, and counseling. Our study is the first study that uses mainstreaming of the genetic testing of OC by clinicians in India. Although no founder mutations were seen, there appears to be a significant difference in North-South Indian populations concerning the prevalence of Ashkenazi Jewish mutations.

Limitations

The patients in the study represent a highly selected population. It included those who were referred by the clinicians and who could avail of the testing. This selection may have its own biases. All patients could not undergo multigene panel testing, and some underwent BRCA only testing.

Future Directions

This study and other contemporary studies point to the significant burden of a germline mutation in carcinoma of the ovary in the Indian population. The oncology community in India has accepted universal genetic testing for *BRCA1/2* genes, and given the scarcity of counselors, this is imperative that all oncologists take part in mainstreaming the test. Locus-specific database specific to the Indian population is the need of the hour.

Conclusion

OC is an aggressive disease, and it has got high genetic predisposition. Knowing the deleterious mutations in various genes can help the patients and their biological relatives. This study reports various pathogenic and VUS mutations in BRCA and non-BRCA genes in the North Indian population. Forty-four percent of P/LP mutations were found with a very low frequency of founder mutations (2%). This study would help in building up the database for the Indian population.

Ethical Approval

The study protocol was approved by the Institute Ethics Committee vide letter number-IEC-511/5.6.20 RP/50/2020

Consent to Participate

Informed consent was obtained from all patients.

Availability of Data and Material

Data regarding this study will be available from the corresponding author (RP) at reasonable request.

Authors' Contributions

R.P; A.U; S.K; L.K; P.M; M.R; S.D; and S.K contributed to the concept design, patient referrals, and conduct of the study. R.G; D.T; and V.L.R were involved in laboratory testing. A. U; R.P did the statistical analysis.

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None.

Conflict of Interest

None declared.

References

1 Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence

and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68(06):394–424
2 Mathur P, Sathishkumar K, Chaturvedi M, et al; ICMR-NCDIR-NCRP Investigator Group. Cancer Stat 2020: report from national cancer registry programme, India. JCO Glob Oncol 2020; 6:1063–1075
3 Lin JJ, Egorova N, Franco R, Prasad-Hayes M, Bickell NA. Ovarian cancer treatment and survival trends among women older than 65 years of age in the United States, 1995-2008. Obstet Gynecol 2016;127(01):81–89
4 Torre LA, Trabert B, DeSantis CE, et al. Ovarian cancer statistics, 2018. CA Cancer J Clin 2018;68(04):284–296
5 Walsh T, Casadei S, Lee MK, et al. Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. Proc Natl Acad Sci U S A 2011;108(44):18032–18037
6 Chen S, Parmigiani G. Meta-analysis of BRCA1 and BRCA2 penetrance. J Clin Oncol 2007;25(11):1329–1333
7 Rebbeck TR, Lynch HT, Neuhausen SL, et al; Prevention and Observation of Surgical End Points Study Group. Prophylactic oophorectomy in carriers of BRCA1 or BRCA2 mutations. N Engl J Med 2002;346(21):1616–1622
8 Friedlander M, Moore KN, Colombo N, et al. Patient-centred outcomes and effect of disease progression on health status in patients with newly diagnosed advanced ovarian cancer and a BRCA mutation receiving maintenance olaparib or placebo (SOLO1): a randomised, phase 3 trial. Lancet Oncol 2021;22 (05):632–642
9 Pujade-Lauraine E, Ledermann JA, Selle F, et al; SOLO2/ENGOT-Ov21 investigators. Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Oncol 2017;18 (09):1274–1284
10 Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17(05):405–424
11 Singh J, Thota N, Singh S, et al. Screening of over 1000 Indian patients with breast and/or ovarian cancer with a multi-gene panel: prevalence of BRCA1/2 and non-BRCA mutations. Breast Cancer Res Treat 2018;170(01):189–196
12 Mehta A, Vasudevan S, Sharma SK, et al. Germline *BRCA1* and *BRCA2* deleterious mutations and variants of unknown clinical significance associated with breast/ovarian cancer: a report from North India. Cancer Manag Res 2018;10:6505–6516
13 Gupta S, Rajappa S, Advani S, et al. Prevalence of BRCA1 and BRCA2 mutations among patients with ovarian, primary peritoneal, and fallopian tube cancer in India: a multicenter cross-sectional study. JCO Glob Oncol 2021Jun;7:849–861
14 Eoh KJ, Kim J, Park HS, et al. Detection of germline mutations in patients with epithelial ovarian cancer using multi-gene panels: beyond BRCA1/2. Cancer Res Treat 2017;•••:50
15 Enomoto T, Aoki D, Hattori K, et al. The first Japanese nationwide multicenter study of *BRCA* mutation testing in ovarian cancer: CHARacterizing the cross-sectional approach to Ovarian cancer geneTic TEsting of *BRCA* (CHARLOTTE). Int J Gynecol Cancer 2019; 29(06):1043–1049
16 Ataseven B, Tripon D, Rhiem K, et al. Prevalence of *BRCA1* and *BRCA2* mutations in patients with primary ovarian cancer - does the German checklist for detecting the risk of hereditary breast and ovarian cancer adequately depict the need for consultation? Geburtshilfe Frauenheilkd 2020;80(09):932–940
17 Implementing rapid, robust, cost-effective, patient-centred, routine genetic testing in ovarian cancer patients | Scientific Reports [Internet]. . [cited 2020 Dec 28]. Available from: https://www.nature.com/articles/srep29506. Accessed March 10, 2022
18 Rajkumar T, Meenakumari B, Mani S, Sridevi V, Sundersingh S. Targeted resequencing of 30 genes improves the detection of deleterious mutations in South Indian women with breast and/or ovarian cancers. Asian Pac J Cancer Prev 2015;16(13): 5211–5217
19 Tassone P, Di Martino MT, Ventura M, et al. Loss of BRCA1 function increases the antitumor activity of cisplatin against human breast cancer xenografts in vivo. Cancer Biol Ther 2009;8(07):648–653
20 Tewari KS, Burger RA, Enserro D, et al. Final overall survival of a randomized trial of bevacizumab for primary treatment of ovarian cancer. J Clin Oncol 2019;37(26):2317–2328
21 Kotsopoulos J, Rosen B, Fan I, et al. Ten-year survival after epithelial ovarian cancer is not associated with BRCA mutation status. Gynecol Oncol 2016;140(01):42–47

"PALLCARE Seva"—A Beacon Amid the Catastrophic COVID-19 Times: A Cross-Sectional Study from a Rural Oncology Institute in Western Maharashtra

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Abstract

Introduction Advanced cancer patients often require clinic or hospital follow-up for their symptom control to maintain their quality of life. But it becomes difficult for the patients to attend the same due to financial, commutation, and logistic issues.

Objective The aim of this study was to audit the telephonic calls of the service and prospectively collected data to understand the quality of service provided to the patients at follow-up.

Materials and Methods An ambispective observational study was conducted on the advanced stage cancer patients referred to the palliative care department at Kolhapur Cancer Center, Kolhapur, Maharashtra. We conducted an audit of the 523 telephonic calls of our service—"PALLCARE Seva" from June 2020 to February 2021. Prospectively, we assessed the quality of service based on 125 telephonic calls ($n = 125$) for this; we designed a questionnaire consisting of 11 items on the 5-point Likert scale for satisfaction by the patients or their caregivers at the follow-up. After a pilot study, the final format of questionnaire was used to collect the data.

Results Of the 523 calls attended, we provided 30.11% patients with dosage change of medications for their symptom management, 16.25% patients have liaised with local general practitioners, and 14.34% of cases had to be referred for emergency management to our hospitals. We provided 23.9% of them with emotional and bereavement support and 6.21% with smartphone-based or video-assisted guidance to the patients and caregivers.

Conclusions Liaison of general practitioners was possible in more than one-tenth of cases. The core components of our service were politeness and caring attitude,

Keywords
- COVID-19
- palliative care
- audit
- quality of care

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helpfulness, handling doubts regarding the illness, and an opportunity to share thoughts from the patients or caregivers. More than three-fourth of the callers have rated their experience as satisfactory and would recommend this service to other patients in need.

Introduction

Within a short period, the pandemic of coronavirus disease 2019 (COVID-19) emerged to become a public health emergency of international concern.[1] As with other healthcare services, routine oncological practices were also affected.[2] Cancer patients per se have a higher risk of contracting COVID-19 infection and are associated with a poorer prognosis if contracted.[3–6] Delay in cancer resection surgeries, neoadjuvant therapy, radiation therapy, and palliative care services had started affecting the quality of life of the cancer patients.[7,8] With the progression of the pandemic, an urgent need for modification in the routine practice was required. The government of India suggested telemedicine as the mode to provide equitable care to the patients.[9] But, there were some practical challenges like the lack of interaction between doctor and patient, accessibility issues, regional effects, care delays, technical issues, and licensing issues associated with it.[10–13] Amid all these challenges, it was important that patient-centered services had to be provided. So, we started a telemedicine support service called "PALLCARE Seva" for our patients.

With regard to this pandemic situation, a quality palliative care service is the one that can maintain the continuum of care throughout the illness trajectory of the cancer patient.[14–16] Most advanced cancer patients prefer to be taken care of and die at home with family members. Advanced cancer patients often require clinic or hospital follow-up for their symptom control to maintain their quality of life.[17,18] But, it becomes difficult for the patients to attend the same due to financial, and logistic issues.[19–21] With this background, we conducted this study intending to audit the telephonic calls of the service and prospectively collected data to understand the quality of service provided to the patients during the follow-up.

Materials and Methods

An ambispective observational study was conducted on the advanced stage cancer patients referred to the palliative care department at Kolhapur Cancer Center, Kolhapur, Maharashtra. It was a retrospective audit of our service "PALLCARE Seva." PALLCARE Seva is a proactive telephonic follow-up and consultation service. The patients who consulted the palliative care physician during the week (Monday to Friday) were called on Saturday by trained personnel to inquire about the improvement in symptoms, new symptoms, and any other issues. This telephonic service line was kept open from 9 AM to 6 PM on all days except for Sundays. This available line served as an assistant to the patients if they needed any help. A specialist palliative care physician supervised the whole program. Those patients who needed care were requested to involve their local physicians for any assistance. We also provided telephonic guidance for local physicians. The main motto was to provide "right care at the right time." Primary outcome measures were demographic distribution of our calls and quality of service.

An audit of the telephonic calls is from June 2020 to February 2021 was conducted. We noted age, gender, area of residence, type of calls, the caller's relationship with the patient, and the site of cancer. In addition, we noted services like dosage change, medication-related queries, emergency referrals, emotional support, bereavement support, liaison with general practitioners, and smartphone-assisted guidance. Prospectively, we assessed the quality of service. For evaluating the quality of service we provided, we designed a questionnaire consisting of 11 items (Annexure 1). The items were related to helpfulness, politeness and caring attitude, handling doubts and information regarding the illness, patient listening to personnel, opportunity to share patient's thoughts, involvement in decision making, explanation of care, explanation about medication, and circumstances seeking emergency help and its mode. The last two items were related to their overall satisfaction level of the service and recommendation of this service to other patients. All these questions were rated based on the "5-point Likert scale" for satisfaction by the patients or their caregivers at the follow-up. This questionnaire was designed by experts in palliative care and quality. Originally written in English, this questionnaire was converted to Marathi (local language) by a team of expert translators. It was back-translated and tested on ten caregivers, and we did final modifications.

Our study population consisted of advanced stage cancer patients seeking palliative care and patients referred for pain and symptom management by various departments within the institute. Of the total 630 calls done throughout 8 months, we excluded 107. The main reasons for exclusion were connection-related issues and nonreceiving of calls from the recipient. Hence, the final sample size included in this study consisted of 523 calls. Assuming the overall quality of service to be good in 50% of the feedbacks, with 10% absolute error and 95% confidence interval, we found the minimum sample size to be 96. Considering the attrition rate of 30%, we found the final sample size to be 124. But we collected 125 feedbacks about this service. For analysis, the 5-point Likert scale was categorized into unsatisfactory, satisfactory, and neutral. All the data collection and phone calls were done by trained personnel under the supervision of the palliative care physicians.

