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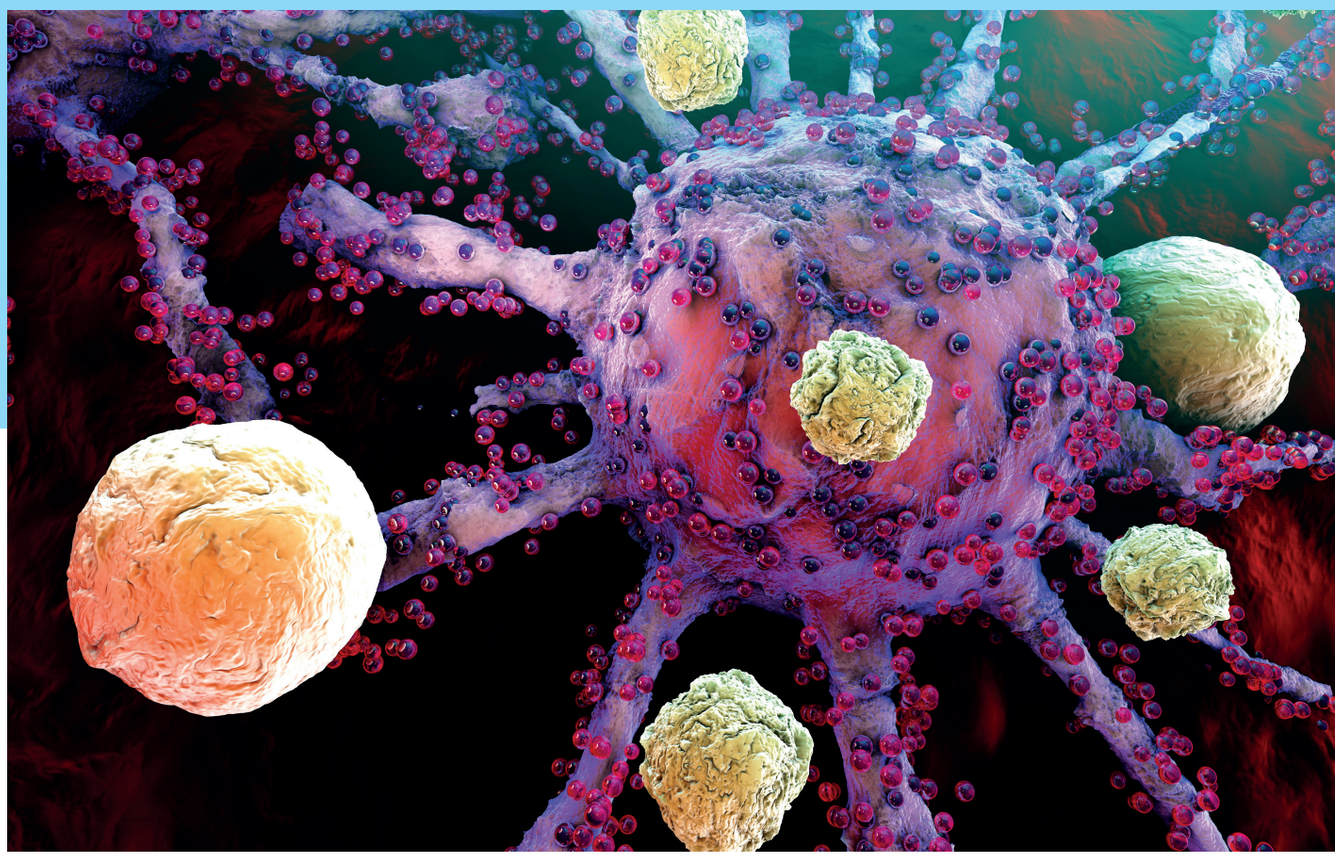

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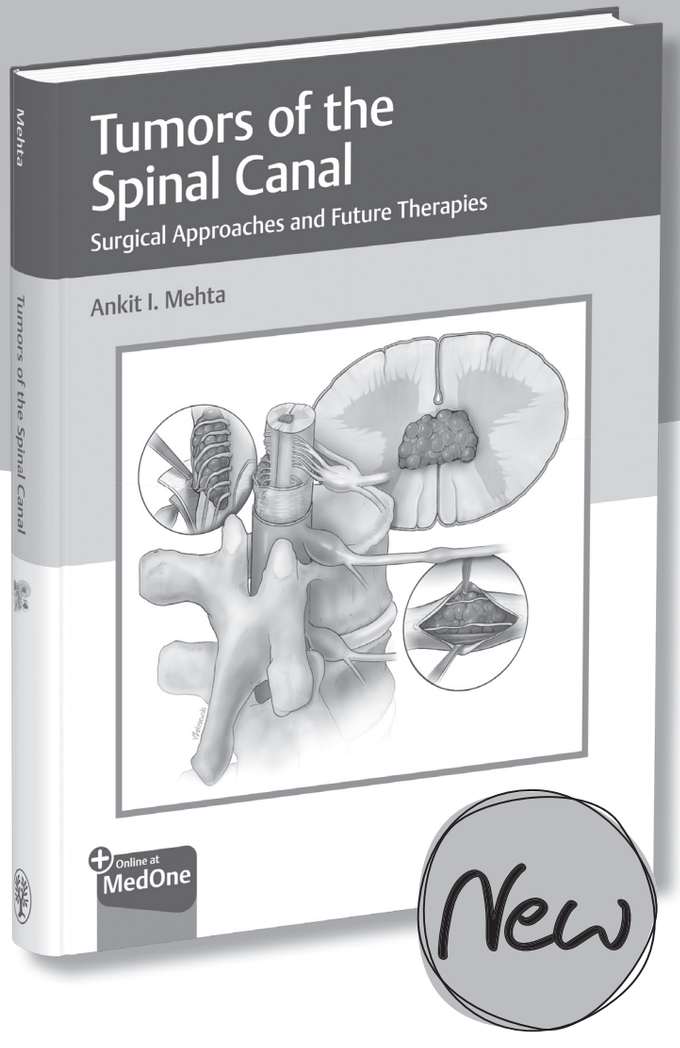
Current and future advances
in the treatment of intradural spinal tumors



Recent strides and future directions in spinal canal tumor treatment are detailed. This review encompasses anatomy, pathophysiology, therapeutic and surgical advances, diverse surgical techniques, and future directions. Readers are provided with the necessary tools to master management of these clinically difficult tumors, medically and surgically.

KEY HIGHLIGHTS

- Treatment algorithms, clinical study summaries, and differential diagnoses presented in reader-friendly tables enhance acquisition and retention of knowledge
- Insightful analyses and pearls on managing complications and improving patient outcomes
- Current research innovations, clinical trials, and groundbreaking future directions

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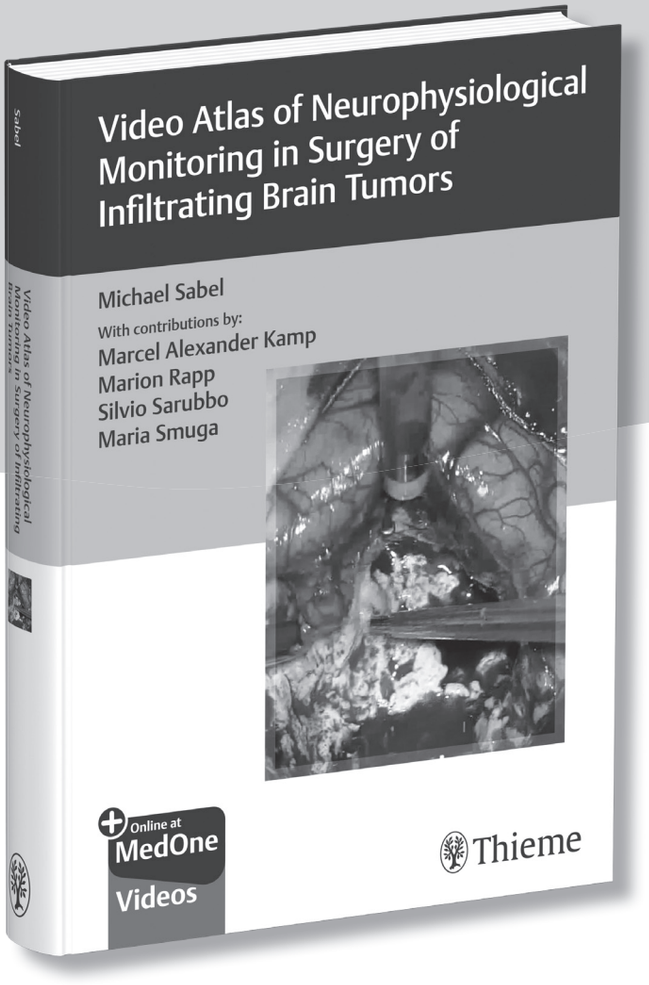
Video atlas on neurophysiological monitoring during brain tumor surgery



Surgical treatment of infiltrating brain tumors is extremely challenging. Videos and text demonstrate practical applications of neurophysiological monitoring to achieve safe removal of infiltrating brain tumors in asleep and awake settings.

KEY HIGHLIGHTS

- Impacted anatomy, physiology of the neural pathways monitored in brain tumor surgery, and key techniques for monitoring and testing during resection
- Instructive illustrations coupled with concise explanations enhance knowledge and facilitate understanding of techniques
- Fifteen videos covering real-life intraoperative cases provide in -depth insights

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National Research Scholars' Meet 2024
December 10-11, 2024

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A001. Development of a Cost-Effective *Bacillus*-Based Biofertilizer and Biocontrol Agent for Wheat

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Keywords
- biofertilizer
- biocontrol
- bacillus
- antimicrobial
- CFS
- cheap media

Background: Chemical fertilizers and pesticides, used for agriculture, disrupt ecosystems by damaging soil quality and reducing crop nutritional value. This study aims to isolate, characterize, and optimize a potent plant growth-promoting bacterium for biocontrol and biofertilizer applications.

Materials and Methods: JPP strain (*Bacillus subtilis*), isolated from mangrove soil, exhibited several beneficial traits, including indole-3-acetic acid production, biofilm formation, high salt tolerance, and promising biocontrol capabilities. Current efforts focus on developing a cost-effective medium for large-scale production of this organism. Blackstrap molasses was used as an inexpensive carbon source, 10 different nitrogen sources were tested, including glutamic acid, aspartic acid, proline, beef extract, glycine, yeast extract, sodium nitrate, ammonium sulfate, and ammonium chloride.

Results: Several antimicrobial compounds such as derivatives of palmitic acid, fatty acid amides, and pyridine, were detected in the cell-free supernatant (CFS) by liquid chromatography-mass spectrometry analysis. In soil-pot experiments, this strain significantly increased shoot and root length in saline soil when bio-primed, compared with control conditions. Optimal growth conditions were identified at pH 7 and 25°C, with 0.1% molasses (approximately 1.35 mg glucose equivalent/L) and 0.5% yeast extract providing the best carbon and nitrogen sources, respectively, for maximizing cell growth and bioactive metabolite synthesis. The resulting medium yielded high cell density comparable to conventional media, and the CFS exhibited antimicrobial activity in well diffusion assays.

Conclusion: The study highlights the JPP strain's potential as a biocontrol agent as well as a cost-effective biofertilizer and for large-scale production and future field trials.

A002. Phytochemical Screening, Evaluation of Antioxidant Potential and Cytotoxic Effects on NIH3T3 Mouse Embryonic Fibroblast Cell Line of *Dalbergia latifolia* Roxb. Leaves

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Keywords
- *Dalbergia latifolia*
- antioxidant
- cytotoxic
- cancer-associated fibroblast
- NIH3T3

Background: Phytoconstituents with potent antioxidant and cytotoxic properties offer promising leads for developing anticancer therapies. *Dalbergia latifolia*, an underutilized tree with diverse ethnomedicinal uses, was investigated for its phytoconstituents, antioxidant activity, and cytotoxicity against NIH3T3 mouse embryonic fibroblasts. The study explores its potential in managing oxidative stress and fibroblast-associated tumor microenvironments.

Materials and Methods: Aqueous and ethanolic extracts of leaves were screened for major phytochemicals. Ethanolic extract was further evaluated for total phenolic and flavonoid content and antioxidant assays (Reducing power assay; Total antioxidant capacity; 2,2-Diphenyl-1-picrylhydrazyl radical; 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid radical). Cytotoxicity assessment at 10 to 200 ug/mL was performed via MTT assay on NIH3T3 cells.

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Results: The ethanolic extract of leaves contained major phytochemicals like alkaloids, phenols, flavonoids, glycosides, quinones, and sterols along with high phenolic (8.1 ± 0.11 mg GAE/g) and flavonoid (3.6 ± 0.05 mg QE/g) content. It exhibited strong total antioxidant capacity (426.3 ± 12 mg AAE/g), reducing power activity (495 ± 6.22 mg BHT equivalent/g), and radical scavenging activity with IC50 values of 18.174 µg/mL for DPPH and 4.63 µg/mL for ABTS radicals. Significant cytotoxicity was observed against NIH3T3 cells with IC50 of 37.75 ± 2.63 µg/mL.

Conclusion: These findings highlight the potential of *Dalbergia latifolia* leaf extracts in developing therapies targeting cancer-associated fibroblasts, key players in tumor progression as well as mitigation of oxidative stress. Further research on site-specific cancer cell lines, such as breast and pancreatic cancers, where fibroblast-driven tumor microenvironments are critical, is recommended to establish its therapeutic potential.

A003. Augmenting Solid Tumor Immunotherapy via CAF Reprogramming Using Injectable Hydrogel

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#Presenting Author: Shivali Patkar

Keywords

- cancer-associated fibroblasts (CAFs)
- hydrogel
- solid tumor
- immunotherapy

Background: Adoptive cell therapies have shown great potential against blood cancers, but challenges such as dense extracellular matrix secretion by cancer-associated fibroblasts (CAFs) in the tumor microenvironment prevent the penetration of genetically modified T cells into the tumor. Directly targeting CAFs may promote tumor metastasis and the targeting agents may also affect normal fibroblasts, necessitating alternative strategies.

Materials and Methods: To address this challenge, we have developed a novel injectable hybrid polymeric hydrogel composed of Pluronic F-127 and crosslinked-gelatin for localized reprogramming of CAFs, aiming to reduce systemic toxicity and enhance T cell penetration in solid tumors. The hydrogel was subjected to physical and biological characterization, and biocompatibility was assessed in vivo in immunocompetent mice.

Results: The hydrogels have excellent shear thinning properties and are injectable through a 21G needle, which eliminates the use of surgical implantation. Porosity tests by scanning electron microscopy imaging and rhodamine B isothiocyanate (RITC)-dextran penetration via confocal microscopy revealed that the hydrogels are porous. Biocompatibility assays with splenocytes indicated high cell viability (~80%), and hemocompatibility tests showed no red blood cell lysis on coculture with blood. Additionally, these hydrogels allowed uniform loading of splenocytes. In vivo studies proved that the hydrogel is biocompatible and can be used for in vivo cell delivery.

Conclusion: In conclusion, we have developed a biocompatible and injectable biopolymeric hydrogel, which can facilitate localized delivery of CAF reprogramming agent and genetically modified T cells, offering a promising potential for enhancing solid tumor immunotherapy.

A004. Flow Cytometric Evaluation of Normal Ranges of Surface Immunoglobulin B Cell Subsets in Healthy Controls and Its Clinical Significance

A. Acharya[1#], P. Dhende[1], N. Deshpande[1], S. Ghogale[1], K. Girase[1], J. Patil[1], G. Chatterjee[1], S. Rajpal[1], P. G. Subramanian[1], P. Tembhare[1*]

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Keywords

- CVID
- IEI
- B subsets

Introduction: Common variable immunodeficiency (CVID) is a rare inborn errors of immunity (IEI) characterized by significantly reduced immunoglobulin levels. Euroflow demonstrated the utility of B cell subset levels expressing different surface heavy-chain immunoglobulins (sIg) in the diagnosis of IEI. Data on the normal range of sIg+B-subsets in healthy controls is limited and there is no data from Indian population. Hence, we are using flow cytometer to evaluate normal ranges of sIg subsets in healthy controls.

Materials and Methods: We studied sIg+B-subsets in the peripheral blood of 36 healthy volunteers. Eleven-color flow cytometry assay with antibodies against IgG1 (clone SAG1), IgG2 (clone SAG2), IgG3 (clone SAG3), IgG4 (clone SAG4) IgA1 (clone SAA1), IgA2 (clone SAA2), IgM (clone, G0–127), and IgD (clone IA6–2) along with backbone B cell. Data was acquired on LSR Fortessa (BD Biosciences).

Results: The median percentage of B-lymphocytes was 4.95% (range 1.89–18.62). Median (range) of naive and memory B cells was 70.65% (43.67–86.14) and 25.19% (5.02–53.09), respectively. The median percentage of IgG1+B was 5.78% (range 0.76–15.33), IgG2+B 2.16% (range 0.35–5.63), IgG3+B 1.35% (range 0.10–8.36), IgG4+B 0.31% (range 0.02–1.17), IgA1+B 3.44% (range 0.29–7.73), IgA2+B 1.65% (range 0.20–6.13), IgM+B 84.20% (range 64.32–93.99), and IgD+B 79.18% (range 56.66–96.30). We have diagnosed CVID case that shows 97.30% of all B cells expressing IgD and 94.05% B cells expressing IgM. IgG+/IgA+ memory B cells were markedly reduced. B cells also showed moderate positivity for CD21 but CD81 and CD23 were negative.

Conclusion: We have established a normal range of various surface immunoglobulin expressing B cell subsets in healthy volunteers and demonstrated its clinical utility.

A005. Screening and Characterization of Bacteriophages for Developing Targeted Therapeutic Cocktails against Three Phytopathogens

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Keywords
- bacteriophage
- bacteriophage therapy
- biocontrol
- *Pantoea agglomerans*
- phage cocktail
- phage resistance
- phytopathogens
- *Pseudomonas syringae*
- *Xanthomonas oryzae*

Background: Plant pathogens like *Pseudomonas syringae*, *Pantoea agglomerans*, and *Xanthomonas oryzae* pose significant challenges to agrarian economies, especially in India. *P. syringae* and *P. agglomerans* infect economically important species, while *X. oryzae* threatens rice crops. This research focused on isolating and characterizing phages against these phytopathogens.

Materials and Methods: Different environmental samples—sewage samples and marine samples—were collected from locations all over suburban Mumbai and used as potential sources for phage isolation. After coincubation with the host, spot assay was performed and the plates were inspected for plaques. These plaques were further excised, purified, and enumerated.

Results: Two distinct bacteriophages targeting *P. syringae* were identified, named PS1 and PS2, based on plaque morphology. Using the double agar overlay method, PS1 produced small, turbid plaques with an average diameter of 0.5 cm, while PS2 formed larger, clearer plaques with an average diameter of 0.8 cm. The phage titers were 5.60×10^{18} PFU/mL for PS1 and 9.66×10^{19} PFU/mL for PS2. The clear plaques of PS2 indicate effective lytic activity, suggesting its potential for therapeutic applications, while the turbid plaques of PS1 are characteristic of lysogenic phages, offering insight into their different modes of action.

Conclusion: Future work will focus on characterizing the phages' physical, chemical, and biological properties through electron microscopy and genome sequencing. In vitro and in vivo assays will evaluate their lytic activities, individually and in combination. The goal is to develop a safe, effective phage cocktail to combat bacterial pathogens and prevent resistance.

A006. The Role of BRD4 in Acquiring Drug-Tolerant Persister (DTP) Phenotype in Different Subtypes of TNBC

Kedar Mal Sharma[1,2#], Shagufa A. E. Shaikh[1,2], Siddhi A. Tekalkar[1,2], Nandini Verma[1,2*]
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#Presenting Author: Kedar Mal Sharma

Keywords
- TNBC
- chemoresistance
- DTP
- BRD4

Background: Triple negative breast cancer (TNBC) is the most aggressive and heterogeneous type of breast malignancy and its clinical management relies mainly on chemotherapy. But a large number of patients develop therapy resistance with residual disease, which harbors drug-tolerant persister (DTP) cells. Recent molecular studies have shown that chemoresistance in TNBC tumors is largely driven by epigenetic mechanisms.

Materials and Methods: We have longitudinally modeled cellular state transitions from dormant DTP into proliferating DTP (PDTP) cells representing different TNBC subtypes. To elucidate the role of bromodomain-containing protein 4 (BRD4), we have characterized the DTP population using genetic and pharmacological approaches.

Results: BRD4 is an important epigenetic reader protein and is shown to play key role in the metastasis and development of drug resistance in solid as well as liquid tumors. Here, we have shown that BRD4 messenger ribonucleic acid is selectively overexpressed in TNBC tumors and cell lines as compared with other members of BET family, BRD2 and BRD3. By immunohistochemistry analysis we also found that BRD4 protein is overexpressed in the tumor tissues of TNBC patient compared with non-TNBC ones and has a strong nuclear localization. We found that DTP and PDTP derived from TNBC subtypes shows differential expression of BRD4.

Conclusion: As several BRD4 inhibitors are in clinical trials, these findings can have potential therapeutic implications for TNBC chemoresistant patients.

A007. Elucidating the Therapeutic Potential and Effects of Mitocurcumin on the *Drosophila* Yorkie-Induced Gut Tumor Overgrowth Model

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Keywords
- ROS
- redox homeostasis
- oxidative stress
- apoptosis
- mitocurcumin
- ISC (intestinal stem cells)
- EB (enteroblasts)
- Yki3SA
- escargot-Gal4
- glycolysis

- TCA cycle
- ETC complex
- pentose phosphate pathway (PPP)
- mitochondrial content

Background: Normal cells balance reactive oxygen species production and scavenging to maintain redox homeostasis. Disrupting this balance induces oxidative stress and apoptosis, a potential cancer treatment strategy. Mitocurcumin, a stable, mitochondria-targeted curcumin derivative, may enhance therapeutic effects. This study examines mitocurcumin's molecular impact on a *Drosophila* Yorkie-induced gut tumor model.

Materials and Methods: A *Drosophila* larval gut tumor model was created using the Yki3SA transgene driven by *escargot-Gal4* in intestinal stem cells (ISCs) and enteroblasts (EBs). Larvae were starved, treated with 25 µM mitocurcumin for 12 to 14 hours, dissected, and prepared for immunofluorescence or ribonucleic acid isolation. Mitotic activity and apoptosis were analyzed with H3P and DCP1 antibodies, respectively. Gene expression was assessed via quantitative polymerase chain reaction.

Results: Mitocurcumin treatment reduces mitotic activity and induces apoptosis in ISC-EBs in both control and Yorkie backgrounds. We observe a volumetric reduction in Escargot (Esg)-positive ISC-EB clusters, which are typically large and deformed in the tumor background. Given the altered metabolic demands of cancer cells, we analyzed the metabolic profile of Yki tumors. Our results show no change in glycolysis, but a significant reduction in TCA and ETC complex genes, along with an increase in pentose phosphate pathway (PPP) genes in Yki tumors. Additionally, mitochondrial content decreases in these tumors. We aim to explore if mitocurcumin can alter this metabolic profile.

Conclusion: Mitocurcumin induces stress even in wild-type larval gut by disrupting expression of ETC complex molecules, reduces mitotic activity, and results in increased apoptosis. In tumor-induced larvae, it further downregulates mitotic activity, significantly increases apoptosis, thereby reducing Yki-induced Esg-positive tumor cell load.

A008. Synthesis of a Biomimetic Nanomaterial Using Ghost Membrane for Cancer Theranostic Application

Anuradha Gupta[1,2#], Chetna Patnaik[1,2], Sulagna Rath[1,2], Rohit Srivastava[3], Sunil Shetty[2,4], Abhijit De[1,2*]

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Keywords

- biomimetic nanoformulation
- photothermal agents
- homotypic interaction

Background: Photothermal therapy (PTT) is an emerging cancer nanomedicine. Our group focuses on developing tumor-targeted theranostic photothermal agents (PTAs) using organic near-infrared fluorescent (NIRF) imaging-compatible FF-BSC- L, L-diphenylalanine-conjugated BF2-oxasmaragdyrin and IR820 dyes. Integrating biomimetic principles into PTAs facilitates enhanced homing through active nanomaterial uptake within tumors. We aim to design a biomimetic PTA nanomaterial that enables homotypic binding interactions for cancer theranostic applications.

Materials and Methods: The synthesis of cancer cell ghost membranes (CCGMs) was optimized for EpCAM-overexpressing radioresistant breast cancer cells, with encapsulation of PTAs achieved via electroporation. Physical characterization of CCGMs was performed using dynamic light scattering (DLS), transmission electron microscopy (TEM), and zeta potential analysis to evaluate hydrodynamic size, morphology, and surface charge, respectively. Immunoblotting confirmed cancer cell-specific markers on CCGM-NIR dye nanoconjugates, and in vitro efficacy was assessed using the MTT assay.

Results: The optimized CCGM protocol produced an enriched membrane fraction with retained membrane-anchored proteins (EpCAM, Cadherin) while depleting significant mitochondrial and cytoplasmic components. DLS and TEM analyses revealed a biconcave cup-shaped structure with a mean diameter of 120 ± 20 nm, while zeta potential measurements indicated a surface charge of -13.1 ± 3 mV. PTA uptake was assessed via fluorescence confocal microscopy, and in vitro PTT efficacy was evaluated using both EpCAM-positive and -negative cancer cells.

Conclusion: The NIRF PTA dye-encapsulated biomimetic nanoformulation enables active tumor targeting via homotypic binding to the EpCAM moiety on cell surfaces, potentially broadening the application of PTT therapy for deep-seated tumors.

A009. Mechanics of Extracellular Matrix Dictates Radiation Response of Breast Cancer Cells

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#Presenting Author: Priyanka Kashid

Keywords

- stiff ECM
- mechanotransduction
- radio-resistance

Background: Extracellular matrix (ECM) stiffening is a peculiar feature of breast tumor microenvironment that facilitates cancer progression. Stiff ECM is instrumental in potentiating survival, proliferation, contractility, invasion, and stemness of cancer cells. However, the role of ECM rigidity in regulating radio-response of cancer cells remains largely intangible and needs in-depth investigation.

Materials and Methods: To recapitulate the stiffness of normal breast stroma (0.5 kPa) and metastatic breast tumor (5 kPa), stiffness-tunable hydrogels were fabricated. To elucidate the impact of ECM stiffness on radio-response, various functional and molecular assays including cell survival, apoptosis, migration, immunofluorescence, cell cycle

profiling, immunoblotting, and ribonucleic acid sequencing were performed.

Results: Breast cancer cells cultured on stiff scaffold (5 kPa) when irradiated, showed significantly decreased apoptosis with concomitantly higher clonogenic survival elevated proliferation and migration compared with that of on soft scaffold (0.5 kPa). Furthermore, immunofluorescence and immunoblotting confirmed low levels of γH2AX in irradiated cells under stiff ECM conditions than soft ECM, suggesting attenuated deoxyribonucleic acid damage. Collectively, these findings confirm poor radiation response in cells experiencing stiff ECM. To delineate the molecular mechanisms underlying observed ECM stiffness-induced radio-resistance cells were subjected to transcriptomics, which revealed differential regulation of genes involved in cell cycle regulation, cell death, organelle assembly, and biogenesis.

Conclusion: We demonstrate that breast cancer cells acquire radio-resistance when exposed to stiff ECM, which is reflected from mechanotransduction-driven survival advantages postirradiation. We further show that these changes are associated with differential transcriptional regulation of the canonical and noncanonical pathways, which warrants further investigation; opening new avenues for mechanotargeting.

A010. Ets21c Governs Blood Cell Homeostasis and Innate Immune Response in *Drosophila*

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Keywords
- Ets21c
- hematopoiesis
- AMP (antimicrobial peptides)
- stress-induced hematopoiesis

Background: A harmonious interplay of signaling mechanisms sustains tissue homeostasis. Unraveling the regulators of tissue homeostasis during development and upon stress response remains a significant challenge. *Ets21c* is a stress-inducible transcription factor implicated in various physiological processes. This study aims to elucidate the role of *Ets21c* in hematopoiesis and immune homeostasis.

Materials and Methods: We use whole animal mutants of ets21c, Ets21c knockdown or overexpression lines for spatial modulation of Ets21c using the UAS-Gal4 system in *Drosophila*. We have analyzed the hematopoietic parameters with antibody markers using immunofluorescence and confocal imaging-based approaches. To look at antimicrobial peptide levels, quantitative real-time reverse transcription polymerase chain reaction-based approach was used in the presence or absence of bacterial infection.

Results: Ets21c overexpression in the lymph gland (LG) progenitors resulted in a significant increase in plasmatocyte and lamellocyte differentiation, accompanied by decreased crystal cell differentiation. Differentiated hemocytes respond to stressors, such as bacterial infections and wasp infestation. Perturbing *Ets21c* levels in the LG affected number of niche cells and extent of differentiation underscoring its essential role in hematopoiesis. Consistent with existing literature, LG-specific *Ets21c* expression was significantly elevated upon bacterial infection. Our investigation upon exposure of *ets21c* loss-of-function mutant larvae to Gram-negative bacteria (*Providencia rettgeri*) revealed that Ets21c is an important regulator of infection-induced emergency hematopoiesis and immune response.

Conclusion: Our study highlights the critical role of *Ets21c* in developmental hematopoiesis. Furthermore, *Ets21c* is pivotal in mounting immune responses by promoting blood cell differentiation and regulating antimicrobial peptide expression during cellular and humoral immune responses.

A011. Redefining Platinum Chemotherapy: A Shift Toward Kinetically Inert Agents

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Keywords
- pt drugs
- kinetic inertness
- plasma stability
- in vivo efficacy
- nephrotoxicity

Background: Despite spectacular clinical success, the efficiency of Pt drugs is masked by intrinsic and acquired resistance and toxic side effects like nephrotoxicity. Finding a strong correlation between the kinetic lability, therapeutic effect, and the deficiencies of Pt drugs, we aimed to design a kinetically inert yet efficacious Pt drug candidate to address the issues of Pt chemotherapy.

Materials and Methods: Compound characterization was done using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, high-performance liquid chromatography, and inductively coupled plasma-mass spectrometry. In vitro studies were performed using MTT assay. In vivo studies were done ethically using mice xenograft models. Docking studies were implemented using AMDock v.1.5.2 with Autodock 4.2. Confocal laser scanning microscopy was utilized for imaging purposes.

Results: A kinetically inert Pt drug candidate compound **4** was synthesized and characterized. The compound showed promising in vitro anticancer activities in a panel of different cell lines, better than cisplatin. Owing to its kinetic inertness, compound **4** was able to overcome multifactorial Pt resistance and possessed notable plasma stability. Noteworthy, compound **4** demonstrated promising antitumor efficacy in vivo both in Pt-sensitive and -resistant models and alleviated nephrotoxicity, which is the major dose-limiting side effect of cisplatin. Mechanistic investigations indicated the multitargeting ability of compound **4**, targeting nuclear deoxyribonucleic acid (DNA) including QDNA, and causing

oxidative stress in cancer cells through mitochondrial dysfunction.

Conclusion: Our results suggested that the development of kinetically inert yet efficacious and multitargeted Pt drug candidates is feasible. This will offer the prospects of overcoming the deficiencies of clinical Pt drugs like resistance and toxic side effects like nephrotoxicity.

A012. Target.AI: Drug Sensitivity Prediction for Targeted Cancer Therapy

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Keywords

- target.AI
- cancer targeted therapy
- artificial intelligence

Background: The aberrant alteration in genes, such as epidermal growth factor receptor (EGFR), resulting from somatic mutations, is associated with driver phenotype, making them a critical target in cancer therapy. This study aims to predict the response of tyrosine kinase inhibitors (TKIs) in cancer patients through an easy-to-use Web server utilizing artificial intelligence (AI) techniques.

Materials and Methods: We developed an automated and scalable server, including modeling, molecular docking, and molecular dynamics (MD) simulations, to elucidate the interactions of EGFR mutants ($N \sim 750$) with TKIs. These EGFR mutation models, molecular docking scores, and MD simulation reveal an interesting correlation between in silico observations and clinical TKI sensitivity. We developed a machine learning (ML) model utilizing features of protein sequence, structure, and dynamics.

Results: The ML model achieves a clinical grade accuracy for the TKI-sensitivity prediction for mutations occurring in the kinase domain of EGFR. Using this model, we characterize the sensitivity of novel EGFR kinase domain mutations previously considered variants of unknown significance (VUS).

Conclusion: We present a new AI model capable of nonlinear interpretation from complex data, allowing us to predict the sensitivity of novel EGFR VUS observed during clinical follow-up of cancer patients. Further refinement and clinical validation of this model may provide valuable solutions to predetermine the drug sensitivity of patients in clinics.

A013. Effect of Bromelain on Transgenerational *C. elegans* on High-Glucose Diet

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#Presenting Auhtor: Sharanyaa Dutta

Keywords

- bromelain
- high-glucose diet
- transgenerational effects
- *Caenorhabditis elegans*

Background: High-glucose diets induce hyperglycemia-associated oxidative stress, inflammation, and neuropathy, exhibiting transgenerational effects. Phytocompounds have recently been shown to modulate glucose-induced stress at epigenetic and metabolic levels. This study aims to elucidate bromelain's ameliorative potential in mitigating glucose-induced physiological and oxidative stress alterations in *Caenorhabditis elegans*.

Materials and Methods: *C. elegans*, with fully annotated genome and short generation time, served as the model organism. F1 and F2 generations were exposed to high-glucose (0–500 mM) and bromelain (0, 0.05, 0.1 mg/mL) diets. Assays included glucose and bromelain toxicity, pharyngeal pumping, thrashing, and antioxidant estimation (DPPH) assay.

Results: Glucose toxicity assay revealed that *C. elegans* exhibited stress at 500 mM glucose in the subsequent F2 generation. Behavioral assays, including pharyngeal pumping and thrashing, showed a decreased pumping rate and an increased thrashing rate over a 1-minute observation, correlating with rising glucose concentrations. DPPH assay demonstrated a color shift from purple to light yellow, indicating enhanced antioxidant activity at higher bromelain concentrations due to DPPH radical neutralization. Nitric oxide assay showed yellow coloration, indicative of oxidative stress. Notably, increased bromelain concentrations reduced NO levels, suggesting bromelain mitigates glycemic stress by modulating inflammation and inhibiting iNOS activity.

Conclusion: Bromelain ameliorates the transgenerational effects of a high-glucose diet in *C. elegans*, mitigating metabolic disturbances in the F2 generation. Enhancements in glucose homeostasis and regenerative health suggest its potential as a dietary intervention to reduce metabolic stress, underscoring its therapeutic significance in managing heritable dietary impacts.

A014. Role of Transforming Growth Factor Beta Induced (TGFBI) in Cancer-Associated Fibroblast and Its Influence on TME

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Keywords

- NSCLC: nonsmall cell lung carcinoma
- NF: normal fibroblast

- CAF: cancer- associated fibroblasts
- TGFBI: transforming growth factor β-induced

Background: Nonsmall cell lung carcinoma has poor survival due to genetic alterations and heterogeneous tumor microenvironment (TME). Cancer-associated fibroblasts (CAFs) represent predominant cells in TME. Preliminary ribonucleic acid (RNA) sequence analysis has shown transforming growth factor beta induced (TGFBI)/BigH3 to be upregulated in lung cancer patient-derived CAFs. Hence, it is important to study the influence of TGFBI in TME.

Materials and Methods: To understand the role of TGFBI in CAFs, we used siRNA targeting TGFBI. Phenotypic changes were observed with respect to migration and contraction properties of CAFs employing transwell migration and collagen contraction assays. Analysis of RNA sequencing was performed to understand genes, and pathways regulated by TGFBI at molecular level.

Results: Higher expression of TGFBI was observed in CAFs compared with NF (normal fibroblasts) at both the cellular and secretory levels. Upon TGFBI knockdown, CAF showed reduced migration in the transwell migration assay. Also, reduced contractile property of CAF was observed upon TGFBI knockdown. From the transcriptomics analysis, we observed that immune-related pathways were upregulated while cell cycle-related pathways were downregulated upon knockdown of TGFBI in CAFs. Validation of differential gene expression was confirmed by quantitative real-time polymerase chain reaction.

Conclusion: Our study suggests that TGFBI may play a vital role in maintaining CAF phenotype and modulating immune response in TME. This study can highlight the understanding of CAF's expressed TGFBI role in TME and overall outcome on tumor growth.

A015. Investigating the Binding Potential of a Novel Peptide Targeting Platinum-Resistant Ovarian Cancer

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Keywords

- peptides
- platinum-resistant ovarian cancer
- peptide-receptor interaction
- GPCR

Background: Peptides have emerged as potential therapeutic agents due to their ability to bind to specific receptors on tumor cells. Using phage display technology, a novel heptapeptide was identified targeting platinum-resistant ovarian cancer. This study aims to identify the specific peptide receptors that are differentially expressed in resistant ovarian cancer populations and explore the functional effects of peptide-receptor interactions.

Materials and Methods: Fluorescence-activated cell sorting and confocal microscopy were performed to assess peptide-receptor interactions. The impact of peptide binding on cell viability, adhesion, and migration will be evaluated using MTT, PI staining, Matrigel, and wound healing assays. In silico target prediction and molecular docking are being conducted to identify putative receptors.

Results: The peptide binding was higher in early platinum-resistant (ER) cells compared with late-resistant (LR) and sensitive ovarian cancer cells. The binding was also significantly higher in tumor cells obtained from malignant ascites of high-grade serous ovarian cancer patients and the patient-derived xenograft model. In silico analysis using the *Swiss-target prediction* tool indicated a 66.7% probability of the peptides binding to members of the GPCR (G-protein coupled receptor) family. Among several GPCRs associated with ovarian cancer, the endothelin type A receptor was identified as a likely target, showing strong polar interactions at the ligand-binding site.

Conclusion: This preliminary study suggests that the peptide has the potential to specifically bind to a subset of ovarian cancer cells. The ongoing investigation aims to identify the peptide-targeted putative receptor(s) and assess the functional consequences of peptide-receptor interaction.

A016. Can Insight: Insights into Cancer Biology through Transcriptome

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Keywords

- transcriptomics
- artificial intelligence
- cancer classification
- cancers of unknown origin

Background: Transcriptomics offers a dynamic snapshot of gene activity, bridging the genomic blueprint and functional outcomes. Ribonucleic acid sequencing (RNA-seq) analysis often involves isolated interpretations, such as cancer subtype prediction or tumor immune profiling. We developed an artificial intelligence (AI)-based model to deliver quick, integrative insights into tumor biology through transcriptomics. Using well-annotated data sets, our model refines cancer classification and predictive features like microsatellite instability (MSI) and tumor mutational burden (TMB), addressing high-dimensional data challenges and enabling personalized oncology applications.

Materials and Methods: Our framework leverages transcriptomic data from TCGA (The Cancer Genome Atlas) RNA-Seq experiments across 28 cancer cohorts. Machine learning models classify tissues of origin, detect malignancies, and stratify samples by cancer type, histology, and molecular traits such as human papillomavirus status, metastasis, MSI, and TMB. Model accuracy and precision are systematically evaluated using statistical methods.

Results: The AI system achieves an average classification accuracy of 90%, outperforming traditional methods in scalability and accessibility. It excels in analyzing cancers of unknown primary (CUP), a clinical challenge, by identifying tissue origins and molecular characteristics. A user-friendly Web server provides real-time predictions and visualizations, making the framework accessible for biological and clinical use without requiring programming skills.

Conclusion: This study integrates transcriptomics and machine learning to address high-dimensional data challenges in pan-cancer diagnostics. It bridges molecular profiling and clinical applications, providing a cost-effective, scalable solution. The framework is particularly impactful for CUP, enabling precise tissue and molecular feature inference.

A017. Understanding Sperm Chemotaxis with a Novel Microfluidics Device

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Keywords
- metabolomics
- chemotaxis
- microfluidics sperm selection
- IVF/ICSI

Background: In natural conception, chemotaxis selectively attracts capacitating sperm with the highest fertilization potential and guides them toward the egg, enhancing fertilization success. In infertile individuals undergoing in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI), conventional sperm selection methods lack this mechanism, potentially reducing success rates. Identifying chemotactic compounds for assisted reproduction remains challenging due to limited tools.

Materials and Methods: Hydrophilic and hydrophobic metabolites extracted from ovulatory-phase oviductal fluid were identified by liquid chromatography-tandem mass spectrometry and analyzed using XCMS. The chemotactic potential of some selected metabolites was evaluated by exposing capacitating sperm to metabolite gradients in a microfluidics device developed in our laboratory. Sperm response was analyzed by assessing directionality and straight-line velocity.

Results: The first compound evaluated, N-formyl-L-aspartate, exhibited a bell-shaped curve, with maximum chemotactic response observed at 0.01 M gradients for rat and human sperm. The second compound, X (name withheld), induced a strong chemotactic response at nanomolar concentrations in both rat and human sperm. The third compound, cinnamyl isovalerate, did not show chemotactic activity but promoted capacitation-like characteristics in sperm, suggesting its potential role in sperm capacitation. Both the fourth compound, tulobuterol, and the fifth compound, ferulic acid, failed to induce chemotaxis, indicating they do not act as chemoattractants.

Conclusion: Our study identified several promising metabolites, with N-formyl-L-aspartate and compound X showing chemotactic responses in both rat and human sperm. These findings suggest the potential of chemoattractants in sperm sorting to selectively isolate good-quality sperm with maximal fertilization potential, thereby improving IVF/ICSI outcomes and ultimately increasing "take-home baby" rates.

A018. Assessing the Comparative Bioactivity of Trastuzumab Biosimilars Using a Cell-Based Inhibition of Proliferation Assay

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Keywords
- trastuzumab
- biosimilars
- biological activity
- bioassay

Background: Trastuzumab is instrumental for the treatment of HER2-positive breast cancers, and growing global demand has led to the emergence of its biosimilars. It is essential to ensure these biosimilars are as safe and effective as the original drug. This study focuses on developing a cell-based bioassay to compare the efficacies of trastuzumab biosimilars with the reference product.

Materials and Methods: To assess the bioactivity of biosimilars, a comprehensive characterization of several HER2-positive breast cancer cell lines was done using Western blotting, reverse transcription polymerase chain reaction (RT-PCR), and flow cytometry to select an appropriate cell line for the bioassay. Further, to evaluate the bioactivity of trastuzumab biosimilars an antiproliferation bioassay was performed using Alamar Blue.

Results: The molecular characterization of various HER2-positive breast cancer cell lines of SKBR3, BT-474, MDA-MB-453, MDA-MB-175, MDA-MB-231, and MCF-7 explored HER2 receptor expression using Western blotting, RT-PCR, and flow cytometry to identify a suitable cell line for a bioassay. The SKBR3 cell line, which abundantly expressed HER2 receptor, was selected for this purpose. An inhibition of proliferation bioassay using Alamar Blue assessed the activity of trastuzumab biosimilars against a reference standard of trastuzumab. Four biosimilars were evaluated and their relative potencies were determined using 4PL nonlinear sigmoid curve analysis. The trastuzumab biosimilars were found to function similarly to the reference standard of trastuzumab based on their relative potencies.

Conclusion: The efficacies of different trastuzumab biosimilars are found to be similar to the reference standard of trastuzumab in terms of potency, thus establishing a robust cell-based bioassay method for trastuzumab biosimilars.

A019. Deciphering the Unexplored Mechanisms of Venetoclax-Azacitidine Combination Therapy Resistance in Acute Myeloid Leukemia (AML)

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Keywords

- acute myeloid leukemia
- venetoclax-azacitidine
- mitocurcumin

Background: Acute myeloid leukemia (AML) being an aggressive blood cancer, requires immediate diagnosis and treatment. The combination therapy of venetoclax-azacitidine (Ven-Aza), although highly effective in newly diagnosed (ND) patients, has grim response in relapsed-refractory (RR) patients. In this study, we have delineated the unexplored intricacies of the acquired resistance to Ven-Aza combination therapy.

Materials and Methods

- Cytotoxicity and apoptosis assays
- Cell proliferation and cell cycle assays
- Cellular reactive oxygen species (ROS), mitochondrial ROS, and JC-1 staining assays
- Immunoblotting
- BH3 profiling assay
- Phase contrast microscopy, electron microscopy, live cell imaging, and confocal microscopy

Results and Conclusion: All Ven-Aza resistant (VAR) AML cells had higher IC50 values for Ven-Aza compared with Ven-Aza sensitive (VAS) cells. They were smaller in size than VAS cells, and had high levels of cellular ROS, mitochondrial ROS, and hyperpolarized mitochondria. They also expressed low levels of BCL2, DNMT1, and mitochondrial fusion proteins; and increased levels of BFL1, MCL1, and mitochondrial fission proteins. BH3 profiling assay demonstrated an enhanced apoptotic dependency on BFL1 and MCL1 in VAR cells. Electron micrographs showed that VAR cells had more mitochondria per cell and decreased mitochondrial length, area, and perimeter compared with VAS cells. Mitocurcumin elevated mitochondrial ROS, collapsed mitochondrial membrane potential, and triggered oxidative stress signaling, thereby activating intrinsic apoptotic pathway in all VAR cells. Our study unravels the contribution of mitochondria in Ven-Aza resistance. We also propose use of mitocurcumin as a leading molecule for the treatment of Ven-Aza-resistant AMLs.

A020. Aggregation-Induced Emission (AIE)-Based Fluorescence Probe for Dual Sensing of Protamine and Trypsin

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Keywords

- protamine
- trypsin
- aggregation-induced emission
- fluorescence sensor

Background: Protamine and trypsin play critical roles in clinical and biological processes, such as neutralizing heparin and regulating enzyme activity, respectively. Nevertheless, current detection strategies face the challenges of having low sensitivity, high costs, and time-intensive procedures.

Materials and Methods: A sensing system based on fluorescence was developed utilizing a cationic derivative of allyl pyridinium tetraphenylethylene (ALPTPE) in conjunction with anionic dextran sulfate sodium (DSS) to detect protamine and trypsin by aggregation-induced emission mechanism. The evaluation of the system's sensitivity and selectivity was conducted across diverse environmental conditions and confirmed through the use of actual human urine samples.

Results: The results showed that ALPTPE formed aggregates with DSS due to charge neutralization, leading to enhanced fluorescence emission. Upon adding protamine, significant decrease in fluorescence intensity is observed due to disruption of the ALPTPE-DSS complex, leading to its disassembly and causing the observed "turn-off" in fluorescence. A notable increase in fluorescence emission is observed upon the addition of trypsin, which can be attributed to the progressive disassembly of the DSS-PrS complex, driven by the enzymatic cleavage of protamine by trypsin. The sensor system showed a linear response range from 0.1 to 10 µg/mL for protamine and from 0.01 to 1 µg/mL for trypsin. The method demonstrated high selectivity toward other proteins and enzymes, exhibiting minimal interference.

Conclusion: This fluorescence-based sensing platform provides a rapid, precise, and highly sensitive approach for the detection of protamine and trypsin, offering an efficient alternative to conventional methods.

A021. Deciphering Drug-Resistance Mechanisms in Fluoroquinolone (FQ)-Resistant DNA Gyrase Mutants in *Mycobacterium tuberculosis*: Paving the Path to the Design of Better Drugs to Combat Drug Resistance (DR)

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Keywords

- fluoroquinolones
- moxifloxacin
- levofloxacin

► drug-resistance
► molecular dynamics (MD) simulations (MDS)

Background: The complexes of fluoroquinolones (FQs) moxifloxacin (MFX) and levofloxacin (LFX) (used in India) with *Mycobacterium tuberculosis* deoxyribonucleic acid (DNA) gyrase mutants—GyrA subunit mutants A90V, S91P, D94A, D94G, D94H, D94N, and D94Y—were simulated through molecular dynamics simulation (MDS) to analyze the mechanisms of drug resistance (DR), to possibly facilitate DR-resistant drug design.

Materials and Methods: Plain and steered (SMD) all-atom MDS facilitated the analysis of DNA gyrase-DNA-drug complexes of the wild-type (WT) and the drug-resistant mutants. Plain MDS yielded occupancies of drug-enzyme noncovalent interactions (NCIs), while SMD simulated the drug's persistence in the binding site, via NCIs.

Results: MFX and LFX require an Mg^{2+} ion bound near the carboxylate group of the drug to bind D94 via a strong electrostatic interaction. The mutants are located at or near this electrostatic interaction and either directly (D94 position mutants) abrogate this bond or create misalignments in orientation that abrogate other NCIs nearby. D94 mutants attract the carboxylate group (via Mg^{2+}) toward D89, altering the drug's orientation in its binding pocket, breaking NCIs, and facilitating the drug's earlier egress from the pocket, compared with the WT. An MFX-derivative was similarly tested, to attempt tighter DNA gyrase-binding, as seen with SMD.

Conclusion: The D94 mutants directly abrogate the key drug-D94 electrostatic attraction, while A90V and S91P disrupt neighboring and auxiliary NCIs. An alternative binding mode for D94 binding that retains the orientations of the drug as seen in the WT, along with contacts to immutable residues, might be beneficial.

A022. Deciphering the Role of Dab2 in Skin Tumor Initiation and Stem Cells in Squamous Cell Carcinoma

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Keywords
► disabled-2
► squamous cell carcinoma
► tumorigenesis
► gene set enrichment analysis

Background: Dab2 is an endocytic adapter protein widely expressed across mammalian tissues. It is involved in endocytosis of receptors of key signaling pathways. Dab2 is upregulated in human and mouse hair follicle stem cells. However, loss of Dab2 has been reported in several cancer types. The role of Dab2 in tumorigenesis and cancer stem cells (CSCs) regulation is still obscure.

Materials and Methods: We have used a chemically induced murine skin squamous cell carcinoma (SCC) model harboring an inducible K14 bound Cre-LoxP system for the conditional knockout (cKO) of Dab2 in the skin. Further, we isolated stem-like cells from 7,12-dimethylbenz[a]anthracene (DMBA)/ 12-O-tetradecanoylphorbol-13-acetate (TPA) treated wild-type (WT) and Dab2 cKO mouse skin at various time points. Moreover, we performed total ribonucleic acid (RNA) sequencing on the sorted stem-like cells.

Results: No significant difference was observed in the percentage of stem-like cells isolated from WT and Dab2cKO mouse skin treated with DMBA/TPA. However, expression profiling revealed positive enrichment of proliferative and metabolic pathways in the WT stem-like cells. Moreover, positive enrichment of epigenetic pathways and deoxyribonucleic acid damage repair pathways and negative enrichment of proliferative and metabolic pathways was observed in the Dab2 cKO stem-like cells.

Conclusion: Loss of Dab2 has been reported in several cancer types, suggesting its role as a putative tumor suppressor. Hitherto, our data showed that loss of Dab2 delays tumor initiation and progression in mouse SCC. Loss of Dab2 alters the expression profile of stem-like cells isolated from DMBA/TPA WT and Dab2 cKO skin, suggesting its role in CSCs regulation during tumorigenesis.

A023. The Role of Cancer Cell Driver Mutations in Tumor Microenvironment

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Keywords
► cancer-associated fibroblast
► tumor microenvironment
► KRAS

Background: Tumor microenvironment (TME) is a complex ecosystem surrounding tumor cells with extracellular matrix and stromal cells including fibroblast, immune cells, and endothelial cells. Nonsmall cell lung cancer (NSCLC) harbors diverse genetic alterations and heterogeneous TME. We want to understand the role of driver mutations in shaping the TME in NSCLC.

Materials and Methods: We performed CIBERSORTx deconvolution analysis to plot the abundance of stromal cell signatures with patient groups mutated with different oncogenes. We generated doxycycline-inducible KRAS knockdown Calu1 and A549 cells to perform different functional assays with normal fibroblast (NF) to understand the impact of KRAS mutations on fibroblast modulation.

Results: The in silico deconvolution analysis using CIBERSORTx for TCGA (The Cancer Genome Atlas) Pan-Cancer Atlas data showed that the KRAS mutant patient group are associated with the most abundant cancer-associated fibroblast gene signature. The collagen contraction assay showed that the mutant KRAS tumor cells grown with NFs can modulate the contractility of fibroblast when compared with KRAS knockdown tumor cells cocultured with NFs.

Conclusion: Oncogenic KRAS in NSCLC plays an important role in the modulation of fibroblast behavior in the TME.

A024. Can RT-PCR Serve as Alternate Gold Standard for Validation of ER/PR Results?

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Keywords
- ER/PR
- RT-PCR
- breast cancer
- IHC

Background: Estrogen/progesterone (ER/PR) are critical for patient management, but no molecular standard exists for result confirmation besides next-generation sequencing. This study aimed to use an alternate approach for the evaluation of ER/PR status in breast cancer cases using messenger ribonucleic acid-based reverse transcription polymerase chain reaction (RT-PCR) testing as a point-of-care tool to serve as an adjunct to conventional immunohistochemistry (IHC).

Materials and Methods: The study included 60 histologically confirmed breast cancer samples stored at the Pathology Department of Tata Memorial Hospital. Formalin-fixed, paraffin-embedded blocks with IHC data confirming the expression of ER/PR were analyzed. Concordance analysis, sensitivity, and specificity were calculated for each marker. Kappa statistics was employed to assess the overall agreement.

Results: Sixty breast cancer samples were examined, all providing conclusive outcomes for ER and PR status. Standardization involved using 10 confirmed negative samples in IHC, establishing a cycle threshold of 34; cycles exceeding 34 were deemed negative, while those below 34 were deemed positive. Moderate concordance rates were observed, with ER achieving 81.67% and PR achieving 73.34%. Sensitivity for ER was 91.66% and PR was 73.91% and specificity for ER was 91.66% and PR was 86.48%, indicating robust accuracy for ER. Kappa statistics demonstrated perfect agreement for ER and substantial agreement for PR between the two systems.

Conclusion: The study demonstrates that RT-PCR-based system exhibits moderate concordance with IHC for ER and PR, possibly due to number of low positive cases seen in IHC. Further understanding and testing are necessary to establish this assay as an independent tool for conducting transcript-based biomarker detection instead of IHC.

A025. Positive Feedback Regulation as a Driver Mechanism Behind Drug Resistance in HER2 Positive Breast Cancer

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Keywords
- drug resistance
- EZH2
- feedback regulation
- HER2
- STAT3
- TRIM-ing

Background: HER2 positive breast cancer (BC) accounts for 20 to 25% BC cases worldwide. The personalized treatment strategy has improved disease outcomes significantly but the clinical challenge of drug resistance continues. In this context of drug resistance, our study focuses on regulatory roles of downstream signaling molecules driven by HER family receptors.