To reduce the recall bias during the feedback, the interval between the last call and follow-up was kept to a minimum. Since the prospective feedback was collected from the caregiver or the patient in random selection, the selection bias was reduced to the minimum possible extent.

Statistical Analysis

The data was collected and compiled using Microsoft Excel. The analysis was done using Epi info (Version 7.2; CDC, Atlanta, Georgia, United States). The qualitative variables were expressed in terms of frequencies and percentages.

Ethics

For the prospective quality feedback of the service, we took a written informed consent form from the person who responded (either the caregiver or the patient). We sought permission of the ethical committee of Kolhapur Cancer Center before the start of the study (ECR/523/ Inst/ MH/ 2014/ RR-20).

We ensured the anonymity of the participants with their due permission.

Results

We have included 523 telephonic calls in this study.

The majority of the callers were in the age group of 40 to 60 years and of the male gender. Since the center caters to majority of rural areas of the district, 53.15% were from rural areas. About 72.28% of the cases were outgoing calls. Head, face, and neck cancers were the most common cancers reported (►**Table 1**).

Of the 523 calls attended, we provided 30.11% of patients ($n = 157$) with dosage change of medications for their symptom management, 16.25% ($n = 85$) have liaised with local general practitioners, and 14.34% ($n = 75$) of cases had to be referred for emergency management to our hospitals We provided 10.99% ($n = 57$) of them with emotional support. We offered 12.91% ($n = 68$) of them with bereavement support, 11.95% ($n = 62$) with new medication for newly arising symptoms or some interventions were advised, and provided 6.21% ($n = 32$) of these cases with smartphone-based or video-assisted guidance (►**Fig. 1**).

Politeness and caring attitude (91.20%), helpfulness (88.00%), handling doubts and information regarding the illness (88.00%), and opportunity to share thoughts (88%) were the most satisfying elements of our service and personnel (►**Table 2**).

About 77.60% ($n = 97$) of the patient's or caregiver's feedback was satisfactory and up to the mark (►**Fig. 2**).

About 88.00% ($n = 110$) of the patients would recommend the service to other patients who require similar type of assistance (►**Fig. 3**).

Discussion

Telemedicine has become an essential component of the present oncology care worldwide due to the effect of the COVID-19 pandemic.[11] This "no contact" approach had to be

Table 1 Demographic characteristics of the patients ($n = 523$)

Demographic characteristics	Frequency	Percentage
Age group		
< 20	16	3.05
21 to 40	105	20.00
40 to 60	212	40.53
> 60	190	36.33
Gender		
Female	203	38.88
Male	320	61.21
Relationship with the patient		
Brother/sister	144	27.53
Parents	16	3.05
Children	129	24.67
Spouse	234	44.75
Area of residence		
Rural	278	53.15
Urban	245	46.85
Type of call		
Incoming	145	27.72
Outgoing	378	72.28
Site of cancer		
Head, face and Neck	276	52.77
Thorax	42	8.03
Gastrointestinal	101	19.31
Genitourinary	62	11.85
Others	42	8.03

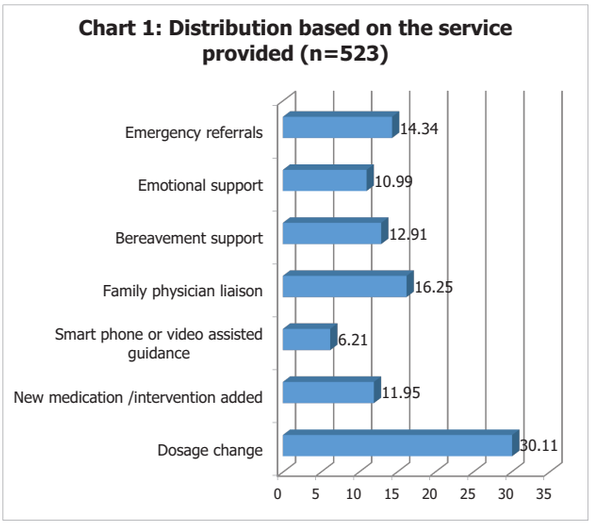

Fig. 1 Distribution based on the service provided ($n = 523$).

Table 2 Prospective feedback of the patient or caregiver about the service provided ($n = 125$)

Questions	Unsatisfactory		Neutral		Satisfactory	
	Number	%	Number	%	Number	%
1. Helpfulness	4	3.20	11	8.80	110	88.00
2. Politeness and caring attitude	3	2.40	8	6.40	114	91.20
3. Handling doubts and information regarding illness	5	4.00	10	8.00	110	88.00
4. Patient listening	6	4.80	12	9.60	107	85.60
5. Opportunity to share thoughts	6	4.80	9	7.20	110	88.00
6. Involvement in decision making process	6	4.80	11	8.80	108	86.40
7. Explanation of care	5	4.00	32	25.60	88	70.40
8. Explanation about medication	5	4.00	26	20.80	94	75.20
9. Explanation about circumstances seeking help and its mode	4	3.20	14	11.20	107	85.60

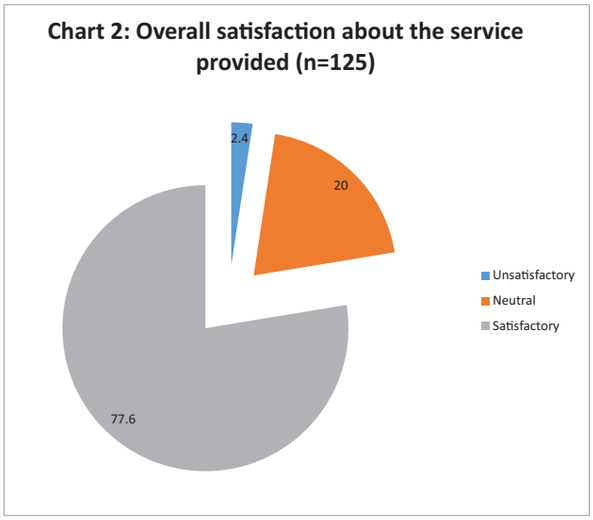

Fig. 2 Overall satisfaction about the service provided ($n = 125$) (%).

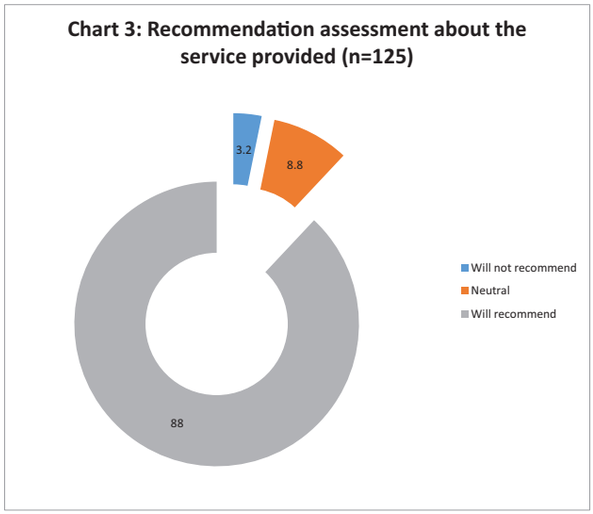

Fig. 3 Recommendation assessment about the service provided ($n = 125$) (%).

inculcated into the guidelines of routine practice in oncological care to break the barrier of transmission of this deadly infection.[12] With this view, we decided to maintain the quality of care for our advanced stage cancer patients to the next level with the help of consultations and started the same over the phone. The basic principles of a palliative care support helpline are direct communication between palliative care physicians with the family; staffing expansion along with training; call line supporting clinicians, families, and the patients; monitoring of call volume throughout the week; and being an ideal choice for crisis response. We started our service with these basic principles, but, unfortunately, due to limited resources, we opened the call line only during the outpatient consultation hours (2 PM to 6 PM). Based on the interim audit of these calls (2 months), we decided to open this line from 9 AM to 6 PM with adequate staff training and tele-help support for any crisis.

Change of dosage of the medications for pain and other symptom management was most commonly encountered over these telephonic calls. The results of our study were similar to a survey conducted by Adhikari et al.[22] This study unfolded their experience of telemedicine consultations of their institution. Majority of the calls (63.62%) were related to uncontrolled symptoms experienced by the patients. Similar study findings have been reported by Jiang et al[23] and Plummer and Allan.[24] Due to the ongoing COVID-19 pandemic, patients could not visit in person for any minor symptom management. So, this service helped the advanced stage cancer patients in this regard.

In 16.25% of the cases, we could liaise with the family physicians and handle the patient's issues. These findings were similar to the integrated model postulated by Atreya et al[25] in their study. Despite the pandemic situation, these family physicians worked relentlessly to assist in caring for these patients. Starting from management of minor ailments

with injectable medication to terminal care of these patients with appropriate help by the specialist palliative care physician over the phone. The family physicians were vigilant in managing the symptoms, and in case of crisis, they did emergency referrals to our institute for further management. Emergency referral cases usually involved uncontrolled severe pain despite providing essential medications. These cases also suffer from spinal cord compression, intestinal obstruction, abnormalities in levels of electrolytes, and convulsions. It is imperative to integrate these family physicians into the training of palliative care to strengthen this service in the future. One of the crucial recommendations is the joint statement of the Indian Association of Palliative Care and the Academy of Family Physicians of India to integrate palliative care at all levels of healthcare and liaise with general practitioners and family physicians.[26]

Bereavement support is one of the crucial components of palliative care service. In approximately 12.95% of cases, we provided this support over the phone. In cases with crises such as death of patients causing grief in caregivers or a medical condition hindering the caregiver's health, we offered the best possible support through the specialists over the phone. A retrospective study conducted on the out-of-hours phone calls by Jiang et al[23] reported that 17.8% cases mentioned distress due to the death of the patient over telephonic support and here bereavement support was provided. Emotional support to the patients and their caregivers was provided by letting them ventilate and express their concerns in 10.99% of cases. Mandal [27] described that their telephonic service provided had advantages of providing regular physical and emotional support, reducing problems regarding finance and human resources, and thus improving their quality of life. Jiang et al[23] reported that in 3.5% of their patients, they could provide emotional support over the phone.

As a part of clinical governance, we took feedback from a small sample ($n = 125$) of the patient or caregivers who visited us during follow-ups. We found that politeness and caring attitude (91.20%), helpfulness (88.00%), handling doubts and information regarding the illness (88.00%), and opportunity to share thoughts (88%) were the most satisfying elements of our service and service personnel. Further, approximately 77.40% of the patients were satisfied with the service provided, and 88% reported that they would recommend it to other patients in need. Similar feedbacks were taken by Adhikari et al[22] in their study and found that more than 70% of their patients were satisfactory about the telehealth service provided. Compared with their research, we used video consultations (6.12%) in a minor set of patients due to logistic issues. Also, Plummer and Allan[24] reported that 66% of patients claimed to have a high satisfaction rate.

This study had some limitations. First, even though it was a retrospective study, we took the necessary feedback through follow-ups prospectively in our research. Due to issues regarding logistics and loss of follow-up of the patients, we could only obtain and analyze a subset of the original sample size. The second limitation was that the study design was of observational type. Feedback, complaint redressal, and feedback mechanisms must be set as a quality control check in such services. Future studies should target integrating general practitioners and family physicians into palliative care, education of informal caregivers about the management of minor ailments, etc. The cost-effectiveness of such services has to be evaluated in the future.