Materials and Methods: Trastuzumab (R.I.-80) and neratinib (R.I.-10) drug-resistant HER2+ve BC cell models were established. Immunoblot and immunofluorescence was performed to check differentially expressed proteins of HER2-STAT3 signaling axis. TRIM-ing of candidate proteins led us to the validation of preliminary observations. Bioinformatics analyses were performed to delineate the role of STAT3, driving HER2 drug resistance.

Results: In resistant cell models, heregulin-β1 ligand-driven phospho-STAT3 activation and HER2 TRIM-ing-mediated p-ERK, p-Akt, and p-STAT3 inhibition suggested STAT3 as a downstream target of HER2-HER3 signaling axis. STAT3 TRIM-ing showed significant ($p < 0.05$) abrogation of upstream kinases, indicating STAT3-driven positive feedback loop. Bioinformatic analysis of STAT3 interaction network revealed enhancer of zeste homologue 2(EZH2) as a lead interactor, which was overexpressed and constitutively activated in resistant cell model. EZH2's relevance in HER2-STAT3 axis was verified by inhibiting Akt. Also, STAT3 binding motifs in EZH2 and HER2 promoter region suggested their transcriptional regulation by STAT3, imparting the positive feedback loop.

Conclusion: Our results underscores STAT3-mediated HER family receptor regulation, where EZH2's significant role was also realized. Thereby, the study highlights a STAT3-driven positive feedback loop, impacting HER2 drug resistance.

A026. Influence of TP53 Mutations on Tumor Stromal Interactions in PDAC

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Keywords
- PDAC
- TME
- TP53
- CAFs

Background: Pancreatic ductal adenocarcinoma (PDAC) is characterized by highly desmoplastic stroma and

cancer cell mutations like TP53, the major causes for metastasis and chemoresistance. PDAC is a highly heterogeneous cancer, it is important to understand how different TP53 hotspot mutations might play a role in cell and noncell autonomous-mediated tumor progression.

Materials and Methods: To study the impact of TP53 hotspot mutations on cancer-associated fibroblasts (CAFs), the major stromal population, TP53 KO pancreatic cancer cell was generated by CRISPR/Cas9 technology and transduced with recombinant lentiviruses to reexpress different mutant p53 proteins. Subcutaneous and orthotopic injections were standardized in nonobese diabetic-severe combined immunodeficiency (NOD-SCID) and C57/BL/6J mice, respectively.

Results: Migration assay showed higher migration rates in p53 reexpressed pancreatic cancer cells compared with p53 KO cancer cells. Also, migration of CAFs was seen to be more toward mutant p53 reexpressing cancer cells than p53 KO cells. Increased messenger ribonucleic acid expression of CAF activation markers was observed when cocultured in a contact-dependent manner with p53 reexpressed cancer cells as compared with p53 KO cells. For in vivo studies, successful tumors were obtained within a justifiable period of time from subcutaneous injection in NOD-SCID mice as well as orthotopic injection in pancreas of C57/BL/6J mice.

Conclusion: TP53 mutations in cancer are reported to have different transcriptional signature. Hence, it is expected that different p53 mutations in cancer cells might play differential role in rewiring of stroma. Coculture studies followed by RNA sequencing might determine how tumor microenvironment is impacted based on the p53 status in cancer cells.

A027. Raman Spectroscopy-Based Detection of Chronic Myelogenous Leukemia: Insights from India

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Keywords

- CML
- Raman spectroscopy
- PCA
- LDA
- patients
- healthy volunteers

Background: Chronic myelogenous leukemia (CML) is a myeloproliferative disorder marked by the Philadelphia chromosome, a shortened chromosome 22 formed by reciprocal chromosomal translocation. Diagnostic tools for CML include increased myeloid cell count, immature myeloid cells in peripheral blood, and detection of the transgene from translocation. Initially, the disease is managed with tyrosine kinase inhibitors (TKIs), but resistance to TKIs can lead to disease progression. Resistance detection methods are varied and complex, posing challenges for resource-poor countries with limited health care facilities. A universal resistance detection method, regardless of mechanism, would be beneficial.

Materials and Methods: Raman spectroscopy, requiring minimal sample preparation, captures the biochemical profile of a sample and has shown potential for cancer detection. It can also differentiate between TKI-resistant and sensitive cell lines. In this study, a Raman microscope (Witec α300R, Ulm, Germany) was used to identify biochemical changes in CML by analyzing spectral data ($\lambda = 532$ nm, 8 mW, 1200 grooves/mm, 10 second acquisition, 10 integrations, cm^{-1}) of cells, serum, and plasma from CML patients, compared with healthy volunteers.

Results: Multivariate analysis techniques such as principal component analysis and principal component-based linear discriminant analysis (PC-LDA) were employed for analysis of the spectral data. PC-LDA showed 95 to 100% classification efficiency, depending on the type of sample.

Conclusion: Our findings demonstrate the utility of Raman spectroscopy in the detection of CML. Future directions will focus on checking its potential to segregate TKI-sensitive and -resistant patients in a larger patient cohort.

A028. Identification of Mechanisms by Which LCN2 Promotes Autophagy and Tumor Progression in Colorectal Cancer

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Keywords

- lipocalin2 (LCN2)
- autophagy
- colorectal cancer (CRC)
- tumor progression and therapy resistance

Background: Previous studies of our laboratory have demonstrated that loss of PKP3 led to an LCN2-dependent tumor formation; LCN2 inhibition using monoclonal antibody led to colorectal cancer (CRC) tumor regression and increased 5-fluorouracil (5-FU) sensitivity. It was observed that LCN2-associated increased autophagy led to radio-resistance. LCN2 and autophagy can be potential targets in CRC treatment.

Materials and Methods: Autophagy flux is evaluated in LCN2 overexpressing and knockdown cell lines using Western blotting, immunofluorescence staining, live-cell imaging, and electron microscopy. Validation experiments are performed in the KPC:APC mice model. Wound-healing assays for cell migration, transwell assays for cell invasion, and clonogenic assays for therapy resistance are performed.

Results: Upon IC_{50} treatment of 5-FU, increased LC3-puncta was observed using confocal and electron microscopy. Real-time autophagy flux was studied; cells were transfected with mCherry-EGFP-LC3 probe and autophagy flux was found to be more in 5-FU-treated cells. Using Western blotting, levels of autophagic proteins were found to be increased upon 5-FU treatment in LCN2 high cells. Using purified recombinant wild-type LCN2 pulldown, it was

determined that LCN2 interacts with autophagic proteins like ATG4B. Elevated LC3 levels are observed in colon tumors of KPC:APC mice. Autophagy inhibition using chloroquine significantly reduces the migrative and invasive potential of the cells.

Conclusion: Increased autophagy is observed in LCN2 high cells upon drug treatment. Autophagy is required for LCN2-associated increased cell migration and invasion implying that there might be feedback loop mechanism. Increased LC3 in KPC:APC mice tumor indicates autophagy as potential target in CRC. LCN2 interacts with autophagic proteins suggesting its direct role in regulating autophagy.

A029. Investigation of Cancer Stem Cell-Associated Drug Resistance Genes in Gastric Cancer Cell Lines

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Keywords
- gastric cancer
- cancer stem cells
- 5-FU
- TYMP
- TYMS

Background: Gastric cancer (GC) is challenging to treat due to acquired drug resistance and lack of alternate therapy. Cancer stem cells (CSCs) are major factors imparting resistance. Identifying GC stem cells (GCSCs) and specific genes involved in GCSC-mediated drug resistance is crucial and may be targeted to combat the disease.

Materials and Methods: CSC-like cells were isolated from GC cell lines using side population assay and pulse-chase label retention assay, validated using pluripotent stem cell markers by quantitative polymerase chain reaction. 5-Fluorouracil (5-FU) resistance genes were analyzed in AGS/AGS^{5FU} and KATO III/KATO III-SP. The effect of 5-FU resistance genes will be validated in GC orthotopic mice model.

Results: Using SP assay, KATO III showed 15 to 18% CSC-like cells with increased pluripotent stem cell genes. AGS^{5FU} showed higher percentage of PKH^{+} cells as compared with AGS. PKH^{+} cells exhibit increased expression of pluripotent stem cell markers as compared with PKH^{-} cells, indicating dye-retaining cells showing stem cell-like characters. Expression of thymidine phosphorylase (TYMP) (responsible for the conversion of 5-FU to its active form) and thymidylate synthase (TYMS) (inhibited by 5-FU) were significantly altered between sensitive and resistant cell lines. AGS^{5FU} cells showed approximately 3.8-fold decreased TYMP and approximately 1.4-fold increased TYMS as compared with AGS. GC orthotopic mice models are being developed to study 5-FU resistance in tumor progression.

Conclusion: Our preliminary data suggested that TYMP and TYMS genes could be involved in causing 5-FU resistance in AGS^{5FU} cell line. Deciphering the detailed mechanism by which CSCs can cause 5-FU resistance is under investigation.

A030. Investigating the Role of Transcription Factor Ets21c in the *Drosophila* Hematopoietic Niche

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Keywords
- Ets21c
- ERG
- hematopoiesis
- drosophila

Background: Human ERG oncoprotein, mammalian counterpart of the *Drosophila* Ets21c, has been previously shown to be upregulated during acute myeloid leukemia. The molecular function of Ets21c, a stress inducible transcription factor in regulating developmental hematopoiesis, remains unknown. Here, we delineate Ets21c function in the hematopoietic niche in the lymph gland.

Materials and Methods: We employ *ets21c* whole animal mutants, Ets21c knockdown or overexpression lines to spatially modulate Ets21c expression using the UAS-Gal4 system. We analyze the hematopoietic parameters using various antibody markers employing immunofluorescence and confocal imaging-based approach. The imaging data are then analyzed using ImageJ software.

Results: Ets21c loss of function mutants display a smaller niche as compared with wild-type and their blood cell differentiation was affected. This intrigued us to explore the role of ets21c in the niche. Upon ets21c overexpression, we observed a significant increase in the niche size as well as lamellocyte differentiation, which have been shown to be associated with hyperactivation of insulin signaling. When recombinant human ERG was expressed in the niche specifically, similar results were obtained. We further aim to delineate the role of Ets21c during development focusing on niche and unravel new conserved mechanisms with its mammalian counterpart, ERG.

Conclusion: We have investigated the role of Ets21c in the niche during development. Our results suggest that ERG and Ets21c act similarly in regulating the niche cell proliferation as well as expansion. This study will shed insights on unmasking novel mechanism regulated by ERG and its role during development via Ets21c, its fly counterpart.

A031. Systematic Molecular Profiling of Thyroid Cancer Identifies Therapeutic Targets in Indian Cohort

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Keywords

- thyroid cancer
- integrative sequencing
- RET
- DUOX2
- THRA
- mutation profile
- actionable biomarkers

Background: Thyroid cancer (TC) is the most common endocrine malignancy originating from the parafollicular C cells or follicular cells of the thyroid gland. The incidence rate has steadily increased by 30% in India in the past decade, raising an interest to study the molecular alterations underlying the genome of Indian patients for therapeutically relevant biomarkers in TC.

Materials and Methods: We performed integrative whole-exome and whole transcriptome sequencing of fresh frozen and formalin-fixed, paraffin-embedded tissue samples for genomic characterization of TC. Using computational tools, we characterized the mutational profile of different subtypes of TC followed by molecular pathway analysis and understanding the tumor immune microenvironment. Also, we structurally characterized few novel mutations.

Results: The mutational landscape of TC revealed that somatic *RET* mutation (50%) was predominantly observed in MTC subtype, alongside low frequency of mutations in *RAS* and *BRAF*. Notably, we reported a novel *RET* kinase domain mutation Y900S showing affinity to *RET* inhibitors accessed via docking and molecular dynamics (MD) simulation. The ATC subtype revealed somatic alterations in major hallmark genes like *TP53* (~42%), *BRAF* (~10%), and *RAS* (~27%), along with novel pathogenic mutation in *THRA* (11%). In the PTC cohort, we identified somatic hallmark alterations in *BRAF* (38.6%) and *RAS* (26.3%) along with non-hallmark alterations-driven molecular subtype and a germline *DUOX2* (8.8%) mutation associated with poor prognosis.

Conclusion: Overall, our study provides a comprehensive molecular profiling of Indian TC patients underlying MTC, PTC, and ATC subtypes for underscoring potential therapeutic therapies.

A032. Comprehensive Genomic Profiling of 1,000 Indian Cancer Genomes Identify Opportunity of Precision Medicine: A Retrospective Cohort Study

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Keywords

- pan-cancer
- Indian ethnicity
- genomic profiling
- clinical and therapeutic markers
- computational tools

Background: Cancer patients with targetable biomarkers show much better responses to molecularly guided therapy. Global genomic profiling has cataloged novel biomarkers and driver genes in developed nations, enabling tailored therapeutic strategies. However, Indian ethnic diversity is poorly represented in such global genomic studies. This study aims to determine the landscape of molecularly targeted therapeutics using comprehensive genomic profiling to identify novel therapeutic opportunities.

Materials and Methods: We attempted genomic characterization of 1,000 Indian patient samples across 27

cancer types using whole exome and whole transcriptome sequencing. We developed an integrated genomics analysis computational pipeline and a clinical inference tool, ClinOme, to identify molecularly guided therapeutics.

Results: We identify recurrent high tumor mutation burden, microsatellite instability, and high PD-L1 expression in multiple cancers. Furthermore, we observe cancer hallmark genes such as *TP53* (40%), *PIK3CA* (13%), *CDKN2A* (12%), *KRAS* (8%), *EGFR* (7%), *BRAF*, *RET*, *ERBB2*, *MET*, *ALK*, *FGFR3*, *KIT*, *STK11*, *FGFR2*, and *ERBB3* at below 5% frequency in Indian cohort. Altogether, approximately 42% of Indian patients harbor therapeutically relevant alteration. However, we observe that only 8% of Indian patients have access to all therapeutic options based on Drugs Controller General of India approvals.

Conclusion: Our study provides strong evidence for disproportional somatic molecular biology associated with ethnic descent, further underscoring novel therapeutic opportunities in one of the world's largest ethnic populations.

A033. Early Diagnosis and Stratification of Oral Sequential Carcinogenic Changes in Hamster Buccal Pouch Model: A Serum Raman Spectroscopy Approach

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Keywords

- Raman spectroscopy (RS)
- oral cancer
- hamster buccal pouch (HBP)
- principal component analysis (PCA)
- principal component-based linear discriminant analysis (PC- LDA)

Background: Oral cancer, a prevalent malignancy with high mortality due to late diagnosis, often complicates treatment and worsens prognosis. Early detection is crucial, and Raman spectroscopy (RS) offers insights into biochemical changes preceding morphological alterations. Due to limitations in studying early oral malignancy stages in humans, the hamster buccal pouch model was used.

Materials and Methods: Forty-five female Syrian Golden hamsters were divided into three groups, consisting of 15 hamsters each: treated group with 0.5% DMBA, vehicle control, and untreated control for 14 weeks. Weekly retro-orbital blood collection and serum separation were done. Raman spectra were acquired, preprocessed, and analyzed using principal component analysis and principal component-based linear discriminant analysis for classification.

Results: Visual changes recorded show changes in the pouch due to treatment. No significant changes in weight were observed between the different groups. Stratification of the treated group was observed as early as the 4th week (~70%), with classification efficiency increasing to 80% in week 7 and 87% in the 10th week using a three-model approach. A two-model approach showed similar trends: 80% in week 4, 87% in week 7, and 93 and 87% in week 10 when compared with control and vehicle-control groups, respectively.

Conclusion: The stratification at distinct stages might be correlated with histopathological changes in the buccal pouch of hamsters. These findings suggest that serum RS can detect biochemical alterations during carcinogen treatment, while also highlighting its potential as an early diagnostic tool for oral cancer detection.

A034. Developing Novel Polymeric Biomaterials as Nonviral Gene Delivery Vectors for Enhancing Transfection in Immune Cells

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Keywords

- nonviral gene delivery
- transfection
- hard-to-transfect immune cells
- β-amino ester copolymers
- nanoparticles

Background: Gene delivery to immune cells boosts immune system's ability to combat diseases like cancer or autoimmune disorders. Viral vectors are effective, but have high immunogenicity, manufacturing issues, costly, and safety issues. Nonviral vectors are promising alternative, but struggle with transfection efficiency, heterogeneity, and immune activation risks.

Materials and Methods: To address this challenge, we have developed a novel polymeric biomaterial-based nonviral gene delivery system and assessed its gene delivery efficiency in hard-to-transfect immune cells (T cells and macrophages). We have synthesized a library of novel β-amino ester copolymers, which self-assembled into nanoparticles upon complexing with genetic material (GFP-pDNA).

Results: This novel β-amino ester copolymers were successfully synthesized via Michael addition of functionalized acrylates, amino-alcohols, and hydrophobic amines (lipids) in different molar ratios and with subsequent capping using different end-groups (1°, 2°, 3°, heterocyclic amines). Higher molar ratio of amino-alcohols with respect to lipids (1:0.5) in polymer (end-group heterocyclic amine) enhances the transfection efficiency of GFP-pDNA in J774A.1 macrophage cells by fourfold. In contrast, altering the end-cap group to amine chemical moieties (1°, 3°, and heterocyclic) in β-amino ester polymer improved the transfection efficiency of GFP-pDNA in primary murine splenocytes and human T lymphocytes (Jurkat cells).

Conclusion: We systematically varied polymer backbone hydrophobicity and end-cap structure to probe structure-function relationships that enhance the transfection efficiency. We screened them in a high-throughput manner in various immune cells for enhanced gene delivery. We will use the selected nonviral formulation that shows high transfection efficiency for application in cancer immunotherapy.

A035. Elucidating the Crosstalk between Epithelial Tumors and the Hematopoietic Organ, Lymph Gland in *Drosophila*

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Keywords
- crosstalk
- *Drosophila*
- gut tumor
- hematopoiesis
- ImpL-2
- lymph gland

Background: The hallmarks of cancer include tumor-promoting inflammation. Many epithelial tumors cause systemic effects in distant organs by secreting factors in circulation resulting in severe syndromes like cancer cachexia. These secreted factors could have an impact on the stem cell homeostasis and hematopoiesis, thereby contributing to systemic inflammation, which is fairly undercharacterized.

Materials and Methods: We use *Drosophila* as a model organism to understand in vivo interorgan communication between the tumor and the lymph gland. The *UAS-Gal4* system has been employed to model Yki^{3SA}-induced gut tumors using the stem progenitor-specific *esg-Gal4*. We use antibody-based markers to ascertain various hematopoietic phenotypes using immunofluorescence and confocal imaging-based approach.

Results: Our results demonstrate that the lymph gland of the larvae bearing gut tumor has a smaller hematopoietic stem cell niche as compared with the control and also higher differentiation of three blood cell types. Transcriptomic analysis of circulating hemocytes suggests the blood cells have a higher gene expression of tumor secreted factor ImpL2, an antagonist of insulin signaling that bind to *Drosophila* insulin (dILP2). Our data shows that systemic upregulation of ImpL2 results in a smaller lymph gland niche due to abrogation of insulin signaling causing an imbalance in blood cell homeostasis.

Conclusion: This study provides evidence that there is active crosstalk between the tumor cells and the lymph gland wherein there is hematopoietic remodeling characterized by smaller niche and increased differentiation leading to systemic inflammation.

A036. Pharmacological Activation of GPER1 (G Protein-Coupled Estrogen Receptor) Inhibits Prostate Cancer Progression

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Keywords
- GPER1
- chemoprevention
- prostate cancer
- TRAMP mice

Introduction: Prostate cancer (PCa) offers opportunities for chemoprevention due to its long latency period. GPER1 is being explored for its therapeutic potential in various cancers. In PCa cells, GPER1 activation by its agonist G1, leads to the inhibition of proliferation in vitro and in vivo. However, its role as a chemopreventive target in PCa is unexplored.

Materials and Methods: Prostatic GPER1 (pGPER1) expression was assessed in human data sets and PCa and benign prostatic hyperplasia (BPH) tissues. TRAMP (transgenic adenocarcinoma of mouse prostate) mice prostates were assessed for GPER1 expression using quantitative real-time reverse transcription polymerase chain reaction and flow cytometry. Stable GPER1-silenced clones were generated using shRNA in RWPE-1, LNCaP, and PC3 cell lines. Proliferation, migration, invasion, RT2 profiler, and zymography assays were performed. TRAMP mice subcutaneously administered with different doses of G1 were assessed for their tumor progression.

Results: A decreased frequency of GPER1-positive cells in high-grade PCa compared with BPH tissues was observed. A similar observation was found in the public expression data sets. In TRAMP mice, the frequency of pGPER1-positive cells and expression was found significantly increased at the HGPIN (high-grade intraepithelial neoplasia) stage and decreased at the poorly differentiated carcinoma (PDC) stage compared with respective age-matched controls. GPER1-silencing led to an increase in migration and invasion while proliferation remained unchanged in vitro. Further, GPER1 regulated epithelial to mesenchymal transition through miR200a-ZEB2-E-Cadherin loop and other metastasis-associated genes. GPER1 activation with G1 in TRAMP mice prevented progression of HGPIN to PDC.

Conclusion: GPER1 signaling offers a protective advantage by preventing PCa progression.

A037. Proteomics-Based Identification and Validation of Potential Biomarkers in Head and Neck Squamous Cell Carcinoma

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Keywords
- ELISA
- HNSCC
- iTRAQ
- MRM
- PRM
- SWATH

Background: HNSCC (head and neck squamous cell carcinoma) accounted for 1.5 million cases globally in 2020 and has poor survival rates in advanced stages. Early detection is critical for better outcomes. Proteomics provides a powerful tool for identifying biomarkers, enabling early diagnosis, prognosis, and targeted therapeutic strategies to combat resistance and metastasis.

Materials and Methods: Serum samples from oral cavity cancer patients at different time points of treatment will be enriched with acetonitrile. Mass spectrometry-based proteomics will be utilized for discovery and validation of proteins. Final biomarker verification using enzyme-linked immunosorbent assay (ELISA) will be performed, and the data will be correlated with survival analysis to assess prognostic relevance and clinical applicability.

Results: Serum enrichment was successfully optimized to ensure high abundant protein depletion, preparing the samples for advanced proteomic analysis. Preliminary evaluations confirm their suitability for iTRAQ and SWATH workflows. These complementary approaches will enable the identification and quantification of differentially expressed proteins. Selected candidate proteins from this analysis will undergo targeted quantification using multiple reaction monitoring or parallel reaction monitoring. Subsequently, ELISA will validate these findings, correlating protein expression levels with patient survival outcomes to uncover clinically significant biomarkers.

Conclusion: This study aims to identify and validate robust serum biomarkers for oral cavity cancer using advanced proteomic approaches. The integration of proteomic findings with survival analysis has the potential to enhance early diagnosis, improve prognostic assessment, and contribute to the development of personalized therapeutic strategies.

A038. The Impact of Physical Activity on Childhood and Adolescent Cognitive Growth and Its Link to Obesity

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Keywords
- physical activity
- obesity
- cognitive development
- age group
- gender

Background: The impact of physical activity and obesity on human brain has long been a debatable matter. There are several studies that either accept or refute the relation. It is influenced by several factors like age, gender, and socioeconomic status. This research aims to dwell on these topics.

Materials and Methods: An observational analytical study was conducted. A questionnaire was circulated among parents of children between 3 and 18 years of age. Incomplete forms and children suffering from previous diseases were excluded. The variables were compared, analyzed, and interpreted by using SPSS software.

Results: Considering the exclusion criteria a total of 292 responses were considered. A bivariate cross-tabulation was made comparing variables such as age group, gender, and socioeconomic status with engagement in physical activity. It was found that kindergarten children (81.8% of the kindergarten children) participated more in physical activity as compared with higher age groups. More boys (79.4%) participated in physical activity as compared with girls (60.7%). A weak correlation of –0.111 was found between engagement in physical activity and time taken to complete a topic. Children with higher than normal body mass index (BMI) took more time to finish a topic.

Conclusion: The findings implied that younger children engaged more in physical activity. Males were found to be more physically active as compared with females. Children with sedentary lifestyle and higher BMI were found to take longer time to understand a concept.

A039. Integrative Approaches for Biomarker Discovery and Drug Repurposing in Gastric Cancer

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Keywords
- gastric cancer
- biomarkers
- therapeutic targets
- drug repurposing

► network biology
► molecular docking
► virtual library

Background: Gastric cancer is a leading global health concern, with high morbidity and mortality rates. Early diagnosis and targeted therapies are crucial for improving outcomes. This study focuses on identifying reliable biomarkers and therapeutic targets through innovative strategies, contributing to early detection and advancing therapeutic approaches in gastric cancer research.

Materials and Methods: This study integrated network biology, gene enrichment, pathway enrichment analyses, molecular docking, virtual screening, molecular dynamics simulations, and Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) energy calculations. Gastric cancer genes were identified through public databases and text mining. Network analysis pinpointed hub genes, while docking simulations validated drug–target interactions. Combinatorial scaffold synthesis generated virtual compounds.

Results: Network analysis highlighted key therapeutic targets with ACTB identified as potential drug target. Pathway enrichment analysis revealed the critical roles of these genes in carcinogenic pathways. Screening 626 Food and Drug Administration-approved drugs highlighted norgestimate and nimesulide as promising candidates for repurposing in gastric cancer therapy. Further, 56,160 virtual compounds derived from scaffold libraries were screened, with 76 compounds prioritized based on favorable interactions with hotspot residues such as GLU214 and LYS18. MM-PBSA energy calculations validated the binding stability and suggested the therapeutic potential of these compounds.

Conclusion: This study highlights the importance of computational approaches in gastric cancer research. The identified hub genes, drug candidates, and novel compounds lay the groundwork for future experimental validation and clinical application. It underscores the potential of repurposing existing drugs and developing therapies for gastric cancer treatment.

A040. Exosomes as Conduits of Radiation-Induced Bystander Effects

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Keywords
► bystander effect
► exosomes
► radiation

Background: Radiation-induced bystander effects describe effects observed in nontargeted cells due to signals released from irradiated cells. Exosomes are secretory nanovesicles and are key mediators of intercellular communication. Radiation alters the release and cargo of exosomes; however, their precise function in bystander signaling and the underlying molecular mechanisms remains obscure.

Materials and Methods: Here, we have employed various techniques like immunoblotting, transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), acetylcholine esterase activity, and zeta potential to qualitatively and quantitatively characterize the exosomes isolated from nonirradiated and X-ray irradiated cells. Exogenous addition of radiation-altered exosomes was also done to observe changes in bystander cell phenotype.

Results: The cup-shaped morphology and purity of the isolated exosomes were validated by TEM examination and immunoblotting, respectively. Radiation increased exosome release in a dose-dependent manner, as demonstrated by acetylcholine esterase activity and NTA-based quantification. Interestingly, NTA data also revealed that radiation altered the size of exosomes. Furthermore, zeta potential measurements indicated enhanced colloidal stability of radiation-altered exosomes. Notably, the exogenous addition of these exosomes resulted in a range of alterations in the phenotype of bystander cells, including enhanced migration, invasion, and reduced proliferation, as well as an enhanced uptake by the nonirradiated cells.

Conclusion: These findings hint toward the role of ionizing radiation in modifying the release of exosomes as well as in altering the exosomal profile. These radiation-altered exosomes also play a crucial role in transmitting radiation effects to the nontargeted bystander cells resulting in changed phenotype.

A041. Exploring the Anticancer Potential of *Pterospermum acerifolium* and *Dalbergia sissoo*

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Keywords
► breast cancer
► network pharmacology
► cancer therapeutics
► phytochemicals

Background: Breast cancer is the second leading cause of mortality, necessitating exploration of potential targeted therapies. Indian medicinal plants exhibit anticancer potential with minimal side effects, and hence, present promising avenues for drug discovery. *Pterospermum acerifolium* and *Dalbergia sissoo* are recognized for their pharmacological attributes, although their anticancer benefits are underexplored in breast cancer.

Materials and Methods: Anticancer efficacy of crude leaf extracts of *P. acerifolium* and *D. sissoo* was evaluated using Gene Ontology, KEGG pathway analysis, protein–protein interaction networks, and compound-target-pathway analysis. Comprehensive systems pharmacology framework was established that linked *P. acerifolium* and *D. sissoo* with breast cancer and breast cancer bone metastasis. In vitro anticancer activity of crude leaf extracts of *P. acerifolium* and *D. sissoo* was accessed on T47D and MDA-MB-231 breast cancer cell lines.

Results: *In silico* analysis identified 3 compounds and 12 targets for *P. acerifolium*, whereas 4 compounds and 25 target genes for *D. sissoo* demonstrating multiple targets, and involvement of multiple pathway networks in breast cancer metastasis. In vitro studies revealed dose-dependent inhibition of proliferation of T47D and MDA-MB-231 cells. Comparative analysis indicated that *P. acerifolium* exhibited superior anticancer activity than that of *D. sissoo*.

Conclusion: The study highlights anticancer potential of *P. acerifolium* and *D. sissoo* extracts, paving the way for discovery of novel compounds and development of alternative therapeutic strategies for breast cancer treatment. Further, we will be identifying active ingredients of phytochemical extracts and evaluate their molecular and cellular effects on breast cancer.

A042. Screening for Antimicrobial Resistance in Mumbai Locality and Developing Phage Therapy against Multidrug-Resistant Pseudomonas Sp.: A Pilot Study

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Keywords

- antimicrobial resistance
- bacteriophage therapy
- biocontrol
- multidrug resistant
- *Pseudomonas aeruginosa*
- *Pseudomonas syringae*

Background: The overuse of antibiotics in health care, agriculture, and animal husbandry has led to multidrug-resistant (MDR) strains. As antibiotics lose efficacy, bacteriophage therapy is emerging as a promising alternative. A pilot study in Mumbai was conducted to assess local resistance levels using two sampling sources and to isolate bacteriophages against pathogenic MDR strains obtained.

Materials and Methods: All isolates, including *Pseudomonas syringae*, were tested for resistance using 3 to 4 antibiotics via disc diffusion. Potential MDR isolates were further screened with a broader antibiotic set, followed by 16S rRNA sequencing for identification. Environmental samples were also used to isolate bacteriophages targeting MDR strains.

Results: The resistance levels for different antibiotics were seen to be ranging between 28.57 and 37.5% of total isolates, excluding gentamicin, which had 0% resistant isolates. Five potentially MDR isolates were found during preliminary screening and secondary screening. The one exhibiting the highest MDR profile (8 of 10 antibiotics tested) was identified to be *Pseudomonas aeruginosa* (via 16s rRNA amplicon sequencing method). *P. syringae* showed resistance to tetracycline; however, still showed sensitivity toward gentamicin, vancomycin, and amoxicillin. Further, two bacteriophages each against *P. aeruginosa* and *P. syringae* were isolated from different water bodies showing clear zones of lysis.

Conclusion: The lytic activity of the bacteriophages against their specific host organisms in vitro could prove to be a promising candidate for their application into therapy for the treatment of plant and animal infections caused due to these bacteria.

A043. Understanding the Role of LCN2 in Therapy Resistance and Tumor Progression

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Keywords

- LCN2
- therapy resistance in CRC
- EGFR recycling
- genetic mouse model of CRC

Background: Therapy resistance is a major challenge in colorectal cancer (CRC) treatment, leading to low survival rates. Lipocalin 2 (LCN2) is a secreted glycoprotein, which is upregulated in CRC. One of the mechanisms through which LCN2 imparts therapy resistance in CRC is by promoting the expression of ETS1 transcription factor, resulting in an inhibition of ferroptosis. The focus of this project is to understand the mechanisms by which LCN2 induces ETS1 expression. Further, we aim to generate a genetic mouse model for *KRAS* mutated CRC expressing high LCN2 levels.

Materials and Methods: LCN2 overexpressing and LCN2 knockdown cells were generated from HCT116 and DLD1 cells, respectively. Protein levels were assessed by Western blot analysis and localization was checked by immunofluorescence assay. To generate tumors in mice, intraperitoneal injection of tamoxifen was given and mice were monitored for 10 to 12 months.

Results: LCN2 overexpressing CRC cells show an increase in colony formation and tumor growth upon 5-fluorouracil (5-FU) treatment, indicating the role of LCN2 in 5-FU resistance. LCN2 overexpressing cells exhibit increased epidermal growth factor receptor (EGFR) activation and enhanced EGFR membrane localization upon 5-FU treatment. Moreover, inhibiting EGFR in LCN2 overexpressing cells leads to a decrease in cell survival. In the genetic mouse model, the tumor tissue was confirmed by hematoxylin and eosin staining and further characterized by staining for epithelial markers.

Conclusion: The study aims to understand the role of LCN2 in therapy resistance and tumor progression, potentially aiding in the development of effective CRC treatment.

A044. Structural Elucidation of the SAM Domain of EphB4 Receptor Tyrosine Kinase

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Keywords
- EphB4
- circular dichroism
- Sterile α motif

Background: Eph receptor tyrosine kinase intracellularly consists of a juxtamembrane region, kinase domain, and Sterile alpha motif (SAM) domain with the juxtamembrane and SAM-kinase linker regulating kinase activity. Since SAM domain forms the part of this allosteric regulation network, thus mutations in the SAM domain can disrupt this allosteric regulation. Noting the importance of SAM domain, we have analyzed the cancer-associated EphB4-SAM mutations using biophysical techniques to study receptor activation.

Materials and Methods: EphB4 mutations retrieved from CBioPortal were evaluated for pathogenicity by using in silico pathogenicity predictors. Mutation predicted to be pathogenic were further evaluated using stability predictors. Mutations predicted to pathogenic are introduced by site-directed mutagenesis, and structural impacts were evaluated using circular dichroism, fluorescence, and X-ray crystallography.

Results: In silico analyses of SAM domain mutants E914K, A931T, and D946N revealed destabilizing effects, while S964T was stabilizing. Among these mutants, two D946N and S964T are in the conserved region. Thermal denaturation experiments showed all mutants were destabilizing when compared with the wild-type (WT). Far-ultraviolet circular dichroism data showed strong negative ellipticity at 208 and 222 nm, indicating α-helix dominance in both WT and mutants, though helical content was decreased in mutants. Intrinsic fluorescence assays showed a 320-nm emission for WT and mutants, with a blue shift in S964T, indicating a more hydrophobic environment due to the serine-to-threonine substitution.

Conclusion: Thermal denaturation and circular dichroism data confirm mutant destabilization and loss of helical content. Intrinsic fluorescence shows that S964T increases hydrophobicity and affects protein structure and stability.

A045. Investigating the Effect of Mitocurcumin on Metastatic Potential of Nonsmall Cell Lung Cancer (NSCLC)

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Keywords
- metastasis
- mitocurcumin
- mitochondrial dynamics

Background: Despite only 0.01% of tumor cells successfully metastasizing, metastasis accounts for 90% of cancer deaths. Mitochondria have emerged as a promising therapeutic target, as mitochondrial dynamics are linked to key metastasis traits, including motility, invasion, microenvironment modulation, plasticity, and colonization. Mitocurcumin-1 (MiC), a curcumin derivative with two TPP moieties, selectively accumulates in cancer cell mitochondria. By inhibiting TrxR2 and modulating reactive oxygen species (ROS), MiC demonstrates potential anticancer activity. ROS in turn affects mt-dynamics, hence we aim to evaluate whether MiC modulates metastasis in lung cancer.

Materials and Methods: A549, H1299, and LLC-1 lung cancer cell lines were studied. IBIDI inserts facilitated wound healing assays, while Boyden chamber assessed migration and invasion (Matrigel coated). Confocal microscopy analyzed mitochondrial and lysosomal structures, and Western blot evaluated protein levels. Frozen cell samples were sent to MedGenome for transcriptome analysis. Nonobese diabetic-severe combined immunodeficiency and C57BL/6 mice will be used for in vivo MiC evaluation.

Results: MiC treatment delayed gap closure in wound healing assays and reduced cell migration and invasion in Boyden chamber experiments. Mesenchymal markers such as N-cadherin, vimentin, and Twist were downregulated. Mitochondria exhibited a more rounded structure, with increased colocalization of mitochondria and lysosomes. Transcriptome analysis further revealed modulation of metastasis-related gene expression in MiC-treated cells compared with controls.

Conclusion: Lung cancer cells' ability to migrate and invade reduces upon MiC treatment. MiC downregulates the early metastatic markers. MiC treatment leads to degradation of mitochondria via mitophagy in human nonsmall cell lung cancer cell lines (A549 and H1299). MiC treatment modulates migration-related genes

A046. Exploring the Role of 26S Proteasomal Chaperone PSMD9 in Maintaining Nucleolar Architecture and Homeostatic Cellular Physiology

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Keywords
- PSMD9
- nucleolar architecture
- nucleolar stress
- p53 levels
- NPM1

Background: PSMD9 is a proteasomal chaperone involved in the assembly of the 19S regulatory particle of 26S proteasome. Overexpression of PSMD9 in breast cancer is correlated with radio-resistance. Additionally, PSMD9 is important in maintaining nucleolar architecture and wild-type p53 levels. This study aims to understand the functional aspect of the same.

Materials and Methods: To assess the abundance of proteins of interest, immunoblotting was performed. To

inspect direct physical interaction, coimmunoprecipitation assays were performed. For visualization of cellular morphology and protein localization, immunofluorescence was performed. For observing cellular dynamics, live cell imaging and fluorescence recovery after photobleaching were conducted. Cell cycle analyses were performed using fluorescence-activated cell sorting.

Results:

• PSMD9 knockouts grow slower, have larger nuclei, and aberrant nucleolar morphology.

• The nucleolar morphology of the knockout cells is restored when rescued with PSMD9 overexpression.

• The abundance of a crucial nucleolar phosphoprotein, NPM1, is low in PSMD9 KO which, however, does not alter nucleolar dynamics.

• PSMD9 KOs have higher abundance of p53, exhibit higher protein synthesis, and are sensitized to nucleolar stress response.

Conclusion: In conclusion, we can say that PSMD9 has a significant functional role in maintaining nucleolar architecture. In absence of PSMD9 cells are stressed, which lead to slow growth and cellular morphological changes. These experiments pave the way for exploring novel noncanonical functions of PSMD9 in maintaining homeostasis and cellular physiology.

A047. Identifying the Genomic Heterogeneity in Head and Neck Cancer Subsites

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Keywords

► head and neck squamous cell carcinoma (HNSCC)
► genomic heterogeneity
► mutation frequency
► subsite variation
► molecular pathways
► Indian population
► targeted therapy

Background: Head and neck squamous cell carcinoma (HNSCC) is one of the most prevalent cancers globally, especially in Southeast Asia, with India contributing to 30% of global cases. Despite advancements in clinical diagnosis, the genetic variation among different HNSCC subsites in India remains underexplored.

Materials and Methods: A meta-analysis of literature specific to Indian HNSCC patients was conducted. Genomic data from different HNSCC subsites were curated from 22 research articles comprising an Indian cohort. Data extraction involved identifying genes with their mutation frequencies across each subsite, followed by pathway analysis to discover common molecular processes.

Results: Significant genes with the highest mutation frequencies were identified: THAP7 (94.11%) in oral cancer, GSTM2 (95%) and NR4A3 (95%) in tongue, PTK2 (80%) in sinonasal, WIF1 (40%) in laryngeal/pharyngeal cancer, and GSTT1 (81.87%) in overall HNSCC. Pathway analysis revealed shared genes across multiple subsites further contributing to common pathways between them.

Conclusion: This study highlights the genomic heterogeneity within HNSCC subsites in India, despite their clinical similarities thereby offering new insights to precise, population-targeted therapies. Furthermore, common genes found across various sites along with their common pathways, point to a shared genetic base that may contribute to cancer metastasis and progression via shared pathways.

A048. Evaluation of Phytochemical, Antimicrobial, and Cytotoxic Properties of Flower Extract of *C. ternatea* Linn

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Keywords

► *C. ternatea*
► antioxidant activity
► cytotoxic activity
► antimicrobial activity
► anythocyanin

Background: *Clitoria ternatea* is a plant of the Fabaceae family. Research and studies are being conducted on *C. ternatea* to ascertain its pharmacological properties. Pharmacological investigations on this plant have shown it has a variety of medicinal properties, including antibacterial, antioxidant, and antiasthmatic activities. There is limited research on detailed properties of the flower extract.

Materials and Methods: Phytochemical qualitative evaluation of the flower extract was performed as per standard protocols. The antimicrobial activity of *C. ternatea* flower crude extracts was tested against *Escherichia coli*, *Staphylococcus Aureus*, and *Pseudomonas aeruginosa*. Antioxidant activity of the extract was estimated by the DPPH method. MTT assay was performed to assess cell viability against A549.

Results: The crude extract from the flower confirmed the presence of alkaloids, terpenoids, cardiac glycosides, fats, and anthocyanins. Antimicrobial activity was tested in the range of (5–0.5 mg/mL). *E. coli* and *S. aureus* showed sensitivity to growth at 0.5 mg/mL, whereas *P. aeruginosa* was resistant and displayed mild sensitivity at 5 mg/mL. Antioxidant activity was studied using the DPPH method. The IC50 of the extract was estimated as 0.05 ug/mL. The IC50 of *C. ternatea* flower extract was found to be 40.42 mg/mL against A549.

Conclusion: The growing shift toward ecofriendly products highlights the potential of modern pharmaceuticals derived from traditional medicinal plants. Preliminary evidence suggests that *C. ternatea* flowers possess significant medicinal properties, notably as a potent antioxidant, with additional antimicrobial and cytotoxic effects. These findings justify further research into its therapeutic applications.

A049. Spargel (Drosophila PGC1-α) Regulates Blood Cell Homeostasis in the *Drosophila* Lymph Gland

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Keywords
- *Drosophila*
- stem cells
- hematopoiesis
- Spargel
- mitochondria

Background: Tissue homeostasis relies on a balance between stem cell maintenance and differentiation, with disruptions potentially causing cancer or tissue degeneration. Here, we aim to elucidate the role of Spargel, a target of the histone acetyltransferase GCN5. Spargel regulates mitochondrial biogenesis and here we investigate its role in regulating lymph gland hematopoiesis.

Materials and Methods: We have employed whole-animal mutants of Spargel, Spargel knockdown and overexpression lines wherein we can spatiotemporally modulate Spargel expression using the UAS-Gal4 system. We use various hematopoietic antibody markers for immunofluorescence and acquisition with confocal imaging for determining hematopoietic phenotypes that are analyzed using ImageJ software.

Results: Our study shows that whole-animal mutants for Spargel showed abnormalities in blood cell homeostasis, indicating its role in hematopoietic regulation. Targeted manipulation of Spargel expression revealed that its depletion in specific lymph gland cells led to an increase in crystal cell differentiation, while overexpression suppressed crystal cell lineage. Furthermore, Spargel overexpression caused a notable reduction in niche size, which may disrupt overall homeostasis within the lymph gland. We are currently characterizing the hematopoietic phenotypes caused upon perturbation of Spargel in different cell subsets along with studying how mitochondrial structure and dynamics change in the lymph gland upon Spargel perturbation.

Conclusion: Our study suggests that Spargel plays a crucial role in maintaining blood cell homeostasis wherein its levels determine niche size and extent of blood cell differentiation. Our future aim would be to determine its impact on mitochondrial dynamics in the lymph gland.

A050. Elucidating the Mechanisms of Resistance to Spontaneous Antitumor Antibodies in Epithelial Malignancies

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Keywords
- antitumor antibodies
- epithelial malignancies
- cancer
- FcRs
- checkpoint inhibitors
- noncanonical

Background: In many cancers, TIL-Bs carry strong prognostic significance and are emerging as key predictors of response to immune checkpoint inhibitors. We hypothesize that the ineffectiveness of antitumor antibodies is partly due to the noncanonical expression of different Fc receptors by the cancer cells as part of their immune escape mechanism.

Materials and Methods: We will correlate expression of FcRs, some immune cells, and epithelial cells where transcytosis of antibodies occurs, with 18S rRNA. An orthotropic mouse model will be established to validate the findings followed by fluorescence-activated cell sorting analysis of the processed tumors to check the prevalence of FcRs. To fulfill this objective, we will first transduce murine transplantable cancer cell lines with lentiviral constructs for expressing murine FcγR and Fcα/μR, individually.

Results: The status of various FcRs was analyzed from four different murine cancer cell lines. Here, we observed a consistent expression of all the receptors across the selected cell lines except in CT26 where there was a mild expression of Pigr and no expression of Fcrn.

Conclusion: To date, no comprehensive study has demonstrated the immunomodulatory functions of these Fc receptors when expressed by epithelial cancer cells. If our hypothesis is factual, then therapeutically, this could lead to something similar to checkpoint inhibitors, where cancers can be treated by monoclonal antibodies targeting these Fc receptors.

A051. Ancestral Sequence Reconstruction of the HtrA Family of Proteins and Their Functional Implications

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Keywords
- evolution
- cancers
- neurodegenerative disorders
- autoimmune disorders
- mutations

Background: Ancestral sequence reconstruction, a computational method, is crucial in identifying the chronology of the mutations of macromolecules across evolutionary time. When the technique is applied to the heat-shock-induced serine proteases, HtrAs, present ubiquitously across almost all taxonomic forms, factors that led to the conservation can be elucidated.

Materials and Methods: First, databases will be built to predict the ancestors. The latter shall then be subjected to biophysical and biochemical assays. Finally, structural comparisons of the extant and the ancestral proteins will be

employed to unravel those residues that might be important for targeting.

Results: So far, ancestors have been predicted of mature HtrA2 and observed to have significant conservation of the key residues—those involved in trimerization, active site triad, and putative substrate binding sites. Apart from these, secondary structural elements do not differ much between the extant and the predicted extinct sequences. Additionally, generation of mutations in some substrate binding grooves in the serine protease domain and the PDZ domain are underway to decipher the subtle differences in the enzymatic activities between the human and the bacterial orthologs. Understanding the former shall be instrumental in analyzing the course of evolution as well.

Conclusion: Despite the availability of structural and sequential information on HtrAs, effective targeting has not been achieved. Thus, from synthesizing the ancestral sequence to the cognate therapeutic intervention, the project aims to attempt to bridge a few gaps in the existing biology of these serine proteases.

A052. CancerBiomeDB: A Knowledge Base on Microbiome Signatures for Pan-Cancer Immunotherapy

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Keywords

- cancer
- gut microbiome
- immunotherapy
- metagenomic sequencing
- database

Background: Gut microflora influences efficacy of cancer immunotherapy. Studies indicate compositional deviations in gut flora leading to development of nonresponsiveness to immunotherapy; however, a clear understanding of gut microflora shifts associated with clinical benefit across cancer types is lacking. We present a comprehensive meta-analysis exploring the relationship between gut microbiota and cancer immunotherapy outcomes.

Materials and Methods: The data were organized into a knowledgebase, including taxa, cancer types, location, clinical outcomes, etc. Data curation, processing, and visualization were performed using R, Python libraries, and iTOL. We developed a database using XAMPP stack with HTML, CSS, and JavaScript for the front end, and PHP and MySQL for the back end.

Results: We analyzed 2,595 articles and deposited extracted information in the database. Statistical analysis revealed that most commonly studied cancer types are gastrointestinal cancer, melanoma, and lung cancer. We identified a total of 279 candidate taxa reported in these studies. Further statistical evaluation revealed eubiotic bacteria, including *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*, were associated with responsiveness to immunotherapy. In contrast, *Escherichia coli*, *Bacteroides thetaiotaomicron*, and *Veillonella* genus causing gut dysbiosis have been linked with resistance spanning different cancer types. Additionally, at family level, *Lachnospiraceae*, *Ruminococcaceae*, and *Akkermansiaceae* were more prevalent in the effective group, whereas *Bacteroidaceae*, *Streptococcaceae*, and *Veillonellaceae* were abundant in the noneffective group.

Conclusion: We present a consensus microbial dysbiosis signature predictive of worse immunotherapy outcomes. We also present an easy-to-use database of gut microbiomes, aiding further research to improve treatment strategies across cancers.

A053. Investigating the Impact of Curcumin on Transgenerational *Caenorhabditis elegans* Exposed to High-Glucose Diet

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Keywords

- high-glucose diet
- *Caenorhabditis elegans*
- neurodegenerative diseases
- transgenerational effects
- curcumin

Background: High-glucose diet has been linked to metabolic conditions and neurodegenerative diseases. *Caenorhabditis elegans i*s considered a model organism due to its well-characterized genetics and short lifespan. This study examines the transgenerational effects of elevated glucose levels on *C. elegans* and evaluates curcumin's potential to ameliorate these neurodegenerative effects.

Materials and Methods: C. elegans (wild-type) were exposed to high glucose (0, 50, 100, 400 mM) and treated with curcumin (0, 25, 50, 100 µM) to evaluate the behavioral and antioxidant effects on the F1 and F2 generations. L4-stage worms from each generation were examined to evaluate the effect of glucose and curcumin's possible protective benefits.

Result: The study observed that a high-glucose diet had effects on *C. elegans*. Behavioral assay like pharyngeal pumping and thrashing were performed and the results showed that with increased glucose concentrations (0–400 mM), the worms showed decreased pumping rate and thrashes. Interestingly, curcumin (0, 25, 50, 100 µM) treatment had reversed some of these effects. Curcumin showed increased pumping and thrashing rate similar to control, indicating its ability in preventing high glucose-induced damage. Enzymatic assay like DPPH had observable color change from purple to yellow indicating a shift in antioxidant activity.

Conclusion: A high-glucose diet affects *C. elegans*, resulting in negative transgenerational effects. Curcumin appears to have potential benefits through lowering oxidative stress and increasing worm health. This reveals that curcumin may be beneficial for mitigating the effects of increased glucose levels.

A054. Studying the Binding Multiplicity of Steroid Hormone Receptors and Ligands in Triple-Negative Breast Cancer

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Keywords

- triple-negative breast cancer
- steroid hormone receptors
- cancer therapeutics
- molecular docking
- MD simulation

Background: Triple-negative breast cancer (TNBC) lacks progesterone receptor (PR), estrogen receptor (ER), and HER2. Thus, therapies targeting these receptors are ineffective, leading to poor prognosis and high recurrence rate. However, TNBCs express androgen (AR), glucocorticoid (GR), and mineralocorticoid (MR) receptors. Thus, we aimed to understand the roles of these steroid hormone receptors in TNBC and identify drugs that bind them.

Materials and Methods: We performed in silico molecular docking experiments to identify natural/synthetic compounds ($n = 117$) that can bind to nuclear and membrane AR/GR/MR. Further, we performed a cross-recognition experiment to identify multitarget potential of these drugs. We performed molecular docking using AutoDock Vina, followed by postdocking analyses (binding affinity and active site prediction criteria) to confirm receptor–ligand interactions.

Results: Analyses identified novel natural/synthetic ligands to bind nuclear and membrane AR/GR/MR with binding affinity similar or more than that of their cognate ligands. Further, we identified additional ligands that show multitarget potential in their interactions with AR/GR/MR (cross-docking). Postdocking analyses identified drugs that bind receptors with high affinity. Further, we will perform absorption, distribution, metabolism and excretion (ADME), molecular dynamics (MD) simulations, and functional cell-based validations in TNBC cells.

Conclusion: We identified several potential ligands that can interact with AR/GR/MR, and hence, may make TNBC amenable for endocrine therapy. Moreover, study results may serve as paradigm shift in cancer therapeutics by making available ligands that can function even in the absence of their cognate receptors in TNBC—a potential drug-repurposing approach.

A055. Development of Saliva-Based Biosensor for Noninvasive Detection of Oral Cancer

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Keywords

- biosensor
- biomarker
- early detection
- oral cancer
- oral health
- pathogens

Background: Oral cancer has poor response rates and patients show poor survival outcomes. Therefore, early detection is necessitated to improve treatment outcomes. A majority of diagnostic techniques are either invasive or costly, limiting their utility. This study aims to develop an economical, noninvasive biosensor for quantifying levels of pathogenic bacteria as a means of detecting oral cancer.

Materials and Methods: We are developing an aptamer-based biosensor that would quantify levels of certain proteins secreted by *Fusobacterium*, *Porphyromonas*, and others, which are associated with oral cancer. The biosensor is prepared using aptamers against these proteins, conjugated to gold nanoparticles and attached to screen-printed electrodes. We will use saliva sample for detecting the protein levels.

Results: We have synthesized gold nanoparticles and obtained preliminary spectrometric standardization results. We have isolated *Fusobacterium*-like bacteria as one of the isolates and are currently confirming its identity. We are also standardizing the aptamer-gold nanoparticle-conjugated screen-printed electrodes (SPE) for the detection of bacterial proteins secreted in liquid growth medium. Once successful, we will test the applicability of the biosensor for the detection of pathogen-secreted proteins in whole saliva of pathologically normal and patients with oral cancer, possibly of varying stages.

Conclusion: The development of a biosensor using pathogen as a biomarker may serve as a promising, cost-effective, noninvasive, rapid, and reliable approach for the detection of oral cancer, potentially improving early detection and outcomes, especially in settings with limited resources.

Editorial

Prostate Cancer Before 50: A Wake-Up Call

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Prostate cancer is the second most common malignancy in males and the fourth most common malignancy overall globally.[1] Prostate cancer is a disease occurring in older males ($\geq$ 60 years); however, recent studies show an increased trend in young patients (age $\leq$ 50 years).[2–4] While the majority of prostate cancers in younger patients are less aggressive than those in older patients, emerging evidence indicates that biological factors, particularly genetic alterations, rather than chronological age alone, determine disease aggressiveness.[5] Younger patients with prostate cancer may present with biologically aggressive variants driven by specific molecular features rather than chronological factors. Studies demonstrate that aggressive variants occur in approximately 15 to 20% of early-onset cases.[6]

We present a case of a 46-year-old male patient, presented with persistent backache and was found to have metastatic prostate cancer with widespread sclerotic skeletal metastases and spinal cord compression. Decompressive laminectomy with biopsy confirmed metastatic poorly differentiated adenocarcinoma of prostatic origin. Next-generation sequencing identified an SLC45A3(1)–ERG(4) gene fusion, a rearrangement associated with unfavorable outcomes and representing a distinct molecular subtype.[6,7]

Initial serum prostate-specific antigen (PSA) was markedly elevated. Gallium-68 (^{68}Ga)–prostate-specific membrane antigen (PSMA) positron emission tomography-computed tomography confirmed extensive skeletal and nodal metastases. The patient underwent bilateral orchidectomy followed by palliative radiation and systemic therapy including abiraterone with prednisolone, enzalutamide, and three cycles of ^{177}Lu–PSMA radioligand therapy.