Conclusion

Change in dosage and addition of new medication were the most common issues handled over the phone. Liaison of general practitioners was possible in more than one-tenth of cases. We successfully provided both bereavement and emotional support for the patients and their caregivers whenever appropriate. We could also use newer technologies such as video consultation and smartphone-based assistance for patient care in a few cases. The core components of our service were politeness and caring attitude, helpfulness, handling doubts regarding the illness, and an opportunity to share thoughts with the patients or caregivers. More than three-fourth of the patients were satisfactory and would recommend this service to other patients in need.

Conflict of Interest
None declared.

References

1 Wilder-Smith A, Osman S. Public health emergencies of international concern: a historic overview. J Travel Med 2020;27(08): taaa227
2 Kumar A, Rajasekharan Nayar K, Koya SF. COVID-19: challenges and its consequences for rural health care in India. Public Health Pract (Oxf) 2020;1:100009
3 ElGohary GM, Hashmi S, Styczynski J, et al. The risk and prognosis of COVID-19 infection in cancer patients: a systematic review and meta-analysis. Hematol Oncol Stem Cell Ther 2020:S1658-3876 (20)30122-9
4 Curigliano G. Cancer patients and risk of mortality for COVID-19. Cancer Cell 2020;38(02):161–163
5 Al-Quteimat OM, Amer AM. The impact of the COVID-19 pandemic on cancer patients. Am J Clin Oncol 2020;43(06):452–455
6 Yang L, Chai P, Yu J, Fan X. Effects of cancer on patients with COVID-19: a systematic review and meta-analysis of 63,019 participants. Cancer Biol Med 2021;18(01):298–307
7 Gosain R, Abdou Y, Singh A, Rana N, Puzanov I, Ernstoff MS. COVID-19 and cancer: a comprehensive review. Curr Oncol Rep 2020;22(05):53
8 Palka-Kotlowska M, Custodio-Cabello S, Oliveros-Acebes E, Khosravi-Shahi P, Cabezón-Gutierrez L. Review of risk of COVID-19 in cancer patients and their cohabitants. Int J Infect Dis 2021; 105:15–20
9 Mohfw. gov [Internet].. Telemedicine Practice Guidelines. [Updated Apr 2020; Cited Jan 2021]. Accessed July 12, 2022 from: https://www.mohfw.gov.in/pdf/Telemedicine.pdf
10 Kichloo A, Albosta M, Dettloff K, et al. Telemedicine, the current COVID-19 pandemic and the future: a narrative review and perspectives moving forward in the USA. Fam Med Community Health 2020;8(03):e000530
11 Scott Kruse C, Karem P, Shifflett K, Vegi L, Ravi K, Brooks M. Evaluating barriers to adopting telemedicine worldwide: a systematic review. J Telemed Telecare 2018;24(01):4–12
12 Giani E, Laffel L. Opportunities and challenges of telemedicine: observations from the wild west in pediatric type 1 diabetes. Diabetes Technol Ther 2016;18(01):1–3

13 Seto E, Smith D, Jacques M, Morita PP. Opportunities and challenges of telehealth in remote communities: case study of the Yukon telehealth system. JMIR Med Inform 2019;7(04): e11353–e11353
14 Al-Mahrezi A, Al-Mandhari Z. Palliative care: time for action. Oman Med J 2016;31(03):161–163
15 Cruz-Oliver DM. Palliative care: an update. Mo Med 2017;114 (02):110–115
16 García-Baquero Merino MT. Palliative care: taking the long view. Front Pharmacol 2018;9:1140
17 Jabbarian LJ, Maciejewski RC, Maciejewski PK, et al. The stability of treatment preferences among patients with advanced cancer. J Pain Symptom Manage 2019;57(06):1071–1079.e1
18 Umezawa S, Fujimori M, Matsushima E, Kinoshita H, Uchitomi Y. Preferences of advanced cancer patients for communication on anticancer treatment cessation and the transition to palliative care. Cancer 2015;121(23):4240–4249
19 Pai RR, Nayak MG, Sangeetha N. Palliative care challenges and strategies for the management amid COVID-19 pandemic in India: perspectives of palliative care nurses, cancer patients, and caregivers. Indian J Palliat Care 2020;26(Suppl 1): S121–S125
20 Abu-Odah H, Molassiotis A, Liu J. Challenges on the provision of palliative care for patients with cancer in low- and middle-income countries: a systematic review of reviews. BMC Palliat Care 2020;19(01):55
21 Woo JA, Maytal G, Stern TA. Clinical challenges to the delivery of end-of-life care. Prim Care Companion J Clin Psychiatry 2006;8 (06):367–372
22 Adhikari SD, Biswas S, Mishra S, et al. Telemedicine as an acceptable model of care in advanced stage cancer patients in the era of coronavirus disease 2019 - an observational study in a tertiary care centre. Indian J Palliat Care 2021;27(02):306–312
23 Jiang Y, Gentry AL, Pusateri M, Courtney KL. A descriptive, retrospective study of after-hours calls in hospice and palliative care. J Hosp Palliat Nurs 2012;14(05):343–350
24 Plummer S, Allan R. Analysis of a network-wide specialist palliative care out-of-hours advice and support line: a model for the future. Int J Palliat Nurs 2011;17(10):494–499
25 Atreya S, Patil C, Kumar R. Integrated primary palliative care model; facilitators and challenges of primary care/family physicians providing community-based palliative care. J Family Med Prim Care 2019;8(09):2877–2881
26 Jeba J, Atreya S, Chakraborty S, et al. Joint position statement Indian Association of Palliative Care and Academy of Family Physicians of India - the way forward for developing community-based palliative care program throughout India: policy, education, and service delivery considerations. J Family Med Prim Care 2018;7(02):291–302
27 Mandal N. Telephonic communication in palliative care for better management of terminal cancer patients in rural India: an NGO-based approach. Ann Oncol 2019;30(November):ix144–ix145

'PALLCARE Seva'-A pharos amidst the catastrophic COVID times: An cross sectional study from rural oncology institute in Western Maharashtra Confidential

Audit: (To be filled by the in charge person of the calls)

Patient's details:
Age:
Gender: Male/Female
Residence:
Phone number:
Type of calls: incoming/outgoing
Caller's relationship with the patients:
Site of cancer:

Quality of service provided by us during telephonic consultation (Patient/ caregiver feedback)

Marathi version of the questions for feedback of the service:

अनु क्रं	प्रश्नाचे स्वरूप	अति उत्तम	उत्तम	चांगले	ठीक	सुधारण्याची गरज	सल्ला / मत
१	जेव्हा आपण फोनवर बोलला तेव्हा ज्या व्यक्तीशी आपण बोलला, ती उपयुक्त होती का?						
२	आपल्याशी बोलणारी व्यक्ती (कर्मचारी / डॉक्टर) यांची भाषा सभ्य आणि काळजी युक्त होती का?						
३	आमच्या तज्ज्ञ डॉक्टर्स / स्टाफ यांनी आपल्या आजारासंदर्भात संपूर्ण शंका व माहिती काळजीपूर्वक एकूण घेलती असे वाटते का?						
४	आमच्या तज्ज्ञ डॉक्टर्स / स्टाफ यांनी तुम्हाला एक रुग्ण / किंवा काळजी घेणारी व्यक्ती म्हणून आपल्या गरजा ऐकल्या असे तुम्हाला वाटते का?						
५	आपणास असे वाटले आहे का की आमच्या तज्ज्ञ डॉक्टर्स / स्टाफ ने आपले विचार किंवा काळजी / आपण ज्याची सेवा करत आहात त्या व्यक्तीस सामायिक करण्याची संधी दिली आहे का?						
६	आमच्या तज्ज्ञ डॉक्टर्स / स्टाफ ने आपल्या उपचारासंदर्भात घेतलेल्या निर्णयामध्ये तुम्हाला सामील केले होते / आहे का?						
७	आमच्या तज्ज्ञ डॉक्टर्स / स्टाफ ने तुमच्या घरी घ्यावयाची काळजी हे समजावून सांगितले आहे का?						
८	आमच्या तज्ज्ञ डॉक्टर्स / स्टाफ ने तुम्हाला औषधोपचार व डॉक्टर्सना परत कधी भेटायचे हे समजावून सांगितले आहे का?						
९	आमच्या तज्ज्ञ डॉक्टर्स / स्टाफ ने तुम्हाला केव्हा व कोणत्या परिस्थितीमध्ये त्वरित आरोग्य सेवेची मदत घ्यावी याबद्दल सल्ला दिला होता का?						
१०	आरोग्य सेवा पुरवण्याच्या आमच्या टेलिफोनिक सेवेबद्दल आपण समाधानी आहात का?						
११	आपणास असे वाटते का, की आणखी काही माहिती उपयुक्त ठरली असती?						

Congenital Epulis

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Congenital epulis is a benign tumor that occurs in newborns. It was first described by Neumann in 1871; hence, it came to be known as Neumann's tumor. It is also known as granular cell rhabdomyoma, congenital myoblastoma, or congenital granular cell tumor.[1] The female infant is usually affected but has no familial tendency. It not only arises from alveolar median ridge of the maxilla and mandible but can also originate from tongue, palate, skin, etc. Therefore, the etiology and cell of origin of this tumor are still not clear.[1,2] It is hypothesized that this tumor arises from several sources: undifferentiated mesenchymal cells, odontogenic epithelial cells, neuroendocrine progenitor cells, pericytes, histiocytes, fibroblasts, or myofibroblast. The steroidal hormones were believed to play a role but this was disproved due to the absence of estrogen and progesterone receptors in the tumor tissue.[3]

A newborn female presented with a large protruding mass in the mouth causing difficulty in breathing and breastfeeding (►**Fig. 1)**. A probable diagnosis of congenital epulis was made based on clinical examination. Surgical excision of the mass was performed under general anesthesia (►**Fig. 2)**. Postoperative period was uneventful. The specimen was sent for histopathological examination. On gross examination, it consists of single mass covered by gray-brown, firm mucosa with the dimensions of $2.8 \times 2 \times 1\ cm^3$ (►**Fig. 3)**. The outer surface was smooth. The cut section showed whitish and hemorrhagic areas. The nearest resected margin was found to be 0.8 cm.

Microscopy revealed large round-to-oval cells with granular eosinophilic cytoplasm and small eccentric, bland nuclei. There was presence of thin fibrovascular network separating the cells. Necrosis and mitosis were not evident. The overlying epithelium was thin and pseudoepitheliomatous hyperplasia was not evident (►**Fig. 4)**. All the resected margins were not involved by tumor. On the basis of these findings, the final diagnosis of congenital granular cell tumor was made. Immunohistochemistry (IHC) was not performed as the microscopy findings were characteristic.

It is a rare non-neoplastic soft tissue lesion. Prenatal diagnosis can be done by ultrasonography mainly in the last week of pregnancy.[4] This can help in counseling the parents about the possible complications and treatment of the tumor mass. It should be removed postnatally if the size of the mass is big as it can cause difficulty in breathing and feeding.[2,4]

Surgical excision is the only treatment, though spontaneous regression has also been observed in a few cases. It does not have malignant potential and recurrence rate.[4]

It presents as single polypoidal protruding mass covered by smooth mucosal surface and firmly attached to the gum by a broad base. The histopathology findings are characteristic. On IHC, the cells are positive for vimentin and negative for S-100 unlike in adults.[1,5]

It is important to diagnose it both prenatally and postnatally. This will help the oncologist involved in planning the possible intervention and thereby decreasing the morbidity and mortality. The parents can also understand the nature of the mass by consulting the oncologist.

Conflict of Interest
None declared.