Despite initial responses, the patient experienced progression with rising PSA levels and worsening neurological symptoms. Repeat laminectomy and biopsy 2 years after initial diagnosis revealed squamous cell carcinoma, indicating histological transformation, a recognized mechanism of therapeutic escape through lineage plasticity.[8]

Clinicians should be careful with younger symptomatic individuals, especially if they have unexplained back pain or vertebral lesions. Current guidelines (United States Preventive Services Task Force, National Comprehensive Cancer Network, European Association of Urology)[9,10] do not recommend routine PSA screening below the age 50. However, selective evaluation may be appropriate in symptomatic or genetically predisposed individuals, balanced against the established limitations of PSA screening including modest impact on disease-specific mortality and minimal effect on all-cause mortality.[11]

The presence of *SLC45A3–ERG* fusion suggests genetically driven disease with implications for prognosis and treatment. A molecular profiling for mutations in *TP53*, *PTEN*, and *RB1* (negative in this case), may inform therapeutic strategies.

Early-onset cases with aggressive features warrant prompt multidisciplinary care, genetic counseling, and consideration for clinical trial enrolment, particularly when standard treatments show resistance.

We propose that selective early PSA screening may be appropriate in specific circumstances like symptomatic individuals (bone pain, urinary obstruction, or neurological symptoms suggestive of metastatic disease), men with strong family histories, and those with known genetic predispositions (*BRCA2* mutations or Lynch syndrome). This approach maximizes the likelihood of detecting clinically notable disease.

Future research should focus on identifying biomarkers to stratify risk in young men and developing screening strategies that maximize benefit while minimizing harm. Until such evidence emerges, clinical judgment should guide evaluation of symptomatic individuals while avoiding unnecessary screening.

Patient's Consent
Patient consent has been obtained.

Conflict of Interest
None declared.

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Immunotherapy Makes Inroads in Head and Neck Cancer Treatment

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Abstract

Immunotherapy has transformed the treatment landscape of metastatic head and neck squamous cell carcinoma (HNSCC), but its role in curative-intent settings remained elusive—until now. Recent data from two pivotal phase III trials, NIVOPOSTOP and KEYNOTE-689, mark a turning point by demonstrating statistically significant improvements in disease-free and event-free survival, respectively. However, the magnitude of benefit remains limited, subgroup efficacy is unclear, and overall survival data are immature. Given the logistical complexity and potential for overtreatment, these results warrant cautious interpretation. Future strategies must prioritize biomarker-driven selection, real-world feasibility, and long-term survival outcomes before immunotherapy can claim a definitive role in curative HNSCC.

Keywords

- head and neck cancer
- HNSCC
- immunotherapy
- neoadjuvant therapy

Introduction

Head and neck squamous cell carcinoma (HNSCC) represents one of the few areas in oncology where the accelerated advancements and accompanying disappointments associated with immunotherapy have been most striking. Despite its biological plausibility and success in the metastatic disease, the integration of immune checkpoint inhibitors (ICIs) into curative-intent strategies for locally advanced HNSCC has been a frustrating journey of failed promises. After years of negative trials, recent phase III data from NIVOPOSTOP[1] and KEYNOTE-689[2] offer new hope. But hope, as we have learned repeatedly, should not substitute for rigorous evidence.

This article examines whether these trials represent a genuine inflection point in the curative treatment of HNSCC or whether we are once again overestimating modest gains, especially in the absence of mature overall survival (OS) data and realistic global applicability.

A History of Missed Opportunities: A Trial Graveyard

For nearly a decade, trials attempting to incorporate ICIs into curative HNSCC therapy have been largely unsuccessful. JAVELIN Head & Neck 100,[3] arguably the most ambitious early effort, added avelumab to chemoradiotherapy (CRT) but failed to show benefit—a hazard ratio (HR) of 1.21 for progression-free survival was observed, pointing toward potential harm. KEYNOTE-412[4] fared little better: the addition of pembrolizumab to CRT showed a nonsignificant trend (HR 0.83), narrowly missing statistical significance and falling short of the prespecified threshold for success.

Several other trials underscored the same theme. In the REACH study,[5] the cisplatin-fit group paradoxically did worse with the ICI arm (HR 1.27). The PembroRad trial,[6] replacing cetuximab with pembrolizumab in patients unfit for cisplatin, showed an HR of 1.05. Simply put, these trials

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did not fail because of poor design alone—they failed because the approach itself may be biologically unsound.

Even in the adjuvant setting, where one might expect better immune reconstitution after surgery, the results were disappointing. IMvoke010[7] (atezolizumab postsurgery and radiotherapy [RT]) reported an HR of 0.94, essentially a null trial. The NRG-HN004[8] study of durvalumab in cisplatin-unfit patients showed an HR of 1.33—again suggesting that simply swapping chemotherapy for immunotherapy is not a viable shortcut ►**Table 1**-**2**.

So Why Did These Trials Fail?

Immunotherapy is Not Just Plug-and-Play

The key mistake was assuming that immunotherapy's success in lung cancer or melanoma could be imported wholesale into HNSCC. This disease is biologically distinct—characterized by intense stromal immunosuppression, T-cell exhaustion, and frequently human papillomavirus (HPV)-driven oncogenesis that does not necessarily correlate with immunogenicity.[9,10]

Moreover, the context of delivery matters. Combining ICIs with high-dose cisplatin and radiation—both profoundly immunosuppressive interventions—may neutralize the very immune activation ICIs require. Concurrent administration may have been the wrong strategy altogether.

Another cardinal flaw was the absence of biomarker-driven stratification. Across many clinical trials, programmed death-ligand 1 (PD-L1) combined positive score (CPS) or HPV/p16 status were often not recorded, not incorporated into therapeutic strategies, or lacked adequate power to determine benefit. Consequently, any potential advantage in a subset was diluted in the broader analysis.

The Turning Point? Dissecting NIVOPOSTOP and KEYNOTE-689

NIVOPOSTOP,[1] presented at ASCO 2025, was the first study to demonstrate a statistically significant improvement in disease-free survival (DFS) with adjuvant nivolumab after surgery and postoperative CRT in patients with high-risk HNSCC. The 3-year DFS was 63.1% with nivolumab versus 52.5% with CRT alone (HR 0.76; $p = 0.034$). Importantly, benefits were seen regardless of p16 or PD-L1 status, though subgroup sizes were small.

Compliance was excellent: 91% completed RT, $>80\%$ received adequate cisplatin doses, and 75% of patients belonging to the nivolumab arm completed at least 10 cycles. Fewer grade ≥ 3 treatment-related adverse events were observed in the nivolumab group (39%) compared with the control group (49%), referring to overall grade ≥ 3 adverse events (AEs), not immune-related ones. OS data remain immature.

What makes NIVOPOSTOP[1] noteworthy is not just the positive result but that it learned from prior failures. It avoided concurrent administration of ICIs with CRT and instead placed immunotherapy in the adjuvant slot—a setting where the immune system may be less suppressed and more responsive.

KEYNOTE-689,[2] now published in *New England Journal of Medicine* (June 2025), assessed the use of pembrolizumab in both the neoadjuvant and adjuvant settings for resectable, locally advanced HNSCC. At a median follow-up of 38.3 months, patients treated with pembrolizumab achieved a 3-year event-free survival (EFS) of 59.8%, whereas the control arm reported 45.9% (HR 0.73; 95% confidence interval, 0.58–0.92). Notably, the benefit was even more pronounced in biomarker-enriched subgroups: CPS ≥ 10 (HR 0.60), CPS ≥ 1 (HR 0.70).

Surgery completion rates were similar between the two arms ($\sim$88%), alleviating prior concerns about neoadjuvant ICI interfering with operability. In the pembrolizumab arm, 24.5% of patients experienced grade 3 AEs, compared with 22.5% in the control arm. This robust benefit led to Food and Drug Administration approval on June 12, 2025, for perioperative pembrolizumab in CPS ≥ 1 resectable HNSCC.[11]

Should We Be Celebrating Yet? Let's Pause and Reflect

Despite the excitement surrounding NIVOPOSTOP[1] and KEYNOTE-689,[2] several cautionary issues need to be addressed before calling this a paradigm shift.

Surrogate Endpoints

Neither NIVOPOSTOP[1] nor KEYNOTE-689[2] reported mature OS data. We are again anchoring major practice changes on DFS or EFS—surrogate endpoints that often fail to predict survival in HNSCC. A 10 to 15% EFS improvement may not translate into a meaningful OS benefit, especially in a disease where salvage treatments are possible and quality of life matters.

Subgroup Ambiguity

Benefit in PD-L1–negative or HPV-positive subgroups remains unclear. These are substantial populations. Approving and funding treatment across the board risks overtreatment in many and benefit for few.

No Clarity on Timing

In KEYNOTE-689,[2] is the benefit coming from the neoadjuvant part, the adjuvant part, or both? While the trial succeeded overall, the individual contributions remain undefined, limiting the ability to streamline protocols.

Feasibility in the Real World

Weekly cisplatin, multiple infusions of ICI, and real-time PD-L1 testing—all increase complexity. In low- and middle-income countries, where most HNSCC cases occur, such logistics are often prohibitive. Unless simplified, these regimens will remain a luxury for the few.

No Quality-of-Life Data

Functional outcomes—swallowing, speech, and nutrition—are crucial in HNSCC, especially after multimodal treatment. None of these trials report validated quality-of-life

Table 1 Comparison of major phase III immunotherapy trials in curative-intent HNSCC

Feature	JAVELIN 100 (Avelumab)[3]	KEYNOTE-412 (Pembrolizumab)[4]	KEYNOTE-689 (Pembrolizumab)[2]	NIVOPOSTOP (Nivolumab)[1]
Phase	Phase 3	Phase 3	Phase 3	Phase 3
Setting	Locally advanced, resectable HNSCC	Locally advanced, resectable HNSCC	Locally advanced, resectable HNSCC	Resected, high-risk HNSCC
Control arm	CRT (cisplatin + RT)	CRT (cisplatin + RT)	Surgery → adjuvant RT ± cisplatin	Surgery → CRT (cisplatin + RT)
Experimental arm	CRT + avelumab (before, during, after)	CRT + pembrolizumab (during, after)	Neoadjuvant pembrolizumab → surgery → adjuvant pembrolizumab + RT ± cisplatin	CRT → adjuvant nivolumab
IO start timing	Lead-in before CRT → concurrent CRT	Start with CRT	Neoadjuvant before surgery	Adjuvant after CRT
Primary endpoint	PFS	EFS	EFS	DFS
Primary HR (95% CI)	0.81 (0.62–1.06)	0.83 (0.68–1.03)	0.73 (0.58–0.92)	0.76 (0.60–0.98)
Primary result	Did not meet primary endpoint	Did not meet primary endpoint	Met primary endpoint	Met primary endpoint
OS HR (95% CI)	0.90 (NS)	Trend favorable (immature)	0.72 (0.52–0.98)	Immature, trend favors NIVO
PD-L1 subgroup	PFS HR 0.59 (0.34–1.02)	EFS HR 0.67 (CPS $\geq$ 20)	13.7% mPR improvement (CPS $\geq$ 10)	No clear differential by CPS
Biomarker enrichment	No	No	Stratified CPS $\geq$ 10	No
Safety	Higher immune AEs	Higher immune AEs	Manageable, consistent	Favorable, fewer grade $\geq$ 3 TRAEs
Trial status	Stopped early (futility)	Completed (failed)	Completed (positive)	Completed (positive)
Publication year	2021	2023	2025	2025
Main summary	Poor timing, no biomarker	Tight alpha, poor timing	Smarter timing, EFS success	First positive adjuvant IO trial in HNSCC

Abbreviations: AE, adverse event; CI, confidence interval; CPS, combined positive score; CRT, chemoradiotherapy; DFS, disease-free survival; EFS, event-free survival; HNSCC, head and neck squamous cell carcinoma; HR, hazard ratio; IO, immuno-oncology; mPR, major pathologic response; NS, not significant; OS, overall survival; PD-L1, programmed death-ligand 1; PFS, progression-free survival; RT, radiotherapy; TRAE, treatment-related adverse event.

Table 2 Failed immunotherapy trials in curative HNSCC

Trial name	*N* (patients)	Arms	EFS/DFS/PFS	OS	Remarks
REACH (cisplatin-fit group)[5]	~300	CRT + durvalumab vs. CRT alone	HR 1.27 (PFS)	Not reported	Durvalumab added to CRT in cisplatin-fit patients showed worse outcomes (HR 1.27), raising concerns about ICI synergy with concurrent chemoradiation
PembroRad[6]	133	RT + pembrolizumab vs. RT + cetuximab	HR 1.05 (PFS)	Not reported	Replacing cetuximab with pembrolizumab in RT for cisplatin-ineligible patients did not improve outcomes, highlighting limited efficacy of ICIs in this context
IMvoke010[7]	682	Postop RT + atezolizumab vs. RT alone	HR 0.94 (DFS)	Not reported	Adjuvant atezolizumab post-surgery and RT did not improve DFS. The trial failed to demonstrate added benefit in a setting theoretically favorable for immunotherapy
NRG-HN004[8]	251	RT + durvalumab vs. RT alone (cis-unfit)	HR 1.33 (PFS)	Not reported	Durvalumab added to RT in cisplatin-unfit patients resulted in worse outcomes (HR 1.33), reinforcing the challenge of integrating ICIs in frail populations

Abbreviations: CRT, chemoradiotherapy; DFS, disease-free survival; EFS, event-free survival; HNSCC, head and neck squamous cell carcinoma; HR, hazard ratio; ICI, immune checkpoint inhibitor; OS, overall survival; PFS, progression-free survival; RT, radiotherapy.

outcomes, which undermines the relevance of their clinical gains.

The Way Forward—Smarter, not Just More Trials

As we plan the next wave of studies, we must prioritize strategic designs over sheer enthusiasm. The following principles should guide future research:

- Biomarker-driven design: Trials must prospectively stratify by PD-L1 CPS, HPV status, and ideally, immune gene signatures or circulating tumor deoxyribonucleic acid.
- Minimal residual disease (MRD): Leveraging MRD or pathologic response to guide adjuvant ICI could help limit overtreatment. Adaptive trial designs are sorely needed.[12]
- Deescalation and organ preservation: Could ICIs allow for reduced radiation fields or avoidance of mutilating surgery in responders? Trials must test this explicitly.

Conclusion

After a long and disappointing journey, immunotherapy has finally shown signs of life in curative HNSCC. But let us be clear: these are small, cautious steps forward—not a revolution.

The survival benefits, while statistically significant, are modest and built on surrogate endpoints. The subgroups that benefit remain murky. The real-world feasibility is questionable, particularly in resource-limited settings. And the long-term impact on quality of life—perhaps the most important outcome in this disease—is still unknown.

If we are to move forward, let it be with scientific humility and practical wisdom. Let us resist the urge to rubber-stamp another expensive, complex treatment protocol based on short-term gains. Instead, we should demand clarity on who benefits, how much, and at what cost.

Immunotherapy in curative HNSCC has earned a seat at the table. But it will take more than two trials to justify putting it at the head.

Patient's Consent

Patient consent is not required.

Conflict of Interest

None declared.

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Role of Nurses in Cancer Education, Screening, and Detection in the Community: Narrative Review Addressing the Existing Lacunae and Scope in India

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Abstract

Across the world, nurses are an important component in both patient and community health care and are excellent ambassadors for community education and awareness endeavors. Cancer is on a rise and creating awareness on the causes, signs, and inculcating the importance on timely detection and accordingly an early treatment-seeking behavior can be very useful in reducing the incidence and can contribute to decreasing the morbidity and mortality associated with the ailment. Effective and correct education play a crucial role in community awareness and nursing interventions in tobacco cessation counselling and cervical cancer screening have been documented from across the world. Efforts to improve the community knowledge, advocacy for cancer screening, and the development of new technologies for cancer screening will allow for improvements in cancer screening over time. This need-of-the-hour narrative review addresses the role nurses can play in cancer education, screening, and detection in the Indian community. In addition to this, the existing lacunae and ways to fill the gap for the betterment of the fraternity and the society at large are also addressed. It is expected that this review, which is the first on the topic from India, will benefit the fraternity and the society at large.

Keywords
- cancer awareness
- screening
- community
- education
- nurses

Introduction

According to recent data published by the world's leading cancer nodal center, the Global Cancer Observatory (GLOBOCAN),[1] in 2020, India was the third leading contributor to the overall global number of cancer cases.[2] Recent predictions were that 1,461,427 cases of cancer occurred in India in 2022.[3] With a crude rate of 100.4 per 100,000, and information that 1 in 9 persons in India will develop cancer at some point in their lives, it is estimated that cancer will be huge

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health issue and will levy immense stress on the existing health care system.[3] What is significantly worrying is that, unlike in the developed countries, majority of Indian patients arrive late for treatment, resulting in high incidence-to-mortality ratio of 0.68 to 0.38.[4,5] On a summative view, illiteracy and lack of awareness, limited access to quality cancer care, and inability of the patient to afford optimum treatment have all contributed to the skewed incidence-to-mortality ratio.[5]

From an Indian health care provider's view, when compared with the first decade of the millennium, there have been immense improvements in upgrading the system with conscious attempts focused on establishing facilities and providing medical infrastructures in the underserved areas and communities across the length and breadth of the nation.[6] However, providing an array of medical services to India's population of 1.4 billion, which currently is the World's largest, is a herculean task that needs bridging the gaps.[6] This is strikingly true in cancer diagnosis and care where several prominent factors contributing to the challenges: inadequate diagnosis and treatment facilities; insurance and public funding; ill-equipped health care system; dearth of medical personnel; huge rural versus urban and socioeconomic disparities; and regional differences in easy access to health care.[5] This issue is further exacerbated by the cultural norms that discourage seeking medical attention and the propensity for patients and their families to conceal cancer diagnoses out of shame, lack of cancer knowledge, and apprehensions of news spreading among the general population.[5] Cumulatively, all these factors have affected the low-income lesser educated people and those living in rural areas immensely.[5]

Importance of Cancer Education in Reducing Incidence

Multiple preclinical, clinical, epidemiological, and community-based studies have proved that cancer is a multifactorial disease and that genetic susceptibility, environmental variables, and lifestyle choices all have a role in its etiology.[7] Therefore, reducing risk factors, such as tobacco use, alcohol consumption, poor diet, low levels of physical activity, workplace and environmental carcinogens, radiation exposure, immunization against hepatitis B and human papillomavirus (HPV),[8] and prevention of infection with *Helicobacter pylori* and schistosomiasis, can prevent one-third or more cancer incidences and cases.[9] From n historical perspective, in the landmark review, Van Parijs had emphasized the role of lifestyle factors in carcinogenesis.[10] The review also emphasized the importance of increasing general public knowledge and initiating behavioral changes, such as altering specific risk behaviors (like quitting smoking),[11,12] inculcating self-examination skills (like breast self-examination [BSE]),[13] and encouraging early cancer detection among the general population.[10] These preventive measures are now well accepted and have been advocated across the world.

From cancer prevention perspective, although complete prevention is impossible in some types of malignancies (pancreatic, brain, etc.),[14,15] there are strategies to lessen one's vulnerability to some forms.[16] It is estimated that 30 to 50% of all cancer cases might be avoided and that cancer prevention is economically viable and an effective long-term approach to prevent and control cancer.[17] Cancers affecting the oral cavity, breast, uterine cervix, lung, and colorectum are preventable with lifestyle modification, awareness of warning signs, and regular screening, as specified by guidelines considered to be vital and from years of study.[18] In short, the famous adages "*prevention is better than cure*" and "*a stich in time saves nine*" are apt as they summarize that timely diagnosis is crucial to the success of complete cure of cancer.[18] If people are conscious of their own personal risk factors, they can begin taking steps toward cancer prevention much sooner for the betterment and for family. In lieu of this, primary prevention (like lifestyle modification, vaccination) [19] and secondary prevention (screening and early diagnosis of oral, breast, uterine cervix, colorectum, lung, and prostate cancers)[20,21] are considered to be an essential part of cancer control and are being advocated affirmatively in some societies of many countries.

Nurses in Cancer Education and Screening

Globally, nurses who constitute 59% of the health workforce are the primary component of direct patient care, and in many countries, some are also involved in administrative endeavors in the health care sector both in hospital and community settings.[22] In 2013, as a component of their Global Action Plan for noncommunicable diseases (NCDs), the World Health Organization (WHO) put forth a recommendation for nations to expand the role of nurses and allied health professionals[23] and proposed objectives in the prevention and management of NCDs.[24] Importantly, specific emphasis was placed on overcoming obstacles that hindered the effective contribution of these professionals, which needs to be adequately addressed in the future. On a practical note, numerous prospects are available to fully leverage the expertise of nurses specialized in oncology, allowing them to significantly address the challenges posed by the burden of cancer.[9,25]

On a realistic note, the most critical abilities that the nurses have are the capacity to create strong professional connections with patients, family members, and other caregivers due to their job nature and involvement in patient care. Nurses are in a unique position to spread the word about the importance of cancer prevention in several situations because of their expertise in health care aspects and ability to develop trustworthy relationships (WHO, 2020).[22] In her inspiring review, Frank-Stromborg highlighted the value of nurses in both community-based and in-patient settings for primary and secondary cancer prevention, emphasizing the role of oncology nurses.[26] Following this, numerous programs were initiated in many developed countries, and reports indicate that nurses have played an important role in the fight against cancer, and that nurse educators in particular have been instrumental in effectively planning and carrying out tobacco cessation counselling and cancer awareness programs.[27]

With regard to nurse-led cancer screening, seminal studies by Ansell and colleagues[28] demonstrated that an intervention program to reduce barriers to breast and cervical cancer screening in Chicago inner city facilities for low-income, high-risk African-American women was successful.[28] In this 18-month nurse-delivered interventional study, nurses and public health personnel recruited women for free breast and cervical cancer screening.[28] The objective of the intervention was to comprehend barriers to breast and cervical cancer access, knowledge, initial and follow-up screening, and treatment behavior and adherence. The 18-month intervention program with 7,654 low-income women helped identify 84 cases of breast cancer and 9 cases of cervical cancer.[28] A post-intervention survey revealed that study participants were more knowledgeable about breast and cervical cancer and were more aware of the program than at baseline. The most important observation was that over 90% of women with breast abnormalities kept their referral appointments clearly indicating the effectiveness of the nurse-led program in cancer prevention and in inculcating the behavior of adherence to safety screening.[28]

From an Indian perspective, Gajalakshmi and coworkers investigated the effectiveness of training village health nurses (VHNs)[29] in identifying cervical abnormality by visual inspection and in Pap smear training.[29] The investigators trained 101 VHNs from different villages in Tamil Nadu and sent to their native places.[29] In the following 2 years, 6,459 were screened and the observations of the trained VHNs and gynecologists were found to be 95% consistent in identifying abnormal cervix and 80% consistent in sampling for Pap smear. The study affirmatively indicated that training the nurses would be a really effective means in cancer screening.[29]

Following this, studies from Tshwane, South Africa[30]; Cardiff, United Kingdom[31]; Malawi[32]; Brazil[33]; Belfast, United Kingdom[34]; Ireland[35]; and Ras Al Khaimah in United Arab Emirates[36] have all shown that nurse-led community-based endeavors had a positive role and were vital in creating cancer awareness and highlighting importance of screening in the society. According to a systematic review of health promotion interventions to increase breast cancer screening in countries across different continents, positive outcomes were achieved, including women's perceptions of breast screening, BSE, and knowledge of breast screening.[37] Following this, another systematic review that addressed primary care nurses' knowledge of cancer screening recommendations, as well as the frequency of early cancer diagnosis–related discussions with patients, found differences across study areas, possibly due to measurement bias and differences in clinical duties in the health systems they were serving.[38] In the recent past, studies have been conducted to ascertain the knowledge and teaching of cancer awareness[39–65] and the details are listed in ►**Table 1**.

Nurses in Cancer Education in India

Nurses, as in the rest of the globe, form the backbone of the Indian health care system. In India, nurses, who make up

Table 1 Details of studies with results indicating observations on cancer awareness in nurses

Cancer (reference)	Objective	Type of study	Sample size	Observation and Inference
Cervix (Shekhar et al, 2013)[39]	Assessment of knowledge, attitude, and practices	Cross-sectional	262	Majority (77%) were aware of Pap smear, 50% knew about its ability of precancerous lesion detection. While 23.4% recognized HPV as a risk factor, only 7% nurses had undergone screening. 85% never did Pap smear for patients. 90% did not refer for Pap testing; viewed as doctor's job.
Breast (Fotedar et al, 2013)[40]	Knowledge of risk factors, early detection methods, and screening practices	Cross-sectional study	457	Nurses' average knowledge of risk factors was 49%. Knowledge levels varied: poor 10.5%, good 25.2%, very good 45%, excellent 16.3% for risk factors and early detection. BSC nurses had higher knowledge; 54% practiced annual BSE. Less than one-third had recent CBE; 7% had prior mammograms.
Cervix (Thippeveeranna et al, 2013)[42]	Knowledge, attitude, and practice of Pap smear screening	Cross-sectional study	224	Majority (98.6%) knew about cervical carcinoma, but 18.3% lacked risk factor knowledge. Adequate Pap smear knowledge in 88.8%, and 11.6% had prior tests. Common nonparticipation reasons: no symptoms (58.4%), lack of counselling (42.8%), no physician request (29.9%), fear of exam (20.5%).

(*Continued*)

Table 1 (*Continued*) Details of studies with results indicating observations on cancer awareness in nurses

Cancer (reference)	Objective	Type of study	Sample size	Observation and Inference
Cervix (Singh et al, 2012)[41]	Knowledge of cervical cancer and Pap smear screening among staff nurses	Questionnaire-based survey	205	It was observed that 74% recognized Pap smear for cervical cancer detection, but 59% knew its dual role. HPV vaccine known to 18%. While 47% respondents never had Pap smear; 63% did not refer patients. Most (79%) considered speculum exam and Pap smear doctor's tasks. Only 11% had personal Pap smear.
Breast (Paul et al, 2015)[43]	Women's knowledge, risk factor awareness, and screening practices in BSE	Cross-sectional population-based survey	560 nurses	BSE knowledge was only 16%, with 15.6% practicing it once. Well-known risks: alcohol (64.6%) and smoking (64%). Least known: early menarche (17.2%), red meat use (23%). Key recovery factors: doctor's support (95%) and family support (94.5%).
Cervix (Shankar et al, 2015)[44]	Impact of awareness programs in adoption of safe practices in prevention and early detection.	Pre-test and post-test questionnaire	156	Knowledge increased for cervical and breast cancer at 6 months, sustained at 1 year. BSE more adopted than CBE, mammography, and Pap test. Over 60% teachers learned from magazines and 75% from doctors about Pap test. Awareness changed alcohol/smoking habits at 6 months and 1 year. Key reasons for not screening: ignorance (50%), lethargy (44.8%), lack of time (34.6%)
Cervix (Sreeramulu et al, 2022)[45]	Knowledge of cervical cancer and its relationship with genital hygiene	Questionnaire-based survey	87 respondents	Six domains on awareness of physical and genital hygiene, cervical cancer causation and prevention, health education and personal experience of cervical cancer were explored. In the awareness domains, the response was uniformly poor in 45–50% of respondents. Nurses had poor knowledge in every domain of the questionnaire
Breast (Ramakant et al, 2018)[46]	Awareness about breast cancer prevention, early detection, symptoms, and management in urban and rural Indian women and correlation with education and socioeconomic strata	Prospective cross-sectional observation study	270	The medical group had more knowledge, but prevention attitudes and BSE skills were low across all subgroups (rural/urban). Reasons for delays: lack of BSE knowledge, BC symptom unawareness, cancer stigma, and financial issues. To boost awareness: media ads, campaigns (roadside/colleges), discussions. Also, involve Anganwadi workers/nurses for village outreach
Breast (Malik et al, 2020)[47]	Identification of the extent of women in Fiji and Kashmir, India have BCA and practice breast self-examination (BSE) and factors associated	Survey	399 and 1,982 women in Kashmir and Fiji	Among the 1,968 women in Fiji, 57% were deemed to have an acceptable BCA compared with only 7.3% of 395 women in Kashmir. Having some education was associated with having BCA with an odds ratio (OR) of 4.7 (1.7–13) in Fiji and 10 (1.7–59) in Kashmir. Of 1,976 women in Fiji, 40% had tertiary education, while 40% of 392 women in Kashmir had no education at all. The lack of female doctors or nurses with whom to discuss issues was perceived as a problem in both countries.