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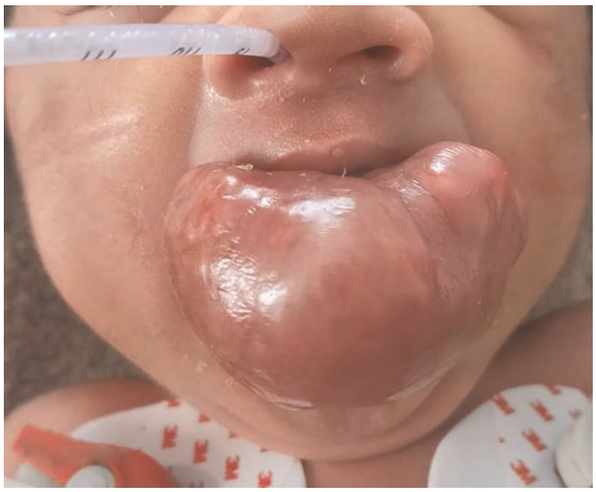

Fig. 1 Pre operative image.

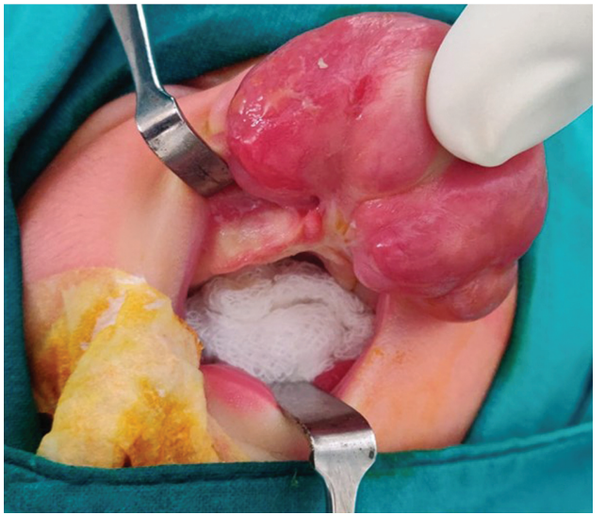

Fig. 2 Intra operative Image.

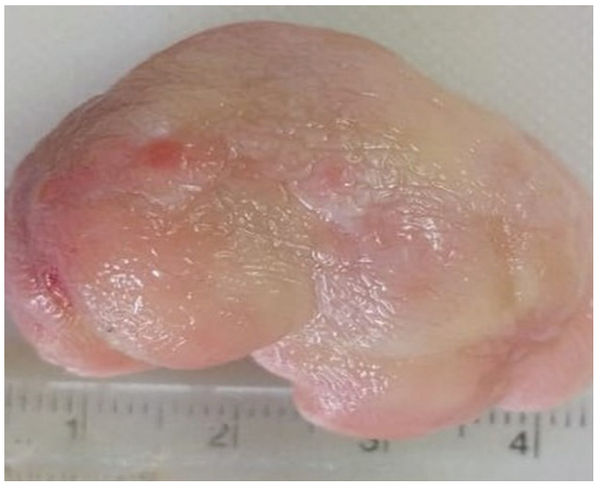

Fig. 3 Gross specimen.

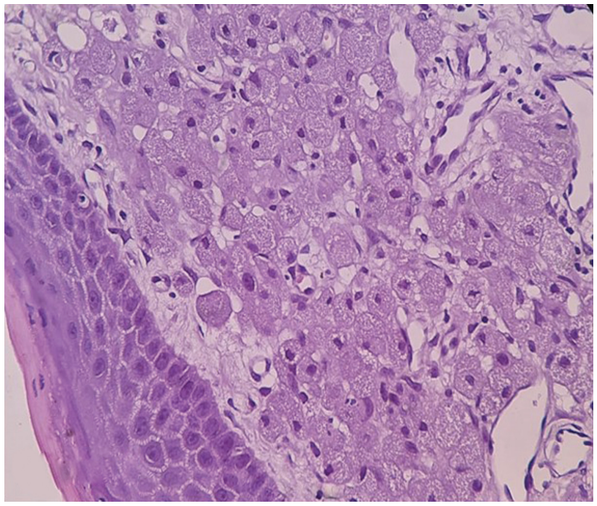

Fig. 4 H&E 40X, showing large round to oval cells with granular eosinophilic cytoplasm and small eccentric bland nuclei.

References

1 Lapid O, Shaco-Levy R, Krieger Y, Kachko L, Sagi A. Congenital epulis. Pediatrics 2001;107(02):E22
2 Chattopadhyay A, Patra R, kumar Vijay. Oral tumors in newborn. Indian J Pediatr 2003;70(07):587–588
3 Conrad R, Perez MC. Congenital granular cell epulis. Arch Pathol Lab Med 2014;138(01):128–131
4 Williams RW, Grave B, Stewart M, Heggie AA. Prenatal and postnatal management of congenital granular cell tumours: a case report. Br J Oral Maxillofac Surg 2009;47(01):56–58
5 Kumar RM, Bavle RM, Umashankar DN, Sharma R. Congenital epulis of the newborn. J Oral Maxillofac Pathol 2015;19(03):407

The True Human Cost of the Novel Coronavirus 2019 (COVID-19) Pandemic

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As of 6th June 2021, 3:00 PM, there were 174,893,444 worldwide reported cases of the novel coronavirus disease 2019 (COVID-19), with 3,774,138 deaths attributed to this pandemic.[1,2] Infection fatality rate (IFR) i.e., the fraction of deaths reported from all laboratory-confirmed cases for the disease, for COVID-19 has thus far been estimated in a broad range from 0.5 to 1.0%.[3,4] The IFR for any disease is influenced by the accuracy of two metrics: the death reporting mechanism and its attribution to the disease, and testing every possible individual who may have contracted the disease.

Both COVID-19 death reporting and testing are contentious issues, with many countries suspected to be underreporting deaths because of errors of omission and/or commission.[5] This is because, in almost all countries, deaths outside the hospital system are inaccurately reported. Many individuals who die from COVID-19-like symptoms do not get a laboratory confirmatory test. Moreover, asymptomatic individuals constitute a large proportion of COVID-19 cases,[6] accounting for as much as 40 to 45% of total SARS-CoV2 infections.[7,8] Many countries with low resources or with limited testing availability do not even test mildly symptomatic individuals due to scarce availability of RT-PCR testing,[9,10] leading to weak attributable death reporting systems. Thus, estimating IFR accurately is extremely challenging.

So just how many people have died of COVID-19 is a question with huge ramifications for public health policymaking.[11,12] Unfortunately, given the times we live in, this is also a question fraught with political overtones, whose answer depending on whom you ask, range from a benign infection no deadlier than the common seasonal influenza to the deadliest disease since the black plague. As always, the truth lies somewhere in between. Recent newspaper and magazine articles in The New York Times (NYT)[13] and The Economist[14] estimating excess COVID-19 deaths for the period March 2020 to May 2021 have received widespread media coverage. However, both are deficient in scientific rigor. The NYT analysis is a simple linear regression model of varying ranges of COVID-19 mortality as extrapolated from hypothesized rates of IFR, one conservative (496,000 excess deaths in India), and the other, an extreme (4.5 million excess deaths in India), with a mid-point spread estimate (2.1 million excess deaths).[15] The Economist analysis, in contrast, while using the same method as that of NYT at its core, also uses certain assumptions to fit its model, using a machine learning methodology,[16] many of which simply do not hold true in the real world. The analysis suggests a point estimate spread of 7 million (conservative scenario) to 13 million (worst case scenario) excess COVID-19 deaths worldwide, 1 million of these from India alone (worst case scenario).[17]

One underreported analysis that has received lesser coverage but is perhaps one of the most credible, is by the Institute for Health Metrics and Evaluation (IHME), at the University of Washington.[18] IHME has perfected and implemented a long-standing methodology for measuring the burden of diseases on a global scale since 1990 through its *Global Burden of Disease* (GBD) study.[19] The GBD estimates worldwide deaths annually due to aging, communicable

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diseases, non-communicable diseases (including psychiatric disorders), and accidental deaths (traffic, personal injuries, occupational etc.). This is thus far the most comprehensive modeling of all-cause mortality worldwide.

In its special COVID-19 mortality analysis, IHME has used the data from its annual GBD modeling methodology from the period of March 1, 2020, to May 13, 2021, to estimate and predict the global burden of deaths specifically due to COVID-19 during this period. Their methodology is broadly as follows:

1) Using their GBD models, they first estimated the expected all-cause mortality in the period March 1, 2020, to May 13, 2021, based on pre-pandemic deaths, i.e., how many people would have died in this period if there was no COVID-19 pandemic.
2) They then collected the total deaths that were actually reported by countries during this period from all-cause mortality.
3) This was subtracted from the total expected deaths in this period in the absence of the COVID-19 pandemic.
4) The excess mortality thus arrived at was further modeled to identify variables including a lag in death reporting, decrease in mortality due to COVID-19, and increase in mortality directly and indirectly due to COVID-19.

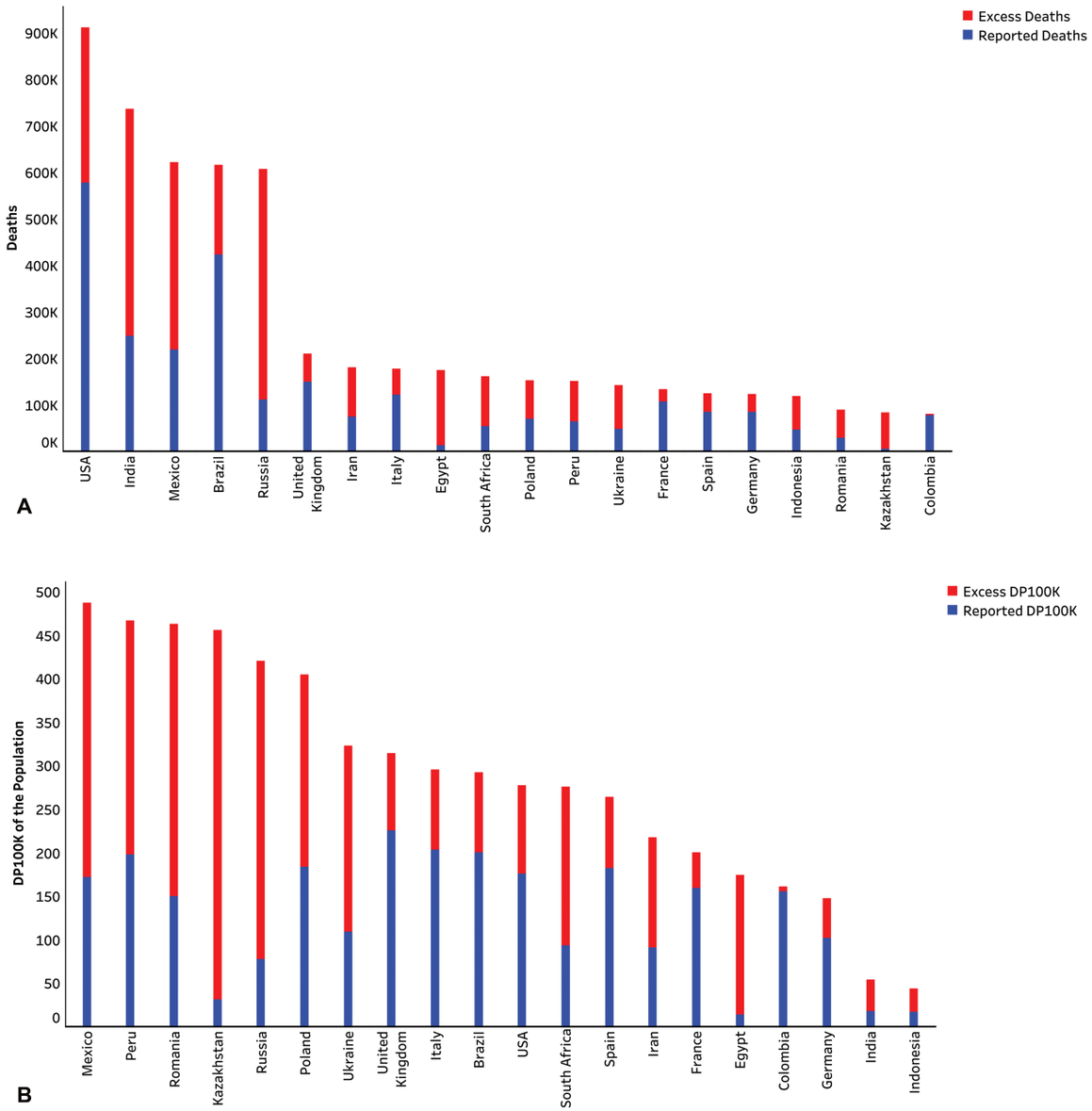

Fig. 1 (A) Blue bar indicates absolute number of reported deaths due to COVID-19. Red bar represents the imputed excess deaths due to COVID-19 in the studied time period. Red and blue bars together constitute cumulative deaths due to COVID-19 in the studied time period for that country (X-axis). (**B**) Blue bar indicates reported deaths per 100,000 of the population (DP100K) due to COVID-19. Red bar represents the imputed deaths per 100,000 of the population (DP100K) due to COVID-19 in the studied time period. Red and blue bars together constitute cumulative deaths per 100,000 of the population (DP100K) due to COVID-19 in the studied time period for that country (X-axis). DP100K: Deaths per 100,000 population.

Their final model thus was able to predict the following different causes of mortality

1) all deaths directly on account of a laboratory-confirmed COVID-19 infection
2) increase in mortality due to
   i. life-saving health care (including disease screening) being delayed or deferred during the pandemic.
   ii. mental health disorders (depression) due to COVID-19 isolation/lockdowns, loss of earnings, and resulting opioid use.
   iii. excessive alcoholism.
3) reduction in mortality due to:
   i. decreased mobility associated with social distancing mandates
   ii. reduced transmission of other viruses (influenza, respiratory syncytial virus, and measles)
   iii. individuals at immediate risk of dying from chronic conditions, such as cardiovascular disease and chronic respiratory disease, dying instead from COVID-19.

The IHME reports the largest number of cumulative deaths worldwide accounting for underreported deaths are from the USA (912,345), India (736,811), Mexico (621,962), Brazil (616,914), and Russia (607,589) (►**Fig. 1A**). This is not surprising because these countries have had the largest epidemics to date. Interestingly, but not unexpectedly, some countries with relatively smaller epidemics saw a large increase in the death rate when accounting for unreported deaths, most of these in South America, Central Asia, and Eastern Europe (►**Fig. 1B**). We divided the excess death count for each country (as estimated by the IHME), with the reported death counts for that country. The excess death ratio thus arrived at revealed that 12 countries had undercounted their deaths by at least as much as their total reported deaths, 6 countries had undercounted by close to half the total reported deaths, while 2 countries had undercounted by a low proportion (►**Table 1**). The IHME estimates the global COVID-19 death rate is 91.7 per 100,000. Vietnam has the lowest total COVID-19 death rate at 0.1 per 100,000, while a staggering 15 countries have total COVID-19 death rates per 100,000 (DP100K) higher than 200 (►**Table 1**, ►**Fig. 1B**). These findings, besides shedding light on the true human cost associated with this pandemic, also reveal an important detail: some countries have done exceedingly well at preventing deaths, while for unknown reasons that we can only speculate upon at this point of time, other countries have been less nimble.[12] Clearly, only data based public health interventions will help formulate policies that can tackle this pandemic to prevent further loss of life.

Table 1 Country-wise reported and excess deaths as per IHME estimates (columns 1–4) and estimated total deaths including per 100 thousand population as estimated by us based on data from the IHME analysis (columns 5–7)

Country	Reported COVID-19 deaths	Excess deaths	Total COVID-19 deaths	Reported COVID-19 deaths per 100K	Total COVID-19 deaths per 100K	Increase in deaths per 100K
Kazakhstan	5,810	78,643	84,453	31.39	456.16	424.77
Russia	111,909	495,680	607,589	77.52	420.85	343.33
Mexico	219,372	402,590	621,962	171.96	487.53	315.57
Romania	29,020	60,599	89,619	149.93	463	313.07
Peru	64,511	87,428	151,939	198.44	467.36	268.92
Poland	69,954	83,672	153,626	184.24	404.59	220.35
Ukraine	48,393	95,022	143,415	109.03	323.12	214.09
South Africa	54,746	106,758	161,504	93.49	275.81	182.32
Egypt	13,970	161,620	175,590	13.92	174.92	161
Iran	75,547	104,940	180,487	91.12	217.69	126.57
USA	578,555	333,790	912,345	176.27	277.96	101.69
Brazil	423,307	193,607	616,914	200.58	292.31	91.73
Italy	122,851	55,293	178,144	203.75	295.45	91.7
United Kingdom	150,815	59,261	210,076	225.66	314.33	88.67
Spain	85,822	38,627	124,449	182.31	264.36	82.05
Germany	84,807	38,170	122,977	102.02	147.93	45.91
France	106,874	27,526	134,400	159.38	200.42	41.04
India	248,016	488,795	736,811	18.16	53.93	35.77
Indonesia	47,150	71,646	118,796	17.43	43.9	26.47
Colombia	78,216	2,752	80,968	155.38	160.85	5.47

Statement of Integrity

The manuscript has been read and approved by all authors and the requirements for authorship have been met, and that each author believes that the manuscript represents honest work.

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Conflict of Interest

None declared.

References

1 GitHub repository for Coronavirus COVID-19 global cases by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins. Updated 09 February 2021. Accessed January 22, 2021 at: https://github.com/CSSEGISandData/COVID-19
2 Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis 2020;20(05): 533–534
3 Pastor-Barriuso R, Pérez-Gómez B, Hernán MA, et al; ENE-COVID Study Group. Infection fatality risk for SARS-CoV-2 in community dwelling population of Spain: nationwide seroepidemiological study. BMJ 2020;371:m4509. Doi: 10.1136/bmj.m4509
4 Perez-Saez J, Lauer SA, Kaiser L, et al; Serocov-POP Study Group. Serology-informed estimates of SARS-CoV-2 infection fatality risk in Geneva, Switzerland. Lancet Infect Dis 2021;21(04): e69–e70
5 Burn-Murdoch JRVaGC. Global coronavirus death toll could be 60% higher than reported. *Financial Times*. April 26, 2020. Accessed April 26, 2020 at: https://www.ft.com/content/6bd88b7d-3386-4543-b2e9-0d5c6fac846c
6 Ward H, Atchison C, Whitaker M, et al. SARS-CoV-2 antibody prevalence in England following the first peak of the pandemic. Nat Commun 2021;12(01):905. Doi: 10.1038/s41467-021-21237-w
7 Oran DP, Topol EJ. Prevalence of asymptomatic SARS-CoV-2 infection. Ann Intern Med 2021;174(02):286–287
8 Almadhi MA, Abdulrahman A, Sharaf SA, et al. The high prevalence of asymptomatic SARS-CoV-2 infection reveals the silent spread of COVID-19. Int J Infect Dis 2021;105:656–661
9 Chakraborty C, Ranjan Sharma A, Bhattacharya M, Lee SS, Agoramoorthy G. COVID-19 vaccine: challenges in developing countries and India's initiatives. Infez Med 2021;29(01):165–166
10 Anjum FR, Anam S, Rahman SU. Novel Coronavirus disease 2019 (COVID-19): new challenges and new responsibilities in developing countries. Hum Vaccin Immunother 2020;16(10):2370–2372
11 Xie Y, Bowe B, Maddukuri G, Al-Aly Z. Comparative evaluation of clinical manifestations and risk of death in patients admitted to hospital with COVID-19 and seasonal influenza: cohort study. BMJ 2020;371:m4677. Doi: 10.1136/bmj.m4677
12 Chaubal R, Kannan S, Khattry N, Gupta S. Worldwide case fatality ratio of COVID-19 over time. Preprint 2021
13 Jin Wu AM Josh Katz, Elian Peltier and Karan Deep Singh. The Pandemic's Hidden Toll: Half a Million Deaths. *The New York Times*. 09–02–2021. Accessed June 13, 2021 at: https://www.nytimes.com/interactive/2020/04/21/world/coronavirus-missing-deaths.html
14 Team TEDM. Counting the dead- Modelling covid-19's death toll: There have been 7m-13m excess deaths worldwide during the pandemic. The Economist. 15 May 2021 ed2021.https://www.economist.com/briefing/2021/05/15/there-have-been-7m-13m-excess-deaths-worldwide-during-the-pandemic
15 Glanz LGaJ. Just How Big Could India's True Covid Toll Be? *The New York Times*. 25–05–2021. Accessed June 13, 2021 at: https://www.nytimes.com/interactive/2021/05/25/world/asia/india-covid-death-estimates.html
16 Team TEDM. Covid-19 death tallies- How we estimated the true death toll of the pandemic: Dealing with potential outcomes, known unknowns, and uncertainty. The Economist. 13–05–2021 ed: The Economist.
17 Team TEDM. More evidence emerges of India's true death toll from covid-19. The Economist. 12–06–2021 ed: The Economist; 2021
18 IHME. Estimation of total mortality due to COVID-19. 13–06–2021, 2021. Updated 13–05–2021. Accessed June 13, 2021 at: http://www.healthdata.org/special-analysis/estimation-excess-mortality-due-covid-19-and-scalars-reported-covid-19-deaths
19 Diseases GBD, Injuries CGBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet 2020;396 (10258):1204–1222

Rare Association of Tuberous sclerosis with Acute Lymphoblastic Leukemia: Case Report with Review of Literature

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Abstract

Acute lymphoblastic leukemia (ALL) is the most common leukemia in children in which 85% of all cases are of B-cell ALL and approximately 15% cases are of T-cell ALL (T-ALL). Recent revolution in next-generation sequencing has uncovered many novel somatic mutations and rearrangements in ALL cells, which have prognostic and therapeutic implications, and it has also led to recognition of germline variants in the same genes with somatic mutations commonly associated with ALL. Apart from increasing the risk of developing ALL, germline variants may influence diagnostic testing, genetic counseling, and response to antileukemic treatment. This emphasizes importance of identification of new germline variants, or association of inherited syndromes with ALL or other malignancies. Down's syndrome, Shwachman's syndrome, Fanconi anemia, Bloom's syndrome, neurofibromatosis, and ataxia telangiectasia are well-recognized conditions associated with ALL. In this communication, we report a rare association of T-ALL with tuberous sclerosis (TS). This is the first reported case, showing association of T cell leukemia and TS with confirmatory genetic work-up.

Keywords

- acute lymphoblastic leukemia
- neurocutaneous syndromes
- tuberous sclerosis

Introduction

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy with peak incidence in children between 2 and 9 years of age.[1] Recent revolution in next-generation sequencing has uncovered many novel somatic mutations and rearrangements in ALL cells, which have prognostic and therapeutic implications.[2,3] Similarly, it has led to recognition of germline variants in the same genes (e.g., *PAX5*, *ETV6*, and *IKZF1*) with somatic mutations commonly associated with ALL (►**Table 1**).[4,5] Apart from increasing the risk of developing ALL, germline variants may influence diagnostic testing, genetic counseling, and response to antileukemic treatment. This emphasizes importance of identification of new germline variants, or association of inherited syndromes with ALL or other malignancies. Down's syndrome, Shwachman's syndrome, Fanconi anemia, Bloom's syndrome, neurofibromatosis, and ataxia telangiectasia are well-recognized conditions associated with ALL.[3] In this report, we describe novel association of T-cell ALL (T-ALL) with tuberous sclerosis (TS), also known as tuberous sclerosis complex (TSC). TSC is autosomal dominant neurocutaneous syndrome characterized by formation of benign hamartoma in various tissues including brain, heart, and kidneys. It is caused by mutations in tumor suppressor gene, either *TSC1* or *TSC2*.