Table 1 (*Continued*) Details of studies with results indicating observations on cancer awareness in nurses

Cancer (reference)	Objective	Type of study	Sample size	Observation and Inference
Cervix (Raj et al, 2023)[48]		Cross-sectional analytical study	118 paramedical professionals	After implementing EI2W, scores improved in all domains except cervical pre-cancer. ANMs showed better knowledge post-EI2W. More experience led to higher cervical cancer awareness. KAP analysis displayed strong reliability: practice (0.726), and knowledge (0.711).
Breast (Dhakal et al, 2023)[49]	Assessment of duration of an educational intervention for a woman's intention to do a breast self-examination (BSE) and mammography screening.	Interventional study	360 females	Initially, attitudes, perceived behavioral controls, and intents were similar between IG and CG for both mammography and BSE, except subjective norms. BSE intentions effective for 4 months, and mammography for 4 and 12 months. Stable attitudes for both tests at 4, 8, and 12 months. Good control persisted for 4 months in both tests. Session maintained BSE and mammography intent for 4 months.
Cervix (Chacko 2022)[50]		Pre-test and post-test design	Community health workers working in selected centers in Najafgarh, Delhi	Initially, community health workers lacked VIA test knowledge. After a structured teaching program, their post-test knowledge scores significantly improved, establishing a positive relationship.
Cervix (Rahman and Kar, 2015)[51]	Assessment of baseline knowledge of cancer cervix, screening, and practice of Pap smear screening	Predesigned, pretested, self -administered multiple responses questionnaire survey	Sikkimese staff nurses in India.	Around 90.4% of nurses knew about cancer cervix, but most did not know it is the commonest site. Among those aware, 79% knew about screening. Only a third knew Pap smears' start age. Age influenced awareness, with older staff more aware. Marital status and religion also impacted awareness. Only 16.6% nurses who knew about Pap smears had one. Most common reason offered for not undergoing Pap smear test were they felt they were not at risk (41%), uncomfortable pelvic examination (25%), and fear of a bad result (16.6%).
HPV vaccine (Shetty et al, 2019)[52]	Knowledge, attitude, and factors associated with acceptability HPV vaccine among undergraduate medical, dental, and nursing students in South India.	Post-test study design	988	In a survey of 988 students, most were familiar with cervical cancer (95%), HPV (89.3%), and genital warts (77.5%). About 59.7% knew about the HPV vaccine, 65.2% intended to receive it, and 68.3% would recommend it. Age influenced vaccine acceptance, with <22-year-olds less likely. Medical students (OR: 1.12), alcohol users (OR: 1.15), and moderate knowledge holders (OR: 1.14) were more likely to intend vaccination. Course and attitude significantly affected vaccine intention on multivariate analysis.
HPV vaccine (Swarnapriya et al, 2015)[53]	Assessment of the knowledge, attitude, and practices regarding cervical cancer screening and HPV vaccination among medical and paramedical students	Cross-sectional study	957 participants belonging to medical, dental, and nursing streams	Out of 957 participants, 430 (44.9%) displayed good knowledge and 65 (6.8%) had received HPV vaccination. Among the unvaccinated, 433 (48.54%) were not willing to take the vaccine. Concerns regarding the efficacy (30.5%), safety (26.1%), and cost of the vaccine (21.7%) were responsible for this.

(*Continued*)

Table 1 (*Continued*) Details of studies with results indicating observations on cancer awareness in nurses

Cancer (reference)	Objective	Type of study	Sample size	Observation and Inference
				Age, gender, family history of malignancy, and mother's education had no influence on knowledge. Compared with medical students, nursing students had better knowledge and students of dentistry had poor knowledge.
Breast (Santhanakrishnan et al, 2015)[54]	Assessment of the knowledge, attitude, and practices (KAP) regarding the breast cancer and its screening methods among staff nurses.	Cross-sectional study	198 staff nurses working in tertiary-care hospital in Puducherry	Family history (40.9%), inadequate breast feeding (29.8%), and lifestyle factors (24.7%) were important risk factors as per participants. About 36.9% mentioned biopsy as a diagnostic test; 73.2% mentioned BSE as a screening test; 67.5% were practicing BSE, but only 5.5% were practicing it regularly. Only 11.6% mentioned CBE as a screening test, and 10.8% had undergone CBE only once. About 18.7% mentioned mammography as a screening test.
Cervix (Goyal et al. 2012)[55]	Evaluation of knowledge, attitude, and practices (KAP) of the nurses on cervical cancer and screening.	Cross-sectional study using self-administered, structured, open-ended, and pretested questionnaire	200 nurses in a teaching hospital in Surat	Majority (88%) were married; most common age of marriage being 21–25 years. Nurses linked multiple sexual partners (61%), sex at an early age (44%), HPV infection (38.6%), and heredity (31%) to cervical cancer. Approximately 70% believed that cervical cancer is preventable, detectable, and curable if detected early. Pap smear was recognized as a major screening technique by 74% nurses. Eighty percent nurses never took cervical screening while 87.5% did not recommend it to others.
Cervix (Kosambiya et al, 2018)[56]	Exploring the knowledge, attitude, and practices of nurses about cervical cancer and screening	Pretested semi-structured questionnaire method	103 nurses of a tertiary care center in Western India.	Majority (98%) were aware about cervical cancer, while 73.8% agreed that it could be prevented. Major symptoms of cervical cancer recognized were irregular bleeding (31.7%) and foul smelling vaginal discharge (34.2%). Nursing academic study (51.3%) was the leading source of all information. Risk factors identified were HPV (15.6%) and poor personal hygiene (14.7%).
Oral health (Philip et al, 2019)[57]	Knowledge, attitude, and practice of nurses regarding oral care for hospitalized patients	Cross-sectional survey	244 nurses working in a tertiary care hospital in Bangalore	The mean oral health knowledge score was 6.74 out of 22. Nurses understood inpatient oral care's significance and oral health's systemic links. Knowledge gaps existed mainly in medication impacts and denture care. Higher qualifications and longer department stay related to better attitudes. Prompt oral health assessment post-admission was common, but protocols and documentation showed inconsistencies.
Cervix (Shah et al, 2012)[58]	Evaluating nursing staff's knowledge on cervical carcinoma. Understanding respondent behavior toward prevention and screening.	Cross-sectional interview-based survey	100 nursing staff from one of the tertiary health institutes of Ahmedabad, India	Only 5 (5%) respondents underwent Pap test

Table 1 (*Continued*) Details of studies with results indicating observations on cancer awareness in nurses

Cancer (reference)	Objective	Type of study	Sample size	Observation and Inference
Cervix (Chawla et al, 2021)[59]	To summarize the knowledge, attitude, and practice toward screening of cervical cancer among health professionals in India	Review	22 studies with a total of 6,811 health professionals	The overall knowledge of cervical cancer among health professionals was 75.15%. The knowledge, attitude, and practice toward screening was 86.20, 85.47, and 12.70%, respectively.
Breast cancer (Oza et al, 2011)[60]	Knowledge, attitude, and practices of nursing staff toward the early detection of breast cancer	Cross-sectional study	250 nurses	Almost 74% knew that early detection of breast is possible and 71% of the nurses would like to go for early detection by mammography. Only 7.2% of nurses had undergone investigation for early detection. 96% of nurses want information regarding the breast cancer and most them by the means of seminar and workshop.
Breast (Sujindra and Elamurugan, 2015)[61]	Assessment of the level of knowledge, attitude, and practice regarding self-breast examination	Cross-sectional study	254 female nursing students	Response rate was 94.5%. Total mean knowledge score was 14.08 ± 3.42. Acceptance of early detection's survival benefit was at 87.5%, while 89.2% knew about BSE for early breast cancer detection and 93.3% considered BSE necessary and 87.5% had done it before. Some (5%) nursing students found BSE embarrassing. Regular BSE practice was low at 33.3% annually.
Cervix (Gedam and Rajput, 2017)[62]	Assessment of the knowledge, attitude, and practices regarding cervical cancer screening and HPV vaccination and to analyze the factors associated	Cross-sectional study	143 nurses and 75 nursing students (total 218)	Among the nurses surveyed, 33.49% had received education about cervical cancer and HPV. 84.4% believed they faced no risk of cervical cancer. 65.60% knew about Pap smear as a screening method. Among married nurses, only 27.28% underwent regular gynecological exams, and 74.47% never had Pap smear. 54.59% were unaware of the Pap smear's appropriate starting age. Knowledge about HPV transmission (33.49%) and HPV vaccine (26.15%) was limited. Almost all (98.62%) nurses had not received the HPV vaccine.
Cervix (Swapnajaswanth et al, 2014)[63]	Knowledge, attitude, and practice (KAP) regarding screening (Pap test) and vaccination for carcinoma cervix and to assess barriers to acceptance of the Pap test.	Cross-sectional, descriptive study using semi-structured, self-administered questionnaire	Among female doctors and nurses in a tertiary care hospital in Bangalore	Higher proportion of doctors, 45 (78.9%), had very good knowledge as compared with only 13 (13.3%) of the nurses, about risk factors for cancer cervix and Pap test ($p = 0.001$). As many as 138 (89.6%) of the study subjects had favorable attitude toward Pap test and vaccination, but 114 (73.6%) of the study subjects never had a Pap test and the most common reason 35 (31%) for not practicing was absence of disease symptoms.
Cancer (Rao et al, 2019)[64]	Assessment of the level of cancer awareness among hospital nurses, identify the knowledge gaps, and to incorporate them into training	Cross-sectional survey	244 nurses in a tertiary care hospital in North Delhi	Out of 244 nurses, 75.81% were aware of cancer and approximately 77.5% recognized cancer as a significant Indian health concern, while 79.9% knew about its rising incidence. About 66.4% understood cancer as a lifestyle disease while 75.4% recognized cancer's preventability, and 78.7% agreed on early detection. 23% thought all lumps were cancerous. Almost 75% knew warning symptoms; nearly 90%

(*Continued*)

Table 1 (*Continued*) Details of studies with results indicating observations on cancer awareness in nurses

Cancer (reference)	Objective	Type of study	Sample size	Observation and Inference
				understood causative factors. It was seen that 21% held cancer myths: 23.4% thought it is contagious, 25% familial, and 13.9% due to curse. Misconceptions included cancer spread by biopsy (17%) or surgery (21.7%).
Cervix and breast (Dhanasekaran et al, 2022)[65]	Educational intervention for medical education reforms to include curricula to strengthen knowledge about cancer screening	Manuscript workshop pre- and post-test method using structured questionnaire	91 nursing students	Ninety-one students attempted both pre- and post-surveys, of which 56 were from Group 1 and 35 from Group 2. Students demonstrated statistically significant improvements in knowledge on cervical and breast cancer screening after participating in the workshop.

Abbreviations: ANMs, auxiliary nurse midwives; BC, breast cancer; BSE, breast self-examination; CBE, clinical breast examination; HPV, human papillomavirus; VIA, visual inspection of the cervix with acetic acid.

30.5% of the health workforce, are a vital part of the country's health care infrastructure and are involved in direct care of people affected with a range of ailments that include road traffic injuries, infectious diseases, mental disorders, maternal and nutritional deficiencies, NCDs especially type II diabetes, hypertension, and arthritis.[66] The other important aspect is that given the low doctor-to-population ratio (1.34 for 1,000 in 2017),[67] coupled with significant absenteeism of medical doctors and their inadequate distribution, the onus of patient care is principally on the nurses.[66–68]

Training

Education plays a vital part in raising awareness and efforts to educate the public, as evidenced by a pre- and post-test study that was beneficial in improving women's knowledge of BSE.[69] The recent findings[70] confirm the effectiveness of a camp-based, organized training program in increasing awareness, selective screening, and timely referral through the utilization of clinical breast examination[70] camps with 1,061 ASHA (Accredited Social Health Activist)[70] workers in Uttarakhand, India.[70] This is a very important observation because ASHA workers perform the job of linking citizens with the government of India's health initiative and can help propagate breast cancer awareness and importance of screening in the rural and underserved areas of society.

A nurse-guided planned teaching on breast cancer and BSE[71] among peri-menopausal women in a selected urban community of Mumbai, India was performed and the results demonstrated that the program increased the women's knowledge and practice of BSE.[71] A 1-day course-based endeavors to improve knowledge on "*Cervical and breast cancer screening*" is also reported to have improved awareness and screening knowledge in the nursing students who participated.[65]

Recently, George and Batra evaluated the efficacy of a community-based, multicomponent, nurse-led intervention program to determine an increase in cervical cancer screening behavior and knowledge in a rural community in Idukki, Kerala, India.[72] The experimental group received small group education, reinforcement, telephone reminders, Pap smear navigation and counselling, and investigator follow-up, whereas the control group received no intervention.[72] Before and twice after the intervention, women's knowledge, attitude, and screening behavior regarding cervical cancer prevention were measured. The results suggest that the interventional group had improved their cancer preventive knowledge, attitude, and screening behavior.[72] A positive relationship between knowledge and screening behavior, as well as a significant correlation between education, age at marriage, and number of pregnancies and knowledge, attitude, and practice regarding cervical cancer prevention was observed.[72] The study confirmed that the nurse-led intervention program improved women's cervical cancer screening behavior and suggested the need for recurrent incentives and reinforcement to incorporate behavioral change and increase rural women's use of screening programs.[72]

Novel Teaching

Traditionally, studies on cancer awareness have principally been based on the contact-tracing learning process followed in the didactic teaching of mentor-based module. However, in the recent past, electronic gadgets have been tried for dissemination of the knowledge and the subsequent sections address the aspects. Sharma and colleagues[73] conducted a random-sample research study in an Indian rural hospital to investigate the feasibility of smartphone-based cervical cancer screening by nurses and health care professionals (through visual inspection under acetic acid).[73] To maintain track of participant information, inspection results, and following care, the nurses in this study used a log of observations and a formal survey at each clinic visit. Concurrently, the cervical area smartphone photographs were forwarded to a specialist for review of the nurse's clinical assessment. The study's findings revealed that nurses with the necessary training can do credible screenings and that timely expert comments can enhance reporting.[73]

Concomitantly, Bhatt and colleagues[74] developed a mobile health (mHealth) prototype, performed training

sessions, and initiated a screening intervention to increase cervical and oral cancer screening rates in three impoverished locations of India.[74] Community health workers used visual inspection with acetic acid and visual oral inspection to test for cervical and oral cancer with the support of nurses.[74] The mHealth prototype was shown to be quite acceptable and capable of promoting cancer screening in low-income rural populations with low health literacy.[74]

In a recent study, Raj and coworkers reported that a 2-week educational intervention to paramedical staff that also included auxiliary nurse midwives (ANMs) improved understanding of cervical cancer.[48] The study conducted by Tata Memorial Centre (Mumbai), India's oldest and leading cancer hospital and training center, focused on knowledge, attitude, and behavior and all of which were assessed through the standard pre- and post-test format.[43] The training focused in five vital domains: disease awareness, HPV, pre-cancer stage, screening methods, and data management.[43] The results indicated that all domains' scores improved except for the pre-cancer domain, confirming that the 2-week intervention was successful in enhancing the knowledge of paramedical professionals and that this approach could be helpful in filling the gaps caused by the lack of human resources in community-based cancer prevention efforts.[43]

Hitt and coworkers researched into how well telemedicine could help fill the gap in care for rural women in Arkansas, United States, by screening them for cervical cancer. Results from 1,504 patients referred from 68 counties showed that they were consistent with those of conventional exams.[75] As a result of its low cost and positive patient reception, telemedicine has the potential to increase health care accessibility for marginalized groups.[75] All these studies indicate that adopting modern electronic-based methods can be useful in creating both cancer education and screening endeavors in hinterlands where the services of cancer specialists are scare.

Cancer Education in Nursing Syllabus in India

Structured teaching during academic teaching through curriculum design is the most appropriate means to inculcate right knowledge on cancer cause, signs, awareness, and good health practice for self as well as to propagate right information to family and others. The Indian Nursing Council has introduced modules on breast, oral, and cervical cancer in the revised syllabus 2020 for undergraduate curriculum for Baccalaureate in Nursing (in Medical Surgical and Community Health Nursing courses). In addition to structured mentor-led didactic class room teaching, emphasis is also placed on practical training to develop necessary clinical skills in performing, assisting, and providing after care for patients undergoing diagnostic procedures like BSE, clinical examination, mammogram, Pap smear, colposcopy, oral examination, and swab taking under the supervision of trained nursing teachers and medical doctors.[18,76]

Concerted efforts are also directed in developing soft skills in the students to contribute as nurse educators in the society and through structured community visit posting in both urban and rural areas. Students are taught to address cancer causes, signs, symptoms, and treatment to the general population in an attempt for them to be health ambassadors in the community. They must be taught the right way to address stigma, fear, and apprehensions and that most cancers are curable if detected early in general public. Special emphasis should be placed in inculcating ethical way of presenting aspects on cancer signs in breast and cervix with cartoons or diagrammatic pictures in a culturally appropriate way. Student's competencies in cancer screening are evaluated through clinical performance assessment, objective structured clinical examination, and effective patient care in practical examinations. The students should also be taught on the ethical aspects and on how to handle moral dilemma in accordance with the bioethics principles.[76,77]

Barriers Nursing Educators Face and How It Can Be Improved

Today, oncology is a highly specialized branch in health care sciences and needs knowledge and training in diverse subspecialties. Considering this, in most developed countries, concerted attempts are directed in training interested individuals in onco-nursing and oncology nurse educator programs through specialized training and mentor-guided teaching in both subject and patient care aspects. However, in India emphasis is on training a larger number of general nurses rather than experts, to satisfy the high demands of the population and specializing advanced nursing training (post basic diploma programs in nursing) is still in its infancy. One of the important reasons for this is that there is scarcity of suitable job prospects for nursing experts and the negligible difference in financial compensation between general and specialist nurses, which discourages many nurses from undertaking specialization.[76]

In India, cancer screening is mostly performed by medical doctors (oncologists, gynecologists, surgeons, and otorhinolaryngologists) and dentists. Studies from both India and abroad have shown that nurses when trained and encouraged can perform the initial cancer screening and refer them to medical doctors for confirmation and advanced care. In the recent past, mobile screening vans for Pap, mammogram, and oral screening along with well-equipped tele-medicine are being used for cancer screening in the society. A well-trained nurse can lead the initial screening endeavors in the community and refer people with possible cancer symptoms to professionals for further confirmation and treatment to medical doctors and tertiary care centers. Concerted and deliberate efforts should be directed toward training nursing students in their academic curriculum by experts in the field to serve the community on graduation.[76]

Challenges in Education, Research, and Training in Cancer Nursing

The Government of India has introduced training module for staff nurses serving at primary health centers on population-based screening of common NCDs. However, this module is

underutilized and efforts should be toward nurses' enrolling and getting trained in cancer screening for oral, breast, and cervix. Periodic knowledge and skill update are essential for nurses and should be attempted through continuing education programs by online or offline methods. Specific efforts should also be directed toward training practicing nurses by organizing credit-based refresher courses and deputing them in oncology care units to get trained under experts and return to serve the community. In the absence/shortage of medical doctors in the rural areas, this re-orientation of nurses' training can be very useful in timely detection of cancer and help patient direct to advanced medical care in tertiary care centers. Efforts are needed to consider these aspects and bridging the gaps.[76,77]

Conclusion

This review offers a succinct summary of the current deficiencies, the contributing factors, and strategies for addressing the gap in cancer education and awareness. It also discusses the different ways in which nurses in India can contribute to the efforts of preventing and detecting cancer in themselves, the community, and clinics. Future research should focus on intentionally prioritizing curriculum development and implementation of cancer prevention programs at both the national and regional levels. To gain a more comprehensive understanding and classification of the duties and tasks performed by nurses in cancer prevention, it is imperative to gather supplementary information from relevant stakeholders and educational resources, as well as provide practical training for the nursing workforce. Additional study will be required to measure the results of nurse education and other treatments for primary and secondary prevention. Efforts to improve cancer screening and prevention should involve utilizing the expertise of nurses in community health and cancer health care systems, both within hospitals and in the community. In conclusion, nursing students and professionals can play a highly significant role in encouraging the widespread adoption of cancer screening guidelines by actively participating in clinical practice, academia, and advocacy after the existing gaps are addressed. Efforts should be focused on supporting and promoting this, as it will provide great benefits to nursing professionals, the fraternity, the community, humanity, and the nation.

Patient Consent

This article is a review of previously published literature and does not involve any studies with human participants or animals conducted by the authors. Hence, patient consent is not required.

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Conflict of Interest

None declared.

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Clinical and Pathological Spectrum of Hepatoblastoma with Emphasis on Treatment-Induced Changes: Experience from Tertiary Care Center

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Abstract

Introduction Hepatoblastoma is a rare pediatric liver tumor. Advances in imaging/surgical techniques and use of neoadjuvant chemotherapy (NACT) in recent times have resulted in improved survival of children with hepatoblastoma. Yet it has dismal prognosis in some children. Unlike other pediatric malignant tumors, pathological tumor regression grading in hepatoblastoma following NACT is not in routine practice. Assessing tumor-induced maturation and delineating it from non-neoplastic liver at resection margin are often challenging in this setting.

Objective We aim to describe the clinicopathological spectrum of hepatoblastoma encountered in our center with emphasis on exploring the role of grading the therapy-induced changes by correlating with existing prognostic factors and patient survival.

Materials and Methods All cases of hepatoblastoma having undergone resection after NACT over 9 years were included. Pathology slides (hematoxylin and eosin/immunohistochemistry) were reviewed. Therapy-related changes were scored and compared with pretreatment extent (PRETEXT)/posttreatment extent (POSTTEXT) staging, alpha fetoprotein (AFP) levels, and patient survival.

Results A total of 15 children diagnosed with hepatoblastoma were included in the study. The median age of diagnosis was 10 months. PRETEXT III was the commonest stage and fetal variant was the commonest histological subtype. Fibrosis, necrosis, maturation, calcification, and ductular reaction were the therapy-induced changes encountered in 93, 80, 60, 53 and 33% cases, respectively. Higher percentage of therapy-induced changes was associated with good prognosis and better survival. Glypican-3 positivity delineated tumor-induced maturation from the non-neoplastic liver.

Keywords
- hepatoblastoma
- child
- liver neoplasm
- neoadjuvant therapy
- immunohistoche–mistry
- prognosis

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Conclusion This study describes the spectrum of hepatoblastoma at a single center and emphasizes that grading therapy-induced changes may have a significant role in patient prognosis and guide further treatment interventions for effective management of patients. Glypican-3 eases microscopic assessment of resection margins in the presence of therapy-induced maturation.

Introduction

Liver malignancies account for 1% of pediatric cancers. Hepatoblastoma (HB) is the most common primary pediatric malignancy of the liver, affecting 1.5 cases per million population annually.[1] Most HBs (>90%) present with markedly elevated serum alpha-fetoprotein (AFP), which plays a major role in the diagnosis, monitoring response to therapy, and patient follow-up.[2] Complete resection of the tumor is critical for cure. However, approximately 60 to 80% of these patients present with unresectable tumor at diagnosis.

Introduction of cisplatin-based neoadjuvant chemotherapy (NACT) has facilitated downsizing of tumor and greatly improved resectability and hence the survival.[3] Currently the 5-year survival rate of HB is up to 80% as compared with 30% 30 years ago.[4] Therapeutic approaches and patient management across the globe largely rely on the risk stratification by international organizations for pediatric malignancies such as Children's Oncology Group (COG), Childhood Liver Tumors Strategy Group (SIOPEL), and Children's Hepatic tumors International Collaboration (CHIC), which are largely based on radiological pretreatment extent (PRETEXT)/posttreatment extent (POSTTEXT) staging, and AFP levels at diagnosis.[5–7] Although controversial, NACT followed by surgical resection is the mainstay of treating[6] HB at most centers. Prognostication and treatment modification based on response to NACT, as adopted for other pediatric malignancies such as neuroblastoma and nephroblastoma, has not been adopted for HB. Various aspects of histological assessment of treatment-induced changes in HB are not well documented in the literature. The phenomenon of therapy-induced maturation of tumor cells in HB poses significant difficulty in accurate assessment of surgical margins.[8] A reliable marker to distinguish tumor cells from normal hepatocytes at the margins is warrantied.

Currently, the role of histological assessment and grading of therapy-induced changes as prognostic/predictive marker in HB is not well established and is not in routine practice.

In this study, we aim to describe the clinicopathological spectrum of HB encountered at our center and emphasize the role of assessing the therapy-induced changes by correlating with known prognostic factors and patient survival.

Materials and Methods

Study Design and Setting

The present study is a retrospective, descriptive study on a cohort of patients diagnosed with HB.

Children diagnosed with HB based on imaging and/or serum AFP levels and who have undergone NACT followed by surgical resection between 2011 and 2019 (9 years) were included in the study. Cases determined as non-HB on histology were excluded. Patient details such as demography, history, AFP levels (at diagnosis, postchemotherapy, postsurgery, and at last follow-up), radiological tumor size (at diagnosis and post-NACT), PRETEXT staging, chemotherapy regimen, and follow-up details were obtained from the hospital database and cancer registry. It was a time bound, retrospective study including all patients diagnosed as HB between 2010 and 2019; the sample size obtained was 15.

Pathological Assessment

Hematoxylin and eosin (H&E) and immunohistochemistry (IHC) slides of all the cases were reviewed, blinded to the original reports. Viable and nonviable areas of the tumor were scored as percentage of the total tumor tissue. Tumor regression grading (TRG) was done as follows.

- **TRG** I: greater than 75% nonviable areas.
- **TRG** II: greater than 25% and ≤75% nonviable areas.
- **TRG** III: ≤25% nonviable area.

The percentages of fibrosis, necrosis, and therapy-induced maturation seen in the tumor were scored separately. Therapy-induced calcification/ossification and ductular reaction were also noted. When no viable tumor cells were seen, it was called pathological complete regression (PCR). Viable tumor areas were classified based on morphology as fetal, embryonal, mixed fetal and embryonal, mixed epithelial and mesenchymal, teratoid, and small cell undifferentiated (SCUD). Margin clearance of less than 1 mm was considered a positive margin.

Immunohistochemistry

Hepatocellular markers, HepPar1 (OCH1E5, PathnSitu) and Arginase1 (SP156, Cell Marque); biliary markers, CK7 (OV-TL12/30, BioGenex), CK19(A53_B/A2.26, Cell Marque), along with β catenin (EP35, PathnSitu) and Glypican-3 (1G12, Cell Marque), were performed for diagnostic and prognostic evaluation. Additional neuroendocrine, mesenchymal, and stem cell markers were performed when required. Area and pattern of staining of each marker were noted carefully.

Primary Outcome

Therapy-induced changes encountered were fibrosis, necrosis, calcification/ossification, and maturation. Higher percentages of therapy-induced changes were associated with

better prognostic factors (reduction in AFP levels and radiological tumor size) and better survival.