Case Report

A 2-year-old girl presented with 2-week history of breathlessness, fever, and generalized swellings around neck and

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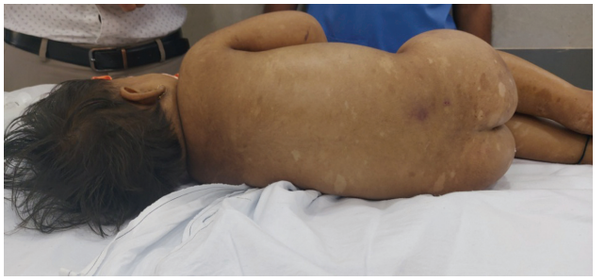

Fig. 1 Hypopigmented macules and shagreen patch on the back of patient.

axilla. On examination, child had dysmorphic facies, microcephaly, and found to have multiple hypomelanotic macules (>3, > 5 mm in diameter) and shagreen patch (►**Fig. 1**), fulfilling two major criteria for diagnosis of TSC. The clinical diagnostic criteria for diagnosis of TSC is shown in ►**Table 1**. She also had cervical lymph node enlargement and moderate hepatosplenomegaly.

On admission, laboratory investigations showed hemoglobin of 8.5 g/dL, platelet count of 9,000 cells/mm^3, and white blood cells of 130,000 cells/mm^3. Peripheral smear revealed a normocytic hypochromic anemia with 94% lymphoblasts. Bone marrow aspiration morphology and flow-cytometry studies showing T cell markers CD1a—0.1%, CD2—93.1%, CD3 (cyto)—98.3%, CD3 (surface)—98.1%, CD4—0.4%, CD5—86.3%, CD7—84%, CD8—47.9%, TCRab—0.1%, and TCRgd—78.5% and precursor markers showing CD34—23.8%, CD117—38.5%, TdT—12.3, and CD99—15.5% confirmed the diagnosis of T-ALL. Karyotype of lymphoblast was normal and fluorescence in situ hybridization for Philadelphia (Ph) chromosome, t(12:21), and MLL rearrangement tested negative. Chest radiograph revealed a mediastinal mass. Radiological studies did not reveal presence of hamartomas anywhere in the body.

Table 1 Clinical diagnostic criteria for TSC

Major features	Minor features
Hypomelanotic macules (≥3, at least 5-mm diameter)	"Confetti" skin lesions
Angiofibromas (≥3) or fibrous cephalic plaque	Dental enamel pits (>3)
Ungual fibromas (≥2)	Intraoral fibromas (≥2)
Shagreen patch	Retinal achromic patch
Multiple retinal hamartomas	Multiple renal cysts
Cortical dysplasias[a]	Nonrenal hamartomas
Subependymal nodules	
Subependymal giant cell astrocytoma	
Cardiac rhabdomyoma	
Lymphangioleiomyomatosis[b]	
Angiomyolipomas (≥2)[b]	

Abbreviation: TSC, tuberous sclerosis complex.
Notes: *Definite diagnosis*: Two major features or one major feature with ≥2 minor features. *Possible diagnosis*: Either one major feature or ≥2 minor features.
[a] Includes tubers and cerebral white matter radial migration lines.
[b] A combination of the two major clinical features (lymphangioleiomyomatosis [LAM] and angiomyolipomas) without other features does not meet criteria for a definite diagnosis.

Induction chemotherapy was started as per Indian Childhood Collaborative Leukemia protocol. Child showed good response to treatment and achieved remission. At this point, whole exome sequencing was carried out on peripheral blood which revealed heterozygous exon 41 c.5226_5252delinsACT mutation in *TSC2* gene on chromosome 16. The mutation is most likely germline mutation as patient was in morphological remission at the time of sampling. We could not do genetic testing on lymphoblasts and on parental samples due to financial constraints. Unfortunately, the child succumbed due to aspiration pneumonia with septic shock during further chemotherapy while leukemia was in remission.

Discussion

Neurocutaneous disorders, also described to as phacomatoses, are a group of congenital disorders with characteristic central nervous system and cutaneous manifestations presenting at different ages. These are rare diseases, with an incidence below 1:2,000 individuals in the general population. They include neurofibromatosis I and II, TSC, von Hippel–Lindau's disease, etc.[6] Owing to their genetic abnormality, they are highly susceptible to the development of tumors both benign and malignant.[7] TSC is a neurocutaneous genetic disorder with an autosomal dominant mode of inheritance and variable expressivity. Clinically cutaneous manifestations (hypopigmented macules, facial angiofibroma, shagreen patch) are most common; however, neurological (epilepsy, cognitive delay, autism) and renal (angiomyolipoma, renal cell carcinoma) manifestations are responsible for most of the morbidity and mortality associated with TSC.[8] De novo genetic mutations in *TSC1* and *TSC2* are known to occur in 65% of the cases. It has general prevalence of 1 in 6,000 to 10,000 newborns.[9] As far as genetic diagnostic criteria for TSC is concerned, the identification of either a TSC1 or TSC2 pathogenic mutation in DNA from normal tissue is sufficient to make a definite diagnosis of TSC. A pathogenic mutation is defined as a mutation that clearly inactivates the function of the TSC1 or TSC2 proteins (e.g., out-of-frame indel or nonsense mutation), prevents protein synthesis (e.g., large genomic deletion), or is a missense mutation whose effect on protein function has been established by functional assessment. Other TSC1 or TSC2 variants whose effect on function is less certain do not meet these criteria, and are not sufficient to make a definite diagnosis of TSC. It has been postulated that 10 to 25% of TSC patients have no mutation identified by conventional genetic testing, and a normal result does not exclude TSC, or have any effect on the use of clinical diagnostic criteria to diagnose TSC. *TSC1* gene, located on chromosome 9q34, and *TSC2* gene, located on chromosome 16p13.3, are tumor suppressor

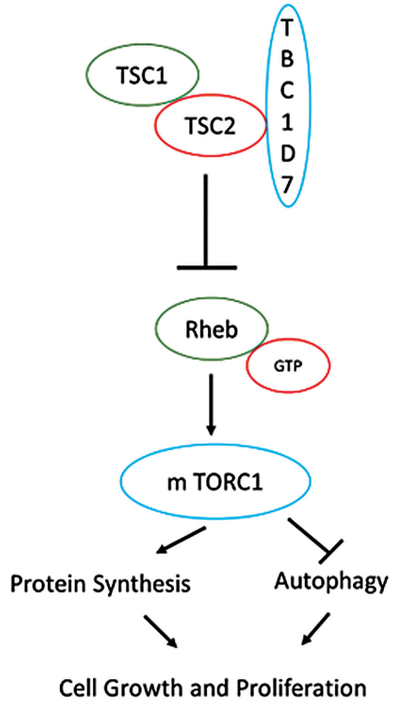

Fig. 2 Role of TSC1 and TSC2 in mechanistic target of rapamycin (mTOR) pathway and tumorigenesis.

genes and encode for proteins called hamartin and tuberin, respectively.[10,11] These two proteins bind each other and form a complex along with a third protein, TBC1D7 (tre2-bub2-cdc161 domain family, member 7). They play a major role in a key pathway in the cells regulating cellular homeostasis. A protein kinase called mechanistic target of rapamycin (mTOR) is involved in various cellular functions including growth, proliferation, and survival. mTOR, in turn, is controlled by Ras homolog enriched in brain (Rheb); when Rheb is activated, protein synthesis is turned on via mTOR signaling, and the cell grows. Both hamartin and tuberin are tumor suppressors, which inhibit mTOR pathway by keeping Rheb in an inactive state (►**Fig. 2**).[12] Inactivation or alterations in these proteins will cause disinhibition of Rheb, leading to uncontrolled activity of mTOR pathway. Thus, somatic mutations provide "second hit" in TSC patients leading to the development of characteristic tumors such as subependymal nodules, retinal hamartomas, angiomyolipomas, rhabdomyomas, intraoral fibromas, pancreatic neuroendocrine tumors, etc.[13] Patients with *TSC1* mutation are more prone for malignant tumor especially renal cell carcinoma compared with those with *TSC2* mutations or no mutation.[14]

Furthermore, mutations in several mTOR pathway component genes are known to cause specific monogenic human genetic diseases and this signaling cascade has been shown to be of relevance for Alzheimer's disease, type 2 diabetes, obesity, and hypertrophy.[15] Deregulation of these genes has also been demonstrated to be associated with sporadic bladder cancer, ovarian and gallbladder carcinoma, non-small cell carcinoma of the lung, breast cancer, pancreatic cancer, astrocytoma, xanthoastrocytoma, oral squamous cell carcinoma, and endometrial cancer.[16]

As discussed earlier, the association of TSC with tumors is well known, but hematopoietic malignancies are not commonly known to be associated with it. To our knowledge, this is the first case of association between TSC and a T-ALL. Occurrence of ALL in TSC by coincidence is less likely, as both the disorders are very rare. Alternatively, it is conceivable that there is causal relationship between TSC and T-ALL. Possibly, somatic mutation in *TSC1* or *TSC2* genes in hematopoietic stem cells may provide second hit, with germline mutation being first hit, to trigger leukemogenesis. Indeed, PI3K–Akt–mTOR signaling pathway is frequently upregulated in T-ALL and is associated with poor prognosis.[17] Similarly, Chiang et al have demonstrated how common β-chain-associated protein facilitates suppression of TSC2 with subsequent Rheb–mTORC1 activation in T-ALL cell line.[18] Moreover, Xu et al found that hypermethylation of *TSC2* promotors led to downregulation of expression of *TSC2* in acute leukemia blasts.[19] This emphasizes role of hamartin and tuberin and mTOR pathway in leukemogenesis and as possible therapeutic targets. Indeed, preclinical studies have shown promising results of activity of mTOR inhibitors in T-ALL.[20] Nonetheless, this needs further exploration with the ultimate goal of its clinical application. We could not study PI3K–Akt–mTOR signaling pathway functional studies in our patient. However, we believe that our observation of this novel and rare association of ALL is of relevance, particularly to stimulate further research.

Conclusion

One should have a high index of suspension for malignancies in cancer predisposing syndromes. We underline the rare development of hematological malignancy in TSC.

Declaration of Patient Consent

The authors certify that they have obtained all appropriate patient consent forms.

Conflict of Interest

None declared.

References

1 Katz AJ, Chia VM, Schoonen WM, Kelsh MA. Acute lymphoblastic leukemia: an assessment of international incidence, survival, and disease burden. Cancer Causes Control 2015;26(11): 1627–1642
2 Pui CH, Nichols KE, Yang JJ. Somatic and germline genomics in paediatric acute lymphoblastic leukaemia. Nat Rev Clin Oncol 2019;16(04):227–240
3 Tebbi CK. Etiology of acute leukemia: a review. Cancers (Basel) 2021;13(09):2256
4 Morganti S, Tarantino P, Ferraro E, D'Amico P, Duso BA, Curigliano G. Next Generation Sequencing (NGS): a revolutionary technology in pharmacogenomics and personalized medicine in cancer. Translational Research and Onco-Omics Applications in the Era of Cancer Personal Genomics 2019:9–30
5 Northrup H, Krueger DA, Roberds S, Smith K, Sampson J, Korf B, Kwiatkowski DJ, Mowat D, Nellist M, Povey S, de Vries P. Tuberous

sclerosis complex diagnostic criteria update: recommendations of the 2012 International Tuberous Sclerosis Complex Consensus Conference. Pediatric neurology 2013 Oct 149(04):243–54
6 Korf BR, Bebin EM. Neurocutaneous disorders in children. Pediatr Rev 2017;38(03):119–128
7 Marjanska A, Jatczak-Gaca A, Wojtkiewicz A, Wysocki M, Styczynski J. Demographical profile and spectrum of multiple malignancies in children and adults with neurocutaneous disorders. Anticancer Res 2018;38(09):5453–5457
8 Portocarrero LKL, Quental KN, Samorano LP, de Oliveira ZNP, Rivitti-Machado MCDM. Tuberous sclerosis complex: review based on new diagnostic criteria. An Bras Dermatol 2018;93(03):323–331
9 Ebrahimi-Fakhari D, Mann LL, Poryo M, et al. Incidence of tuberous sclerosis and age at first diagnosis: new data and emerging trends from a national, prospective surveillance study. Orphanet J Rare Dis 2018;13(01):117
10 European Chromosome 16 Tuberous Sclerosis Consortium. Identification and characterization of the tuberous sclerosis gene on chromosome 16. Cell 1993;75(07):1305–1315
11 van Slegtenhorst M, de Hoogt R, Hermans C, et al. Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. Science 1997;277(5327):805–808
12 Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex. J Pathol 2017;241(02):219–225
13 Borkowska J, Schwartz RA, Kotulska K, Jozwiak S. Tuberous sclerosis complex: tumors and tumorigenesis. Int J Dermatol 2011;50(01):13–20
14 Peron A, Vignoli A, La Briola F, et al. Do patients with tuberous sclerosis complex have an increased risk for malignancies? Am J Med Genet A 2016;170(06):1538–1544
15 Rosner M, Hanneder M, Siegel N, Valli A, Fuchs C, Hengstschläger M. The mTOR pathway and its role in human genetic diseases. Mutat Res 2008;659(03):284–292
16 Kim LC, Cook RS, Chen J. mTORC1 and mTORC2 in cancer and the tumor microenvironment. Oncogene 2017;36(16):2191–2201
17 Lonetti A, Cappellini A, Bertaina A, et al. Improving nelarabine efficacy in T cell acute lymphoblastic leukemia by targeting aberrant PI3K/AKT/mTOR signaling pathway. J Hematol Oncol 2016;9(01):114
18 Chiang YJ, Liao WT, Ho KC, et al. CBAP modulates Akt-dependent TSC2 phosphorylation to promote Rheb-mTORC1 signaling and growth of T-cell acute lymphoblastic leukemia. Oncogene 2019;38(09):1432–1447
19 Xu Z, Wang M, Wang L, et al. Aberrant expression of TSC2 gene in the newly diagnosed acute leukemia. Leuk Res 2009;33(07):891–897
20 Evangelisti C, Chiarini F, McCubrey JA, Martelli AM. Therapeutic targeting of mTOR in T-cell acute lymphoblastic leukemia: an update. Int J Mol Sci 2018;19(07):1878

Acute Promyelocytic Leukemia Masquerading as Sero-negative Polyarthritis: Case Report

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Abstract

Musculoskeletal manifestations as the sole presentation in acute leukemia is rare in adults. Acute promyelocytic leukemia (APML) is a subtype of acute myeloid leukemia (AML) with reported incidence of 10 to 15% of total AML cases. APML presenting as polyarticular arthritis has never been reported in the literature. We present an interesting case of 20-year-old male patient who manifested with polyarticular arthritis mainly of small joints as the initial presentation, followed by pancytopenia and eventually was diagnosed as a case of APML on bone marrow morphology and molecular analysis for *PML-RARα* transcript. He was successfully treated with all-trans-retinoic acid (ATRA) and arsenic trioxide (ATO). Arthritis also resolved with complete remission of APML. Arthritis in a case with pancytopenia should promptly be evaluated prior to treatment with steroids and anti-metabolites. Arthritis can be a presenting manifestation of APML and responds to prompt management of leukemia as in other cases of leukemic arthritis.

Keywords

- acute promyelocytic leukemia
- arthritis
- musculoskeletal

Introduction

The incidence of acute promyelocytic leukemia (APML) is reported to be 0.32 cases per 100,000 population as per the SEER database.[1] APML is classified as acute myeloid leukemia (AML) with recurrent genetic abnormalities, as per the WHO (2008) and is a result of a balanced translocation t (15;17), which leads to the formation of fusion oncoprotein *PML-RARA* that leads to the arrest of normal myeloid differentiation at a promyelocyte stage. With the discovery of all-trans retinoic acid (ATRA) and arsenic trioxide as differentiating agents, the prognosis of APML has improved drastically with complete remission rates of $>90\%$.[2–4] However, such high cure rates are achievable only with timely diagnosis and vigorous management. The main presentation of APML includes fever, generalized weakness, bleeding, and thrombotic manifestations due to coagulopathy.[5] Though available literature has reported various cases of AML developing after rheumatoid or psoriatic arthritis,[6–8] arthritis as an initial presentation in APML has never been reported to the best of our knowledge.

Case Report

This study being a case report, a waiver off consent was applied to the institutional ethics committee (IEC) and the same was accepted by the IEC.

We report a case of 20-year-old male patient, laborer by occupation, who consulted various physicians with complains of fatigue and multiple joint pain, swelling, and

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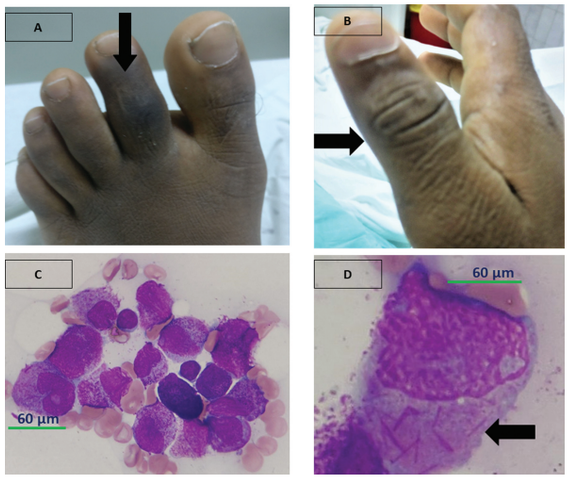

Fig. 1 (**A** and **B**) Arrow highlighting right thumb interphalangeal joint arthritis and dactylitis of the left second toe. (**C** and **D**) Normal hematopoietic elements are replaced by proliferation of more than 90% abnormal promyelocytes having high N:C ratio, irregular nucleus, open chromatin, with 1–2 prominent nucleoli, moderate amount of pale basophilic cytoplasm, with many of them showing multiple Auer rods ("Faggot cells" arrow). Cytochemistry: These abnormal promyelocytes are strongly positive for MPO.

stiffness of joints for 1 month. He was evaluated and treated with prescribed non-steroid anti-inflammatory drugs (NSAIDs) by the general physician; however, due to the persistence of his symptoms he was referred to immunology department of our hospital. General physical examination revealed pallor, no petechiae, ecchymosis, or lymphadenopathy. Musculoskeletal examination revealed right temporomandibular joint pain with jaw and mouth opening of one finger, right acromial tip enthesitis, dactylitis of left second toe (►**Fig. 1A**), left extensor tenosynovitis (►**Fig. 1B**), cervical spine local tenderness (C5-C8) present with restriction of movement in flexion (50°), extension (30°) and lateral rotation bilateral (30°). Systemic examination was with in normal limits.

The laboratory investigations at first visit to the physician showed Hb-12.5 g/dL, total leucocyte count-4500/mm^3, differential leucocyte count-neutrophil-62%, lymphocytes-38%, platelet count-120,000/µL, and peripheral blood smear-no abnormal cells. Baseline investigations at our center are documented in ►**Table 1**. Immunological work up showed elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), anti-nuclear antibody profile, rheumatoid factor, anti-citrullinated peptide antibody (ACPA), HLA-B27 were all negative. The X-ray of involved joints did not show

Table 1 Laboratory profile of the patient

Parameters	Investigations at our center at presentation	Investigations post consolidation chemotherapy
Hb (g/dL)	9.1	11.3
TLC (/mm^3)	**800**	7200
DLC	N5L20	N56L38E2M4
Platelet count (/µL)	**76,000**	2,26,000
S. uric acid (mg/dL)	4.2	NA
S. calcium (mg/dL)	9.1	NA
S. phosphate (mg/dL)	2.5	NA
PT/INR	15.4/1.34	NA
APTT (seconds)	26.7(Control-28.4)	NA
Fibrinogen (mg/dL)	648	NA
ESR (mm/hr) Westergren method	**140**	18
Malarial parasite	Negative	NA
Dengue serology	Negative	NA
CRP (mg/dL)	**13.3**	0.5
ANA	Negative	Negative
ACPA	Negative	Negative
RF	Negative	Negative
HLA-B27	Negative	Negative
Bone marrow examination	Hypercellular marrow with 90% abnormal promyelocytes (MPO+), **Faggot cells**+	No abnormal promyelocytes noted
RT-PCR PML-RARα	**Positive** for Bcr1 transcript	Negative

Abbreviations: ACPA, anti-citrullinated peptide antibody; ANA, anti-nuclear antibody; APTT, activated partial thromboplastin time; CRP, C-reactive protein; DLC, differential leucocyte count; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; INR, international normalized ratio; L, lymphocyte; M, monocyte; N, neutrophil; NA, not available; PT, prothrombin time; RF, rheumatoid factor; TLC, total leucocyte count.

any abnormality (►**Supplementary figs. S1–4)**. Ultrasound of meta-tarso-phalangeal joint of the second left toe showed minimal effusion, which was not amenable for aspiration. Bone marrow and flow cytometry done at our center in view of pancytopenia were diagnostic of APML-macro-granular variant (►**Fig. 1B**, **1D**). RT-PCR for *PML-RARα* was positive for *Bcr1* transcript (**Supplement fig. S5)**. Differentials of APML with leukemic arthritis versus reactive arthritis versus hemorrhagic arthritis were thought of after the reports were available. Hemorrhagic arthritis was ruled out due to normal coagulation parameters, no evidence of bleeding elsewhere and small joint involvement in this case contrary to typical large weight-bearing joint arthritis in hemorrhagic arthritis. The patient was labeled as APML, low risk (SANZ criteria),[3] and was started on ATRA at 45 mg/m^2 per oral daily and ATO at 0.15 mg/kg/day IV infusion with close monitoring of coagulation parameters, electrocardiography (ECG), and serum electrolytes. In view of the limitation of activity due to arthritis and no relief with tramadol, the patient was started on low-dose prednisolone 10 mg daily but the symptoms and signs persisted, dose of steroids was increased to 15 mg and then 20 mg daily with some relief in symptoms. The patient tolerated ATRA and ATO protocol well with no adverse events and no differentiation syndrome. Day 28 marrow showed morphological remission. Musculoskeletal symptoms also gradually subsided and steroid was tapered and was discontinued by day 35 with complete resolution of musculoskeletal symptoms and signs. The patient continued consolidation with ATRA and ATO as per the APL0406 protocol.[4] Bone marrow morphology and RT-PCR for *PML-RARα* from marrow aspirate sample, post consolidation was negative. At 16 months of follow-up, the patient continues to be in remission without any recurrence of joint symptoms.

Discussion

Musculoskeletal presentation has been reported in many malignancies.[9] The most common presentations are myalgia, arthralgia, arthritis, osteolytic bony lesions, and spontaneous fractures.[9] Leukemic arthritis is defined as joint pain and swelling in a diagnosed case of leukemia when other causes of arthritis have been excluded.[10] It is reported to occur in around 12 to 65% cases of acute leukemia in children, whereas in adults the reported prevalence is between 4 and 13%.[11,12] In children, it is reported most in acute lymphoblastic leukemia, while in adults apart from acute leukemia, leukemic arthritis is also reported in chronic myeloid leukemia, adult T cell leukemia, and hairy cell leukemia.[10,13] Pathogenic mechanisms for this type of arthritis described in the literature include direct infiltration of leukemic cells into synovial tissue, synovial reaction to tissue infiltration, immune complex mediated synovitis, and hemorrhage into the joint space due to thrombocytopenia. However, direct infiltration of the synovial tissue is the most common mechanism reported. It mostly presents as asymmetric pauciarticular large joint arthritis presenting as the initial manifestation in acute leukemia, while more symmetric and late presentation in chronic leukemia.[14,15] Involved joints show typical signs of inflammation; erythema, swelling, tenderness mimicking other rheumatic diseases and often cause difficulty in diagnosis at initial presentation. Very few case reports have described the presentation of tenosynovitis or finger joint involvement as in our case.[16,17] A few case reports have reported the development of acute promyelocytic leukemia in patients on therapy for rheumatoid arthritis but even after extensive literature search, arthritis as the presenting symptom of APML has not been reported. In their report, Naithani et al[18] described arsenic trioxide-induced acute flare up of pre-existing rheumatoid arthritis. Akoz et al[19] described atypical presentation of retinoic acid syndrome mimicking septic arthritis. Buyukkurt et al[6] in their case reported the development of acute promyelocytic leukemia in a patient with gouty arthritis who was on long-term colchicine therapy. In our case, we kept the possibility of simultaneous presentation of both rheumatoid arthritis and APML but negative autoimmune work-up and temporal correlation of disappearance of symptoms and signs of arthritis coinciding with remission status of APML and no recurrence thereafter at 16 months of follow-up made the diagnosis of leukemic arthritis evident. In some cases, complete blood count and peripheral smear examination show non-specific findings such as anemia, leukocytosis, or leukopenia, mandating the need of bone marrow examination. Management involves managing the primary disease, joint symptoms, and signs resolve with the remission of disease.