Secondary Outcome

Glypican-3 was found to a reliable immunohistochemical marker to differentiate therapy-induced maturation of tumor cells from the non-neoplastic liver at the resection margin.

Inclusion Criteria

Children diagnosed to have HB based on imaging and/or serum AFP levels and who underwent NACT followed by surgical resection were included in the study.

Exclusion Criteria

Pediatric liver tumors other than HB and cases of HB with unavailable slides/blocks were excluded from the study.

Statistical Analysis

SPSS software version 27.0 was used for statistical analysis.

Data were described using frequency with percentage for categorical variables and means along with standard deviation for continuous variables. The date of diagnosis was considered the entry point. The date of recurrence/metastasis was the end point for disease-free survival (DFS) and the date of the last follow-up/date of death was the end point for overall survival (OS). The *t*-test was used for univariate analyses. DFS and OS were evaluated by using the Kaplan–Meier method and compared using log-rank test, and a *p*-value of ≤ 0.05 was considered significant.

For statistical analysis, the histological subtypes were categorized into four types, fetal, mixed fetal and embryonal, and pure embryonal, which were classified as the epithelial type.

Mixed epithelial and mesenchymal and teratoid were grouped into the mesenchymal type, SCUD, and PCR. Therapy-induced mature/maturing areas of the tumor were considered under fetal variant. The cutoff value was considered at 10% for fibrosis and at 30% for necrosis.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Institutional ethical committee clearance was obtained (ECASN-AIMS-2024–335, dated: July 16, 2024).

Results

Clinical Picture and Demography

Twenty children were diagnosed to have HB and underwent NACT and resection over a period of 9 years. Five children were excluded from the study as their block/slides were not traceable. Fifteen children were included, whose age ranged from 1 to 132 months (median age = 10 months). There were 10 male children and 5 male female children (M:F = 2:1). Ten children (66%) were aged $\leq$12 months, three (20%) were between 12 and 48 months, and two (13%) were older 60 months (72 and 132 months, respectively). Mass per abdomen was the most common presenting symptom (86%), followed by incidental detection in two children and congenital liver mass in one child. Two were born preterm; one had maternal family history of malignancy, the details of the cancer could not be traced. One child was born to a mother with gestational diabetes.

PRETEXT III was the most frequent PRETEXT stage (6 cases, 40%), followed by PRETEXT IV and II (4 cases, 26% each). PRETEXT I was the least common (1 case, 6%). The right lobe of the liver was involved in 8 (53%) cases, the left lobe was involved in 4 (27%) cases, the bilateral lobes was involved in 2 (13%) cases, and 1 (6%) case had multifocal involvement. Serum AFP level at diagnosis ranged from 14.93 to 1,538,000 ng/dL (median = 127,870 ng/dL) and the level after NACT ranged from 9.33 to 15,884 ng/dL (median = 405 ng/dL). There was significant reduction in the AFP levels following NACT (>99% fall in 67% of the cases, $p = 0.03$). One child with the SCUD histological variant *presented with normal AFP level* (14 ng/dL) at diagnosis, which remained within normal range throughout the treatment.

There was significant decrease in radiological size of the tumor after NACT (mean PRETEXT size = 10 cm; POSTTEXT size = 5.3 cm; $p = 0.04$). The demography and clinical and radiological features are further detailed in ►**Table 1**.

Pathological Findings

There were 9 (60%) epithelial tumors (6 pure fetal, 2 pure embryonal and 1 mixed fetal and embryonal variant; ►**Fig. 1A**), 4 (27%) mesenchymal tumors (2 teratoid and 2 mixed epithelial and mesenchymal variants; ►**Fig. 1B**), 1 pure SCUD (6%) variant, and 1 case (6%) of PCR (►**Table 2**). Of the 15 cases, 7 had pretreatment biopsies, of which 6 were epithelial (3 fetal, 2 mixed epithelial and fetal, 1 pure embryonal), and 1 SCUD type (►**Table 2**). Positive margins were seen in five (33%) cases.

Table 1 Patient demography and clinical characteristics

		n = 15	Percentage
Median age (range)	10 (1–132) mo		
Gender	Male	10	66.6
	Female	5	33.3
Symptoms	Mass per abdomen/ abdominal distension	13	86.6
	Incidental detection	2	13.3
PRETEXT stage	I	1	6.7
	II	4	26.7
	III	6	40
	IV	4	26.7
Site	Right lobe	8	53.3
	Left lobe	4	26.6
	Right + left lobe	2	13.3
	Multifocal	1	6.6

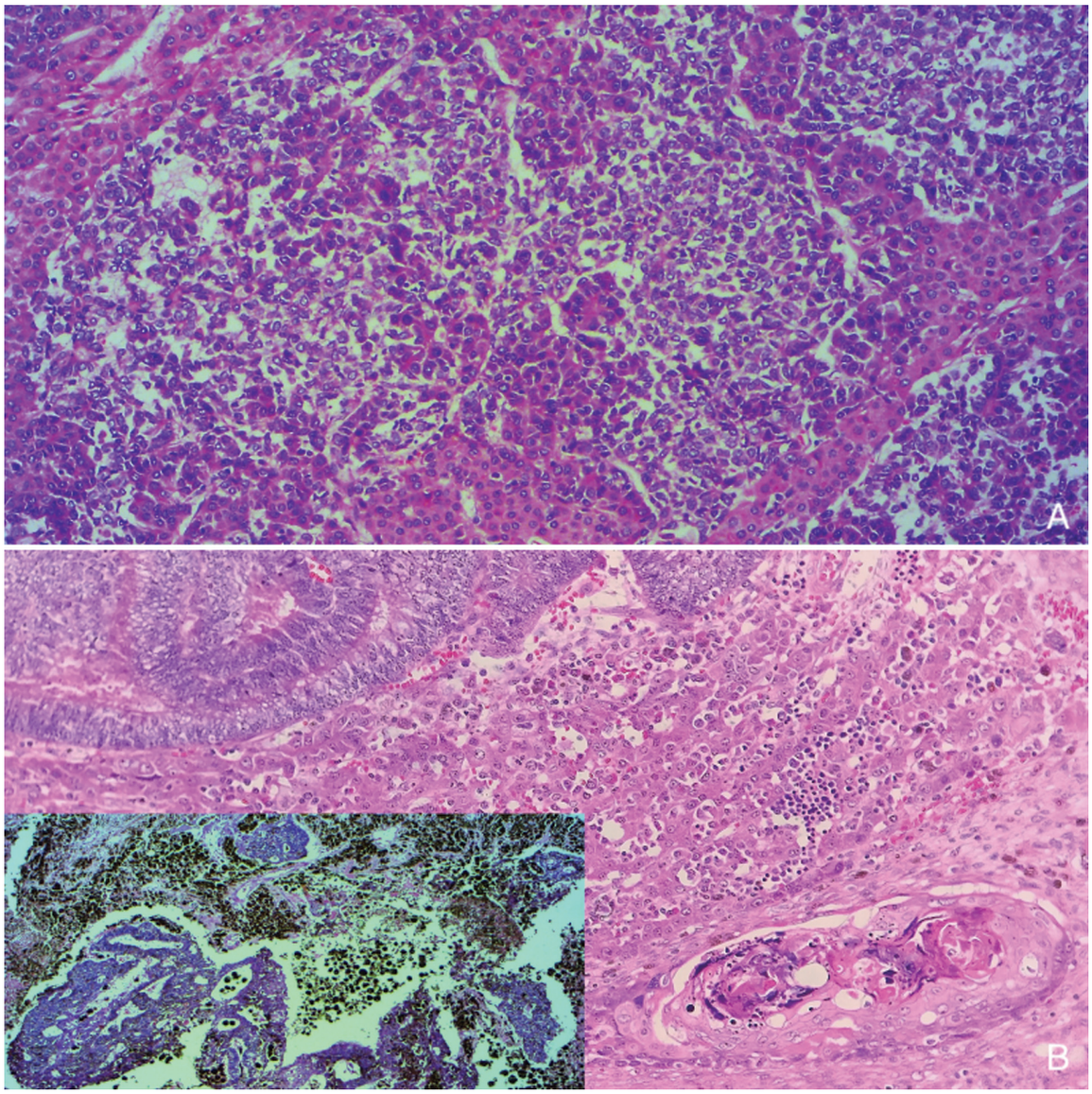

Fig. 1 Photomicrography showing (**A**) embryonal and fetal variant of hepatoblastoma (HB; hematoxylin and eosin [H&E], ×200) and (**B**) teratoid variant with squamous areas; inset showing melanin pigments (H&E, ×200).

Table 2 Distribution of histological subtype

	Prechemotherapy biopsy ($n = 7$)	Post chemotherapy excision ($n = 15$)
Embryonal	1 (14.2%)	2 (13.3%)
Fetal	3 (42.8%)	6 (40%)
Mixed epithelial and fetal	2 (28.5%)	1 (6.6%)
Mixed epithelial and mesenchymal	0	2 (13.3%)
Teratoid	0	2 (6.6%)
Small cell undifferentiated	1 (14.2%)	1 (6.6%)

Therapy-Induced Changes

Grade III tumor regression predominated in our series (53%), while TRG I was the least (20%). Fibrosis was the commonest therapy-induced change (93%), followed by necrosis (83%). Maturation, calcification/ossification (►**Fig. 2A**), and ductular reaction were other therapy-

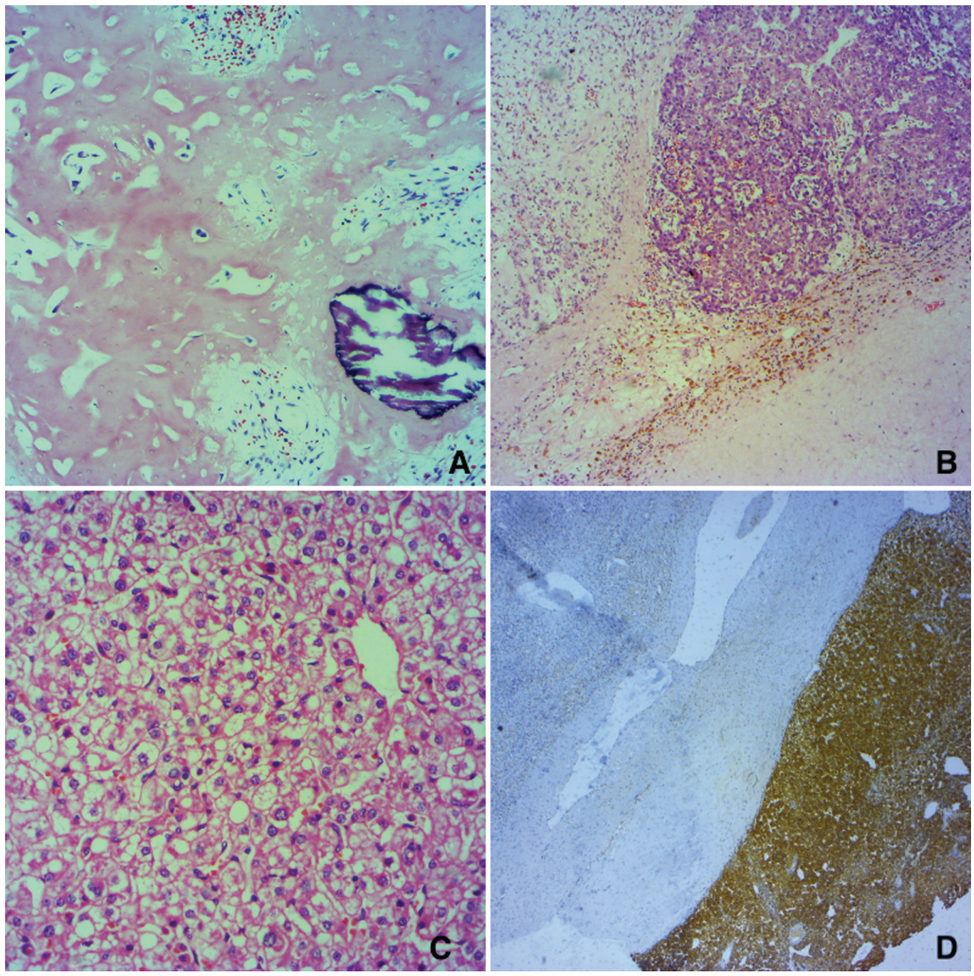

Fig. 2 Photomicrography showing neoadjuvant chemotherapy (NACT) induced changes in hepatoblastoma (HB). (**A**) Osteoid and calcification (hematoxylin and eosin [H&E], ×200). (**B**) Fibrosis with hyalinization and hemosiderin-laden macrophages (H&E, ×100). (**C**) Therapy-induced maturation (H&E, ×400). (**D**) Glypican-3 positivity in maturing areas as opposed to a normal liver (immunohistochemistry [IHC, ×25).

induced changes (60, 53, and 33% cases, respectively; ►**Table 3**).

Fibrosis ranged from zero in SCUD variant to 92% in the case of PCR (mean fibrosis = 26%; ►**Fig. 2B**). The average posttreatment AFP was higher in tumors showing less than 10% fibrosis as compared with tumors showing greater than 10% fibrosis (►**Table 4**).

Histologically, the embryonal variant showed the least amount of fibrosis. However, there was no significant difference in OS and DFS between the two groups (►**Table 5**). Therapy-induced necrosis ranged from 0 to 48% (mean necrosis = 21%). The mean posttherapy AFP level was higher in tumors showing no necrosis as compared with those showing greater than 30% necrosis (►**Table 4**). Likewise, tumors showing no necrosis had lower DFS as compared with tumors showing necrosis; the difference was not statistically significant (►**Table 5**). Therapy-induced maturation was noted in all cases with pure fetal morphology, 50% of mesenchymal type, and 40% of mixed epithelial type. SCUD showed no maturation. Mature areas were often seen at the interface of the tumor with the normal liver and closely resembled a normal liver (►**Fig. 2C**). Interestingly, therapy-induced maturation was directly related to the amount of fibrosis and inversely related to necrosis. Maturation was associated with better survival (►**Table 5**). The most common histological type showing calcification and ductular reaction was the mixed epithelial type. There was significant association of ductular reaction with fibrosis ($p = 0.017$).

Table 3 Histological assessment of therapy-induced changes

$n = 15$	Seen	Not seen
Fibrosis	14 (93.3%)	1 (6.6%)
Necrosis	12 (80%)	3 (20%)
Maturing areas	9 (60%)	6 (40%)
Calcification	8 (53.3%)	7 (46.6%)
Ductular reaction	5 (33.3%)	10 (66.6%)

Table 4 Correlation of therapy-induced changes with known prognostic factors of hepatoblastoma

Therapy-induced change	Fibrosis		Necrosis		Maturation	
Prognostic factors	≤10%	>10%	≤30%	>30%	Yes	No
Predominant histological type	Embryonal	Mesenchymal	Embryonal, mesenchymal	Fetal embryonal	Fetal	Embryonal
AFP at diagnosis (ng/mL)	100,000	370,000	280,000	380,000	270,000	240,000
Postchemotherapy AFP levels (ng/mL)	11,328	1,465	2366	370	2015	2197
Pretext	III, IV	II	III, IV	I, II	III, IV	III, IV
Age (mo)	22	28	17	71	26	23

Abbreviations: AFP, alpha-fetoprotein.

Immunohistochemistry

IHC was performed in 10 cases. β-catenin staining was seen in seven cases (membranous/nuclear). Nuclear β-catenin was seen in embryonal areas only. Membranous β-catenin was observed in the mature areas, fetal area, and non-neoplastic liver. Glypican-3 was positive in all 10 cases, with a variable staining pattern (►**Fig. 2D**). It was strongly cytoplasmic in embryonal, canalicular to moderate cytoplasmic in mature and fetal areas, focal patchy positive in SCUD areas, and negative in non-neoplastic livers.

Patient Follow-Up and Survival

OS and DFS by the Kaplan–Meier analysis was 62.54 ± 13.414 and 53.44 ± 12.603 months, respectively. Nine patients were disease free. Six patients had progression of disease in the form of recurrence in the residual liver (3 cases) and distant metastases (3 cases).

Four patients (2 embryonal, 1 mesenchymal, and 1SCUD histology) succumbed to the disease. Children younger than 3 years and female patients had better OS and DFS when compared with the children older than 3 years and males. Among *children younger than 3 years, 50% showed good response to therapy* with the tumor showing greater than 50% nonviable areas, while among *children older than 3 years, 33% showed a good response*. The difference was not statistically significant ($p = 0.60$).

Histological subtypes, PRETEXT/POSTTEXT staging, therapy-induced changes, or margin positivity showed no significant difference in survival (►**Table 5**).

Table 5 Correlation of clinical and therapy-induced pathological parameters with patient survival

Clinical and pathological parameters		Disease free survival	*p* value	Overall survival	*p* value
Age	≤36 mo	57.22 ± 14.18	0.117	60.75 ± 15.74	0.003
	>36 mo	31.00 ± 7.42		38.00 ± 12.24	
Gender	Male	30.41 ± 7.32	0.95	67.33 ± 24.22	0.95
	Female	67.00 ± 24.49		39.25 ± 6.86	
Calcification	Yes	52.94 ± 17.52	–	68.33 ± 16.56	0.83
	No	30.667 ± 9.60		38.40 ± 8.65	
Fibrosis	≤10%	51.5 ± 16.09	0.2	59.14 ± 16.55	0.2
	>10%	30.00 ± 4.97		40.00 ± 0.00	
Necrosis	0%	17.50 ± 3.18	0.13		
	0–30%	51.5 ± 16.09		–	–
	>30%	43.50 ± 7.42		–	
Maturing areas	No	34.05 ± 8.00	0.09	46.50 ± 4.59	0.18
	Yes	51.5 ± 18.58		52.68 ± 18.07	
Viable areas	>50%	45 ± 17.02	1.02	52.83 ± 18.07	1.026
	≤50%	41.6 ± 7.35		47.00 ± 4.95	
Regression grading	I	35 ± 1.41	1.11	–	–
	II	36 ± 9.67		–	
	III	42 ± 20.02		–	

Discussion

HB is a well-recognized, unique pediatric malignant liver tumor, which recapitulates the normal hepatic ontogenesis.[2] The comprehensive treatment strategy of surgery combined with chemotherapy has greatly improved the overall prognosis of HB over the years. Yet the disease is life-threatening to some with disease recurrence and progression.[9]

Two-thirds of HBs are diagnosed by 24 months.[2] The mean age at diagnosis ranges from 11 to 46 months and was 10 months in our study.[2,10] A male predominance was seen in our study similar to few other studies.[4,10] Our study showed significant better OS in children younger than 36 months and worse OS in children older than 36 months. Similarly, a study by Yang et al[9] showed significant lower DFS in patients older than 54 months. Age is not a risk factor in the COG or SIOPEL guidelines, while the CHIC study states that older children with HB do worse.[6,9]

HB in adults is extremely rare, with less than 75 reported cases so far, and has dismal prognosis.[11,12] The highest age at diagnosis in our study was 11 years.

Elevated AFP[2] is the diagnostic hallmark of HB. The serum AFP level and radiological PRETEXT staging are important for risk stratification and staging of HB.[5,7] As per SIOPEL,[7] PRETEXT I, II, and III with elevated AFP (>100 ng/dL) are considered standard risk, while PRETEXT IV, low AFP, and very high AFP (>1.2 million ng/mL) are high-risk HBs. In our study, the percentage of recurrence and death was higher with PRETEXT IV (75 and 50%, respectively) compared with the combined PRETEXT I, II, and III (27 and 18%, respectively). In another study,[10] PRETEXT I and II had a significantly better DFS and lower mortality when compared with PRETEXT III and IV. The average reduction of the AFP level post-NACT in our cases was 42%, which was comparable to the study by Wang et al.[8] Yang et al[9] showed that reduction of AFP levels by greater than 60% and radiological tumor size by greater than 50% following NACT is an independent prognostic factor for the 3-year DFS.

HB is thought to arise from an embryonal precursor and displays diverse histologies.[13,14] Wnt pathway[15] is believed to be involved in embryonal and mixed histology and the Notch pathway in the pure fetal type. Tumor heterogeneity[16] is claimed to be one of the causes for the varied behaviors of HB. In our study, the pure fetal variant showed the highest survival and lowest mortality and good response to treatment (TRG I), which was similar to the findings in the literature.[5,17] Akin to this, one of our patients with pure fetal variant and lung metastasis at diagnosis responded well to resection and chemotherapy and was disease free. The embryonal, mixed epithelial and mesenchymal, and SCUD variants showed poorer response to chemotherapy (TRG 2 and 3). Likewise, the mesenchymal subtype and the embryonal variant with high mitosis were found to have aggressive behaviors in few studies.[10,18,19] On the contrary, the study by Wang et al[8] showed the mesenchymal component to be associated with better outcome. Although fetal histology had better survival as compared with other histology, statistical significance could not be reached in our studies and many other studies.[8,15,20] The SCUD subtype warrants accurate histological diagnosis as it presents with a normal/low AFP level and has aggressive behavior with worst survival. SCUD in pure or combined with other histological types puts the patient in the high-risk category.[5,6] The SCUD tumor must be differentiated from the malignant rhabdoid tumor by IHC for Integrase interactor 1 (INI1) as it requires distinct management.[9,17] In our study, the child with the SCUD variant with retained INI1 responded poorly to both standard and second-line therapies, and succumbed to the disease within 5 months of diagnosis.

Our study, like the study by Wang et al,[8] did not show a significant survival difference between positive and negative margins. According to the current SIOPEL guideline,[21] the microscopic positive resection margin is no longer a poor prognostic indicator if the tumor has shown good pathological and radiological response to NACT.

Unlike other pediatric malignancies (acute lymphoblastic leukemia, Wilms' tumor), the role of histological assessment and grading of response to NACT in predicting survival and further therapeutic interventions is not established in HB. [20,22,23] Histological assessment of response to NACT could potentially be used to modify adjuvant therapy[9] by intensifying/reducing dose or adding newer drugs for improved outcome in HB. A study by Kiruthiga et al[10] demonstrated that patients with less than 50% of viable tumors following chemotherapy had higher DFS when compared with patients with greater than 50% viable tumors. On the contrary, our study did not show a significant difference in survival between the three TRG groups or with cutoff of 50% for viable tumors (►**Table 5**). However, when each of the therapy-induced changes were considered separately (presence of calcification, fibrosis, necrosis, and maturation), it was seen that patients showing treatment-induced changes had better survival compared with patients without therapy-induced changes (►**Table 5**). In our study, patients with tumor showing greater than 10% fibrosis and greater than 30% necrosis were associated with lower posttreatment AFP levels, better histological type, and higher survival (►**Table 4**). The presence of tumor-induced maturation, necrosis, and calcification showed better survival (►**Table 5**). Similarly, Venkatramani et al[20] showed that the risk of disease progression and death in HB decreases significantly with increasing percentage of tumor necrosis. Necrosis greater than 30% was shown as an independent prognostic factor in their study. The presence of osteoid or calcification post-NACT was considered a sign of maturity[24] and hence considered a good prognostic indicator in HB, similar to Wilms' tumor and germ cell tumor.

Assessing tumor-induced maturation at the resection margin is challenging. In our study, 60% of cases showed a maturation pattern indistinguishable from a normal liver. Glypican-3 positivity in the maturing areas and negativity in the non-neoplastic liver were valuable in delineating two areas (►**Fig. 2D**). While two-thirds of cases in the series by Wang et al[8] showed mature areas mimicking a non-

neoplastic liver, they found β-catenin with CK7 to be useful in differentiating two areas.

Nuclear β-catenin expression was seen in 30% of our cases and only in the embryonal areas. Significant β-catenin staining in the embryonal component was also observed by Bera et al.[16]

Treatment strategies for HB differ across countries. NACT followed by resection[3,5] is followed in most centers. All our patients underwent NACT with four to five cycles of PLADO (doxorubicin and cisplatin) followed by resection and three more cycles of the PLADO regimen postsurgery. The ongoing Pediatric Hepatic International Tumor Trial (PHITT) trial[6,25] is expected to reduce the controversies regarding the treatment of HB.

While treating pediatric cancers, risk stratification of patients is essential for adjusting the doses of adjuvant chemotherapy, adding/deleting radiation therapy and the decision on the extent of surgery. Histology and assessment of response to therapy plays a major role in risk stratification in most of the pediatric solid tumors.[26] Risk stratification ensures fine-tuning of the offered treatment to establish long-term cure while minimizing the side effects of the therapy.[26] HB has rare incidence worldwide. Hence, multi-institutional studies involving larger sample size are desired to conclusively establish a definite relationship of therapy-related changes in HB with the prognosis and pave the way to individualized therapy in these patients. High-throughput studies have revealed many genomic, epigenetic, and transcriptomic alteration in HB[27] in the recent years. Further insights into molecular biomarkers and targets for HB will facilitate newer and less aggressive therapeutic modalities that would heighten HB survivors with improved quality of life after therapy.

Limitations of the Study

HB is a rare neoplasm with a low incidence worldwide. Similarly, due to the study being conducted at a single institute, the sample size of the present study was small and hence no statistical correlation between the treatment-induced changes and the prognosis could be reached. Multi-institutional studies with a larger sample size may throw more light on the prognostic significance of therapy-induced changes in HB and pave the way for a more personalized therapy in these children.

Conclusion

This is a descriptive study of HB encountered at a tertiary care center with emphasis on the role of the histological assessment of therapy-induced changes. Higher percentages of therapy-induced changes in the form of fibrosis, necrosis, calcification, and tumor maturation are associated with better outcome and may have a role in postsurgery therapeutic interventions, leading to personalized medicine and improved therapy for HB. Further inter-institutional studies with larger sample sizes are needed to definitely demonstrate the significance of assessing therapy-induced changes as a prognostic factor in HB.

Authors' Contributions

D.A. contributed to data curation, writing of the original draft, preparation, and editing of the manuscript. M.E. contributed to the conceptualization, methodology, and supervision of the study, and editing of the manuscript. P.K. contributed to the visualization, investigation, supervision, and reviewing of the study. S.S. contributed to the visualization, investigation, supervision, and reviewing of the study. N.V. contributed to the visualization, investigation, supervision, and reviewing of the study.

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Conflict of Interest

None declared.

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Testing Patterns and Prevalence of gBRCA Mutations among Women with Breast Cancer: A Cross-Sectional Observational Study

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Abstract

Introduction Pathogenic germline mutations in BRCA (*gBRCAm*) genes can heighten the risk of breast cancer (BC) among carriers. Economic constraints and patient testing hesitancy challenge adherence to hereditary germline testing guidelines. As a result, clinicians prioritize hereditary BC screening based on patient willingness, affordability, and therapeutic benefit.

Objectives The objectives of the study were (1) to identify the pattern of hereditary cancer germline testing among women diagnosed with BC and (2) to determine the prevalence of *gBRCAm* among the women with BC who underwent hereditary cancer germline testing.

Materials and Methods A retrospective study was conducted at a cancer hospital between October 2023 and January 2024. We aimed to assess the germline testing patterns of physicians in our hospital by examining the clinical profile of patients with BC who underwent hereditary cancer multigene (30 gene panel) mutation testing using next-generation sequencing between January 2021 and December 2023. A simultaneous analysis was performed with a multiplex ligation-dependent probe amplification to detect deletions and duplications in the *BRCA1* and *BRCA2* genes. The classification of the variants as pathogenic and variants of uncertain significance (VUS) was determined by the American College of Medical Genetics and Genomics guideline.