The limitation of this case report is the inability to confirm leukemic arthritis by histopathological diagnosis of the synovial fluid as joint effusion was minimal and hence inability to perform aspiration.

Patient's Perspective

I had visited six to seven physicians prior to consulting this hospital for pain in multiple joints and difficulty chewing food, but there was no relief with various medications given. After I consulted this hospital, my blood tests showed decreased white blood counts for which I was investigated further and then my medications were started; after 15 days, my joint pain and swelling reduced by 50% and after 1 month of therapy, I felt completely normal. I continued therapy for another 6 months and now I am doing my work like before.

Conclusion

Arthritis as a presenting feature of acute leukemia has been well described; however, it has been seldom reported in APML. A high index of suspicion and thorough investigations are required prior to labeling it as rheumatic disorder and initiating treatment with steroids and/or antimetabolites, which may lead to the masking of the underlying etiology. It is imperative to monitor complete blood count and peripheral smear repeatedly in case of doubt and negative serological tests for immune disorders.

Consent

Informed consent was taken from the patient.

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Conflict of Interest
None declared.

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References

1 Guru Murthy GS, Szabo A, Michaelis L, et al. Improving outcomes of acute promyelocytic leukemia in the current era: analysis of the SEER database. J Natl Compr Canc Netw 2020;18(02):169–175
2 Lo-Coco F, Avvisati G, Vignetti M, et al; Gruppo Italiano Malattie Ematologiche dell'Adulto German-Austrian Acute Myeloid Leukemia Study Group Study Alliance Leukemia. Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. N Engl J Med 2013;369(02):111–121
3 Sanz MA, Martín G, González M, et al; Programa de Estudio y Traitmiento de las Hemopatías Malignas. Risk-adapted treatment of acute promyelocytic leukemia with all-trans-retinoic acid and anthracycline monochemotherapy: a multicenter study by the PETHEMA group. Blood 2004;103(04):1237–1243
4 Platzbecker U, Avvisati G, Cicconi L, et al. Improved outcomes with retinoic acid and arsenic trioxide compared with retinoic acid and chemotherapy in non-high-risk acute promyelocytic leukemia: final results of the randomized Italian-German APL0406 trial. J Clin Oncol 2017;35(06):605–612
5 Daver N, Kantarjian H, Marcucci G, et al. Clinical characteristics and outcomes in patients with acute promyelocytic leukaemia and hyperleucocytosis. Br J Haematol 2015;168(05):646–653
6 Buyukkurt N, Korur A, Boga C. Development of acute promyelocytic leukemia in a patient with gouty arthritis on long-term colchicine. Indian J Hematol Blood Transfus 2016;32(Suppl 1):80–81. Doi: 10.1007/s12288-015-0523-4
7 Ki MH, Ho LJ, Min LH, et al. A case of acute myeloid leukemia after adalimumab treatment in psoriatic arthritis. J Rheum Dis 2012;19(02):91–94
8 Tanaka K, Oshikawa G, Akiyama H, et al. Acute myeloid leukemia with t(3;21)(q26.2;q22) developing following low-dose methotrexate therapy for rheumatoid arthritis and expressing two AML1/MDS1/EVI1 fusion proteins: a case report. Oncol Lett 2017;14(01):97–102
9 Morais SA, du Preez HE, Akhtar MR, Cross S, Isenberg DA. Musculoskeletal complications of haematological disease. Rheumatology (Oxford) 2016;55(06):968–981
10 Evans TI, Nercessian BM, Sanders KM. Leukemic arthritis. Semin Arthritis Rheum 1994;24(01):48–56
11 Weinberger A, Schumacher HR, Schimmer BM, Myers AR, Brogadir SP. Arthritis in acute leukemia. Clinical and histopathological observations. Arch Intern Med 1981;141(09):1183–1187
12 Spilberg I, Meyer GJ. The arthritis of leukemia. Arthritis Rheum 1972;15(06):630–635
13 Needleman M. Childhood leukemia mimicking arthritis. J Am Board Fam Pract 1996;9(01):56–60
14 Silverstein MN, Kelly PJ. Leukemia with osteoarticular symptoms and signs. Ann Intern Med 1963;59:637–645
15 Brix N, Rosthøj S, Herlin T, et al. Arthritis as presenting manifestation of acute lymphoblastic leukaemia in children. Arch Dis Child 2015;100(09):821–825
16 Thomas LB, Forkner CE Jr, Frei E III, Besse BE Jr, Stabenau JR. The skeletal lesions of acute leukemia. Cancer 1961;14:608–621
17 Taillan B, Leyge JF, Fuzibet JG, Nectoux F, Ziegler G, Dujardin P. Knee arthritis revealing acute leukemia in a patient with rheumatoid arthritis. Clin Rheumatol 1991;10(01):76–77
18 Naithani R, Mahapatra M, Kumar R, Kumar A, Agrawal N. Arsenic trioxide induced acute flare-up of rheumatoid arthritis in a patient with APL. Ann Hematol 2007;86(02):151–152
19 Ayla Gokmen Akoz. Huseyin Engin & Nefise Oztoprak. Atypical presentation of retinoic acid syndrome that mimics septic arthritis in a patient with acute promyelocytic leukemia. Acta Oncol (Madr) 2007;46(08):1193–1194

Bevacizumab in Oncology: Boon or Bane

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Bevacizumab is a recombinant humanized monoclonal antibody against vascular endothelial growth factors (VEGF). It blocks the angiogenic molecule VEGF, thereby inhibiting tumor angiogenesis. It is approved for a range of solid cancers such as ovarian cancer, colorectal cancer, glioblastoma multiforme, advanced non-squamous non-small cell lung cancer (NSCLC), cervical cancer, renal cell carcinoma, and metastatic breast cancer.

This is in response to the study titled "Real-World Experience in Toxicity with Bevacizumab in Indian Cancer Patients." The authors Patil et al. (2021) conducted a retrospective study on 41 patients with various cancers, who had received bevacizumab with or without chemotherapy. In their study, the incidence of arterial thrombus and hemorrhage was 2% and 10% respectively, whereas the incidence of congestive heart failure and subacute intestinal obstruction was found in 5% of patients.[1] The efficacy of the use of bevacizumab was not evaluated in this study.

Bevacizumab was first approved for metastatic colorectal cancer in combination with chemotherapy. The pivotal clinical trial for the drug in colorectal cancer in a first-line setting was conducted by Hurwitz et al (2004)[2] and Saltz et al (2008)[3] and showed a modest clinical benefit ranging from 3 to 5 months. In the case of NSCLC, the study done by Sandler et al[4] showed an overall survival benefit of merely 2 months; however, the AVAiL study[5] failed to show any benefit in overall survival. For the remaining treatment applications of bevacizumab: metastatic breast cancer, renal cell carcinoma, and glioblastoma multiforme, various clinical trials have failed to show a significant benefit in overall survival.

The benefit noted in progression-free survival has failed to translate into any benefit in overall survival. In the setting of ovarian cancers, GOG-0213 was the only clinical trial to show a benefit in overall survival, whereas other trials such as AURELIA and OCEANS failed to do the same.[6]

Bevacizumab has a range of side effects such as hypertension, proteinuria, intestinal obstruction or perforation, bleeding, thromboembolism, delayed wound healing post-surgery, diarrhea, fatigue, and asthenia. Bevacizumab is also contraindicated in pregnancy given post-marketing reports of embryonal malformations. The other less-frequent side effects include congestive heart failure, posterior reversible encephalopathy, fistulae formation, hypersensitivity reactions, osteonecrosis of the jaw, and increased rates of infections. The highest incidence of potentially serious GI perforations ranged up to 9.2% in colon cancers. The risk of hemorrhage has been reported up to 44.2 % in NSCLC.[6] These are fatal complications that can be potentially life-threatening in patients treated with bevacizumab.

Keeping in mind the various significant side effects and minimal survival benefits, patients for bevacizumab should be selected carefully by an oncologist after weighing both the risks and benefits. It should not be recommended if the risk-to-benefit ratio is moderate or high for a particular patient upon examination and checking their medical history.

Conflict of Interest
None declared.

References

1 Patil PP, Rangaraju RR, Abbas W, Garg S. Real-world experience in toxicity with bevacizumab in Indian cancer patients. South Asian J Cancer 2021;10(02):131–134
2 Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004;350(23):2335–2342

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3 Saltz LB, Clarke S, Díaz-Rubio E, et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. J Clin Oncol 2008;26(12):2013–2019
4 Sandler AB. Molecular targeted agents in non-small-cell lung cancer. Clin Lung Cancer 2003;5(Suppl 1):S22–S28
5 Reck M, von Pawel J, Zatloukal P, et al. Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAil. J Clin Oncol 2009;27(08):1227–1234
6 Garcia J, Hurwitz HI, Sandler AB, et al. Bevacizumab (Avastin®) in cancer treatment: A review of 15 years of clinical experience and future outlook. Cancer Treat Rev 2020;86:102017

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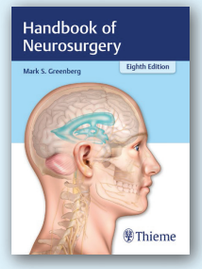

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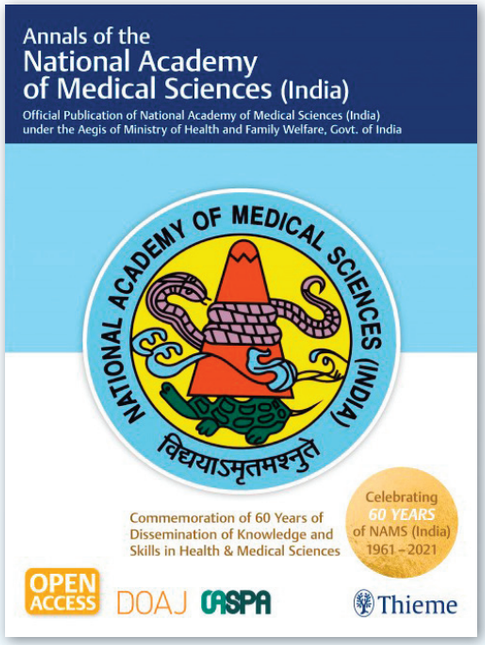

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References:

1.Wynne C, Schwabe C, Batra SS, Lopez-Lazaro L, Kankanwadi S. A comparative pharmacokinetic study of DRL_BZ, a candidate biosimilar of bevacizumab, with Avastin® (EU and US) in healthy male subjects. Br J Clin Pharmacol. 2018 Jul 26;84(10):2352–64. | 2.IPSOS MAT Jun 2020. | 3.MRP of All 100 mg Bevacizumab Brands marketed in India were compared. | 4.MRP of all Brands as on Dec 2020.

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