Results Of the 3,600 patients with BC during this study period, only 325 (9%) underwent germline testing. The testing patterns indicated that the median age of those tested was 48.4 years (standard deviation [SD]: 10.1; range: 20–77), 189 patients (58.2%) were younger than 50 years, and 103 patients (31.7%) had a family history of cancer. Family history of BC was reported in 95 (29.2%) patients. Bilateral BC was noted in 19 patients (5.8%), while ovarian cancer was reported in 9 (2.8%) patients. Triple-negative BC (TNBC), hormone receptor–positive BC, and HER2-positive BC were reported in 52, 42.8, and 17.2% patients, respectively. Pathogenic/likely pathogenic

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(P/LP) germline *BRCA* mutations were detected in 48 (14.7%) patients (*BRCA1* in 29/325 [8.9%] patients and *BRCA2* in 19/325 [5.8%] patients). The highest prevalence was seen among TNBC (36/169, 21.3%) patients. P/LP *gBRCAm* prevalence among those with and without notable family history was 27/103 (26.2%) and 21/222 (9.5%), respectively; age less than 50 years and greater than 51 years was noted in 32/189 patients (16.9%) and 16/136 (11.8%) patients, respectively. VUS was noted in 29 patients (*BRCA1* in 4 patients [8.9%] and *BRCA2* in 25 patients).

Conclusions Measures to ensure equitable access to genetic testing can improve testing rates and enhance patient outcomes through personalized care.

Introduction

Breast cancer (BC) is the most common cancer among women in India, with new cases increasing by 39% from 2010 to 2016.[1,2] In 2019, the Global Burden of Disease (GBD) reported a BC prevalence of 163.3 per 100,000 in India.[3] Among the nonmodifiable risk factors, age, ethnicity, high-risk family history, and genetic susceptibility are critical determinants that significantly contribute to overall susceptibility and likelihood of developing BC.

Germline mutations in the BRCA (*gBRCAm*) gene give rise to *BRCA1* and *BRCA2* variants, heightening the risk of developing BC among their carriers.[4] It has been estimated that approximately 5 to 10% of all BC cases are familial, with germline mutations in the BRCA1 or BRCA2 genes accounting for 15 to 20% of the observed risk.[5] These mutations are more sensitive to certain therapies, necessitating tailored interventions with poly-ADP ribose polymerase (PARP) inhibitors.[6]

The prevalence of *gBRCAm* varies by age, family history of BC or ovarian cancer, and BC types: hormone receptor–positive BC (HR+), triple-negative BC (TNBC), and human epidermal growth factor receptor 2 positive (HER2+). The National Comprehensive Cancer Network (NCCN) broadly endorses testing for women diagnosed with BC based on epidemiological, clinical, and biological factors as well as indications for the use of PARP inhibitors.[7] The prevalence of pathogenic *BRCA1/2* and TNBC is higher in India, with the *BRCA1/2* mutation frequencies ranging from 2.9 to 24% among Indian familial patients with BC.[1,8] Additionally, genetic, ethnic, and cultural diversity within India complicates accurate representation of the *gBRCAm* burden in BC.[9,10] Economic constraints in testing, stigma, and the psychological burden experienced by the patients further challenge adherence to testing criteria such as the NCCN guidelines.[11,12] As a result, clinicians prioritize hereditary BC screening based on patient willingness, affordability, and therapeutic benefit.

The objectives of this study are the following:

- To identify the pattern of hereditary cancer germline testing among women diagnosed with BC.
- To determine the prevalence of gBRCA mutation among women with BC who underwent hereditary cancer germline testing.

Materials and Methods

A retrospective study was conducted at the Basavatarakam Indo-American Cancer Hospital & Research Institute (BIACHRI) between October 2023 and January 2024.

Inclusion Criteria

The study population included all women who had been diagnosed with BC and had undergone testing for germline *BRCA1*/*BRCA2* mutation between January 2021 and December 2023.

Exclusion Criteria

Male patients with BC were excluded.

The data were obtained from the electronic medical records and laboratory records. The collected data included the following: age at the time of diagnosis, family history of any type of cancer and clinical characteristics including type of *BRCA* mutation (*BRCA1* or *BRCA2*); variant category (pathogenic, likely pathogenic, variant of uncertain significance [VUS]); metastatic status; status of estrogen receptor (ER); progesterone receptor (PR); and HER2 status.

Primary and Secondary Outcomes

The primary outcome of the study was the prevalence of *gBRCAm* among women with BC between October 2023 and January 2024. The secondary outcome was to describe the patterns of germline hereditary breast and ovarian cancer (HBOC) testing by analyzing the descriptive characteristics of the patients who were tested. A notable family history, as per the NCCN criteria, was defined as BC at any age and $\geq$1 close blood relative (first-, second-, or third-degree relative) with BC, male BC, ovarian cancer, pancreatic cancer, and prostate cancer.

Statistical Analysis

Data were entered in Microsoft Excel and analyzed using Stata Statistical Software (Release 14, StataCorp LP, College Station, TX, United States). The prevalence of *gBRCAm* was expressed as a percentage with a 95% confidence interval (CI), calculated using the binomial exact test. The analysis was shown for age in mean with standard deviation (SD) and family history of the study population, and the description of *gBRCAm* according to the clinical parameters was expressed in frequency and percentage. Cases with *gBRCAm* were

analyzed using the chi-squared test. A *p*-value of less than 0.05 was considered statistically significant.

Ethical Approval

Ethical approval was obtained from the institutional ethics committee at Basavatarakam Indo-American Cancer Hospital & Research Institute (BIACHRI; IEC/2023/281) on October 30, 2023. All data were anonymized and the study was conducted in accordance with the ethical standards of the institute and the Helsinki Declaration of 1964 and its later amendments.

Testing Methods

Hereditary cancer multigene mutation testing was done using next-generation sequencing (NGS) at BIACHRI. A simultaneous analysis was performed with multiplex ligation-dependent probe amplification for *BRCA1* and *BRCA2*, deletions, and duplications. The genomic DNA was extracted from the peripheral blood samples using the QIAamp DNA Mini Kit (QIAGEN, Victoria, Australia) according to the manufacturer's instructions.[13] The DNA quality was confirmed using a Qubit dsDNA HS Assay kit (Life Technologies) on a Qubit4.0 Fluorometer (Life Technologies).[14]

Next-Generation Sequencing

NGS was performed in the following steps. The library preparation was performed using the Oncomine *BRCA* Research Assay and was sequenced using an Ion GeneStudio S5 Plus Platform (Life Technologies).[15] The resulting sequence reads were initially analyzed for variant detection using the Ion Torrent Variant Caller (available at the Life Technology Torrent Browser Plugin store by aligning to the human genome reference [hg19]).[16] Visual confirmation of the identified variants was accomplished with Integrative Genomics Viewer software from the Broad Institute (Cambridge, MA, United States).[17] Finally, the variants (related to the phenotype) were scored and reported according to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines for sequence variant interpretation.[18]

Multiplex Ligation-Dependent PCR Amplification

Multiplex ligation-dependent PCR amplification (MLPA) was performed using the following kits: P002_BRCA1 for the BRCA1 gene and P045_BRCA2 for the BRCA2 gene (MRC Holland, the Netherlands).[19] An optimized 100 ng of input DNA was utilized for the MLPA reaction and the process was performed at the applied condition for initial denaturation of the sample DNA; a mixture of MLPA probes (BRCA1 and BRCA2 probes) was added separately to the sample. In general, each MLPA probe consists of two oligonucleotides as instructed and designed by the manufacturer. The fragment analysis was performed on the ABI PRISM 3730XL Genetic Analyzer (Applied Biosystems, United States) using LIZ 500 (Applied Biosystems) as a standard size.[20] For statistical analysis, the MLPA ratios (dosage quotient) of below 0.7 or above 1.3 are indicative of a deletion (copy number change from 2 to 1) or duplication (copy number change from 2 to 3), respectively. A dosage quotient of 0.0 indicates a homozygous deletion; a dosage quotient of 0.35 to 0.65 indicates heterozygous deletion, a dosage quotient of 1.35 to 1.55 indicates heterozygous duplication, and a dosage quotient of 1.7 to 2.2 indicates homozygous duplication.

Results

Of the cohort of 3,600 patients with BC that presented to our hospital between January 2021 and December 2023, 325 patients (9%) underwent germline multigene (30 gene panel) mutation testing using NGS for germline BC. Pre- and post-test genetic counseling was provided to all the patients by the consulting oncologist.

Characteristics of Patients Tested

The mean age of the cohort was 48.4 years (SD: 10.1; range: 20–77; ►**Table 1**). A notable family history was present in 103 (31.7%) of the patients tested, including 95 (29.2%) with a family history of BC. Other cancers were reported in 21 patients (6.4%). With regard to molecular subtypes of BC, TNBC, HR+, and HER2+ were reported in 169 (52%), 139 (42.8%), and 56 (17.2%) patients, respectively.

Prevalence of P/LP gBRCA1/2m

Of the patients tested, pathogenic/likely pathogenic (P/LP) gBRCA1/2m were detected in 48/325 (14.7%) patients (►**Table 2**). The afflicted genes were discovered as *BRCA1* in 29/325 patients (8.9%; $p = 0.001$), while 19 patients were found to have BC of *BRCA2* origin (19/325; 5.8%; $p = 0.001$). The majority of P/LP gBRCA1/2m were in patients with TNBC (26/29; 89.7%; $p < 0.001$). The variants of VUS were detected in 29 patients (8.9%); 4 (1.2%) in *BRCA*1 and 25 (7.7%) in *BRCA*2. The P/LP non-BRCA germline mutations detected were two each in RAD50 and MUTYH, and one in MRE1.

Table 1 Patient characteristics

Parameters	Estimate
Age (y)[a]	48.4 (10.1)
Comorbidities	123 (37.8)
Stage 4	64 (19.7)
Bilateral breast cancer	19 (5.8)
Significant family history[b]	103 (31.7)
Other cancers (excluding bilateral breast cancer)	
Contralateral breast	6 (1.8)
Carcinoma ovary	9 (2.8)
Thyroid cancer	3 (0.9)
AML	1 (0.3)
CML	1 (0.3)
Lung cancer	1 (0.3)

Abbreviations: AML, acute myeloid leukemia; CML, chronic myeloid leukemia.
[a]Mean (standard deviation).
[b]Following NCCN criteria: Breast cancer (BC) at any age and ≥1 close blood relative (first-, second-, or third-degree relative) with BC, male BC, ovarian cancer, pancreatic cancer, and prostate cancer.

Table 2 BRCA1 and BRCA2

Variants	*n* (%)	BRCA mutation	
		BRCA1	BRCA2
Pathogenic/likely pathogenic	48 (14.7)	29 (8.9)	19 (5.8)
Variants of uncertain significance	29 (8.9)	4 (13.7)	25 (86.2)

Association of P/LP *gBRCAm* with Patient Characteristics

The P/LP gBRCA1/2m prevalence in patients with ER/PR positive, HER2 positive, and TNBC were 11/139 (7.9; 95% CI 4–13), 4/56 (7.1%; 95% CI 2.6–17) and 36/169 (21.3%; 95% CI 15.7–28), respectively (►**Table 3**). The *gBRCAm* prevalence in patients with a notable family history was 27/103 (26.2%; 95% CI 18.5–35.6). There was no significant association between age of onset and *gBRCAm*.

Discussion

The cross-sectional study aimed to determine the prevalence of *gBRCAm* among BC patients and identify the testing patterns for HBOC. We found a 14.7% prevalence of *gBRCAm* after testing less than 10% of the population over the 3-year study period. The testing patterns showed that patients who underwent HBOC testing typically had a notable family history of breast or ovarian cancers, were diagnosed at a young age, or had TNBC. Upon comparing our findings with other similar studies, 32 (16.9%) young women (<50 years) in our study had a *gBRCAm* compared with 10.3% of the patients with BC in a multicenter study.[21] Germline BRCAm was reported in 27 (26.2%) patients with notable family history, closely matching the 28% reported in the same study.[21] Additionally, *gBRCAm* was detected in 36 (21.3%) patients with TNBC, compared with a range of 14.8 to 62.5% patients with TNBC reported in other studies.[21,22] Applying stringent criteria for HBOC testing was expected to select a BRCA-enriched population and thus reveal a higher positivity rate. Indian studies report a *gBRCAm* prevalence of 18 to 23% in patients with BC meeting the NCCN criteria[22–24] and 8 to 21% in unselected cohorts.[21,23,25,26] In our study, selecting patients with a high likelihood of a positive result yielded a detection rate comparable to that achieved by following the NCCN criteria or testing all consecutive patients with BC. Our perspective is that given the high pretest probability, challenges in applying testing criteria and low uptake of testing services have impacted detection rates. Furthermore, testing only 9% of patients with BC indicated a low overall testing rate. A significant barrier to adequate referral and

Table 3 Association of P/LP *gBRCAm* with patient characteristics

Parameters	*n* (%)	BRCA1 positive (*n* = 29)	BRCA2 positive (*n* = 19)	BRCA1/2 positive (*n* = 48)	*p*-value
Receptor subtype					
HR (ER/PR) positive	139 (42.8)	3 (2.2)	8 (5.8)	11 (7.9)	0.003
HER2 positive	56 (17.2)	1 (1.8)	3 (5.4)	4 (7.1)	0.07
TNBC	169 (52)	26 (15.4)	10 (5.9)	36 (21.3)	< 0.001
Bilateral breast cancer					
Present	13 (4)	1 (7.7)	2 (15.4)	3 (23.1)	0.4
Absent	312 (96)	28 (9)	17 (5.4)	45 (14.4)	
Significant family history[a]					
Present	103 (31.7)	14 (13.6)	13 (12.6)	27 (26.2)	< 0.001
Absent	222 (68.3)	15 (6.7)	6 (2.7)	21 (9.5)	
Age of onset (y)					
≤50	189 (58.2)	22 (11.6)	10 (5.3)	32 (16.9)	0.2
≥51	136 (41.8)	7 (5.1)	9 (6.6)	16 (11.8)	
Disease characteristic					
Metastatic	64 (20.6)	6 (9.4)	6 (9.4)	12 (18.8)	0.3
Nonmetastatic	261 (80.3)	23 (8.8)	13 (5)	36 (13.8)	

Abbreviations: ER, estrogen receptor; HER, human epidermal growth factor receptor 2; HR, hormone receptor; PR, progesterone receptor; TNBC, triple negative breast cancer.

[a]Following NCCN criteria: Breast cancer (BC) at any age and ≥1 close blood relative (first-, second-, or third-degree relative) with BC, male BC, ovarian cancer, pancreatic cancer, and prostate cancer.

underutilization of HBOC testing services is the ethical challenge faced by physicians in recommending tests that many patients cannot afford.[11,27] The cost of genetic testing and subsequent treatments, such as PARP inhibitors and risk reduction surgeries are often prohibitive for most patients. This financial burden creates an ethical dilemma for health care providers who must balance the necessity of these tests with the potential financial strain on their patients. Additionally, informing family members of potentially distressing test results, which are crucial for their screening, presents another ethical challenge.[28] The lack of dedicated genetic counsellors in a culturally diverse country like India complicates the communication of genetic testing benefits in a manner that respects the sociocultural norms and values.[29] High financial costs remain the main barrier to the uptake of genetic testing services, with genetic testing affordable for only 15% of newly diagnosed patients with BC.[30–32] Awareness and interest in genetic counseling and testing are low, with many patients disinclined to screen themselves or their families for hereditary BC.[29,30] Factors such as paternalistic attitudes, sociocultural influences, familial tensions, and a low perceived benefit of testing hinder uptake of genetic testing.[11,12,33,34] Social stigma affecting both patients and future generations, coupled with the absence of laws against genetic discrimination in the workplace or society at large, contributes to the reluctance to pursue genetic testing. To improve testing patterns, enacting laws against genetic discrimination could address policy-level issues and alleviate bias concerns. Insurer support is essential to mitigate the costs of genetic testing and treatment, making these services more accessible. BC awareness campaigns should highlight the unique role of genetic testing in precision treatment, distinguishing it from other diagnostic tests. Establishing training programs in genetic counseling and integrating these services into mainstream health care are vital steps forward in bridging the health care gap and ensuring that patients receive comprehensive care that includes genetic testing.

Limitations

The data for this study were acquired from the hospital records. It reflects the patient population that seeks care at this hospital and may not be representative of the general population. Despite this, the study provides a valuable assessment of testing pattern in our institute and the burden of *gBRCAm* among patients with BC and can be used as a reference for future research.

Conclusion

In our cohort of BC patients, germline mutation testing identified P/LP *BRCA1/2* mutations in 14.7% of cases, with a notable association observed in patients with TNBC and those with a notable family history. Available guidelines and sociocultural and economic constructs are key determinants for the referral and uptake of hereditary germline testing for BC. Measures to ensure equitable access to genetic testing can improve testing rates and enhance patient outcomes through personalized care.

Authors' Contributions

Study concept and design were developed by S.S.A., R.P., N.H., S.R. Literature search was performed by S.S.A. Data acquisition and data analysis were done by S.S.A. and V.A. Manuscript preparation was done by S.S.A., K.K.M.V., N.A.Y., and V.A. Manuscript editing and manuscript review were done by S.S.A., R.P., N.H., S.R., S.K., R.T., N.A.Y., and C.C.K.N. The manuscript has been read and approved by all the authors. The requirements for authorship have been met. Each author believes that the manuscript represents honest work.

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Conflict of Interest

None declared.

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Can ^{18}F-FDG PET/CT Metabolic Tumor Volume Contribute to Better Prognostication in Pediatric Hodgkin's Lymphoma?

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Abstract

Introduction Studies in adults have shown that metabolic tumor volume (MTV) in fluorine-18 fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET)/computed tomography (CT) is a strong predictor of event-free survival (EFS) and overall survival (OS) in Hodgkin's lymphoma, often outperforming clinical scores and molecular predictors. However, there very few studies on pediatric Hodgkin's lymphoma (PHL), with conflicting results.

Objectives This retrospective study was conducted to evaluate the feasibility of MTV assessment in PHL and to assess its prognostic role, given the paucity of data from the developing world and the technical expertise required.

Materials and Methods Children with PHL, treated per EuroNet-PHL-C1 interim guidelines/C2 protocol at our center from 2017 to 2020 who had baseline and interim PET (iPET) scan done at our institution were included. MTV was measured in tumor areas with standardized uptake value (SUVmax) $\geq$2.5. MTV and SUVmax were compared at diagnosis and after two chemotherapy courses.

Results Sixty-one children (male:female = 1.5:1; mean age: 10.10 years) were recruited and categorized into four stages (SI: 11; SII: 15; SIII: 21; and SIV: 14) and three treatment groups (TG1: 16; TG2: 11; and TG3: 34). Based on iPET, 47 and 14 children were adequate and inadequate responders, respectively. At a median follow-up period of 54 months, the OS was 96.7% and the EFS was 85.2%. The median SUVmax and MTV were both found to increase with advancing disease stage with a positive correlation ($r = 0.41$; $p = 0.002$). The difference in the median MTV was statistically significant for SII versus SIII ($p = 0.004$) but not for the median SUVmax ($p = 0.13$). Similarly, the difference in the median MTV was statistically significant for TG2 versus

Keywords
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- metabolic tumor volume
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- pediatric Hodgkin's lymphoma
- prognostication
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TG3 ($p = 0.001$) but not for the median SUVmax ($p = 0.06$). The median MTV in baseline PET/CT with Deauville score–based treatment response groups for adequate and inadequate responders was 98.35 (37.93–298.2) mL and 145 (84.43–463.5) mL, respectively ($p = 0.31$), and for those with events versus no events, the median MTV was 304 (30.45–452.7) mL and 105.35 (37.9–309.2) mL, respectively ($p = 0.82$).

Conclusion Baseline PET/CT MTV showed better correlation than SUVmax in delineating stage and treatment groups. However, MTV in isolation was not sensitive or specific enough in prognosticating treatment response or EFS (relapse or death) in this study setting. The addition of significant clinico-biochemical parameters with MTV for future studies could enhance prognostication.

Introduction

Pediatric Hodgkin's lymphoma (PHL), accounting for 5 to 6% of all childhood cancers, has a peak incidence at younger ages in developing countries, unlike in developed countries.[1,2] With major advancements in PHL treatment protocols, the 5-year event-free survival (EFS) rates have improved dramatically over the last several decades, approaching 90 to 95% overall for all stages in the West.[3,4] Developing countries have progressively bridged the gap, with recent studies in India pegging the 5-year EFS for early-stage PHL at 94%, which is comparable to Western data.[5] There was also marked improvement in the outcomes of advanced-stage PHL to 81.1% 5-year EFS.[6] Despite these excellent 5-year EFS rates, the late morbidities such as second malignancies and cardiovascular risks occur even in those with early-stage disease. Thus, newer PHL treatment protocols call attention to optimal strategies that reduce cumulative therapy, thereby mitigating potential long-term toxicity, while maintaining treatment efficacy.[7] Toward this objective, "risk-based" and "response-adaptive" strategies for PHL are based upon pretreatment prognostic factors and interim evaluation of disease response, respectively.

The "risk-based" strategy involving multiagent chemotherapy, with pretreatment prognostic risk factors defining treatment intensity, is the standard of care for all patients. Radiation therapy is "response-adapted," being reserved for patients with residual disease based on interim positron emission tomography (PET)/computed tomography (CT).[8,9] Although PHL stage, presence of B symptoms (fever, weight loss, night sweats), tumor bulk, erythrocyte sedimentation rate (ESR), and extranodal involvement have significant prognostic values, these are interrelated. Hence, factors with independent prognostic implications have become more difficult to pin down, as the treatment outcomes continue to improve.[10]

Since almost all lymphomas are fluorine-18 fluorodeoxyglucose (^{18}F-FDG) avid, ^{18}F-FDG PET/CT, which integrates morphological and metabolic function information, has now become the primary investigation for lymphoma staging, which translates to risk stratification. The interim PET assessment is also useful in prognostication, with reduction in avidity correlating with treatment response.[11,12] Metabolic tumor volume (MTV), total lesion glycolysis (TLG), and standardized uptake value (SUV) have been reported as diagnostic and prognostic parameters in PET/CT since the late 1990s for several adult cancers.[13] Of these, the most commonly used parameter for the quantification of tumor metabolic activity is the SUV.[14]

In lymphomas, MTV, a parameter extracted from baseline FDG PET/CT has been proposed as a prognosticator at diagnosis, with strong correlation with EFS and overall survival (OS), often outperforming clinical scores, molecular predictors, and interim PET/CT results.[12] MTV and consequently TLG are considered to better reflect metabolic tumor burden and more accurately portend prognosis than maximum standardized uptake value (SUVmax).[14] Several studies have shown that MTV predicts survival in various non-Hodgkin's lymphoma (NHL) subtypes in children[15–17] including a study from India on anaplastic large cell lymphoma (ALCL) by Mathew et al.[15] However, only a few retrospective studies have confirmed this promising role in early HL in adults.[18]

In PHL, a few studies evaluating the prognostic value of SUVmax and MTV in predicting EFS have been published, with rather conflicting results.[19,20] The usefulness of volumetric PET/CT indices is limited by lack of consensus in determining tumor boundary and the need for advanced software.[12,21,22] As there is a paucity of studies from the developing world, this retrospective study was conducted to evaluate the prognostic role and feasibility of MTV assessment in PHL, and compare its performance with SUVmax.

Materials and Methods

Children aged younger than 18 years with classic Hodgkin's lymphoma (HL) diagnosed and treated in the Pediatric Hematology Oncology unit of our tertiary care hospital between the years 2017 and 2020 and who had a diagnostic and interim assessment with PET/CT scan done at the study center were included. Demographic data and basic clinical details were obtained from the electronic medical records.

^{18}F-FDG PET/CT Image Acquisition and Analysis

For baseline and interim PET/CT image acquisition, a minimum of 6 hours of fasting prior to imaging and blood glucose

levels of ≤11 mmol/L was ensured in all included cases. The dose of ^{18}F-FDG was 3 MBq/kg body weight (minimum dose of 14 MBq) as per the European Association of Nuclear Medicine (EANM) guidelines. Image acquisition was commenced at 60 ± 5 minutes after ^{18}F-FDG injection using Biograph 40 Truepoint PET/CT scanner (Siemens Medical Solutions, Illinois, United States). Oral (iohexol) and intravenous contrast (iopamidol) were administered, adjusted for the patient's weight, the former 1 hour before imaging and the latter with a scan delay time of 60 to 80 seconds. The CT parameters for children younger than 13 years and ≥13 years were 120 keV, 60 mA, section width of 3 mm, and pitch of 0.8 and 120 keV, 150 mA, section width of 5 mm, and pitch of 0.8, respectively.

PET/CT scans of the included patients were uploaded on the syngoMMWP VE61A software and were evaluated by a nuclear medicine expert who was blinded to clinical outcome. Following qualitative analysis for visual identification of nodal/extranodal disease and abnormal metabolic activity on PET/CT, quantitative parameters—SUVmax and MTV—were evaluated considering the spherical volumes of interest as necessary, drawn manually, so as to include all areas of lymphomatous involvement. The same method was used in the entire study cohort for calculation of the study parameters as discussed in the following sections.

Study Parameters

Visual Deauville Score

The Deauville score is a 5-point internationally recommended scale, based on visual interpretation of FDG uptake, in the initial diagnostic staging as well as assessment of treatment response of lymphomas. Diagnostic and interim ^{18}F-FDG PET/CT assessment is based on visual Deauville score (VDS) aided by semi-quantitative measures like SUV.[23] VDS for response evaluation in HL in adults has been validated in developed countries as well as India, including this study center.[24]

Standardized Uptake Value

The most commonly used parameter for the quantification of tumor metabolic activity is the SUV. SUVmax is the maximum voxel value of SUV in the tumor. Although observer independent, it is limited by its value being from only one voxel and inherent image noise sensitivity. However, it is the most widely used metabolic parameter in the assessment of PET/CT scans.[12,21,22]

Metabolic Tumor Volume

MTV is a quantitative measure of the total volume of tumor FDG uptake that exceeds a certain threshold defined as SUVmax ≥2.5 or SUVmax ≥40%. MTV is the measurement of the total FDG activity contained by every voxel of the image of the lymphoma lesions, summing up all nodal and extranodal lesions, the voxel activity expressed as SUV.[12,21,22] From both baseline and interim FDG PET/CT scans, SUVmax of the involved area was calculated. MTV was calculated by semiautomated algorithm using an SUV of 2.5.[25,26]

PHL Risk Stratification: Stage and Treatment Groups

All children diagnosed with PHL were risk stratified and treated as per the EuroNet-PHL-C1 interim guidelines/C2 protocol standard arm. Baseline PET/CT was performed. The children were divided into three treatment groups (TGs) based on the stage of the disease, presence of B symptoms, extranodal involvement, and presence of additional risk factors (ESR > 30 mm/h or tumor bulk ≥200 mL). Patients with Ann Arbor stage IA, IB, or IIA without risk factors or extranodal involvement were assigned to TG1, those with stages IA, IB, and IIA with extranodal involvement or additional risk factors as well as Ann Arbor IIB and IIIA were taken as TG2 with stages IIBE, IIIAE, IIIBE, IIIB, IVA, or IVB treated as TG3.[27,28]

Treatment Protocols

All children received two courses of OEPA (Vincristine [oncovin], etoposide, prednisolone, Adriamycin) and then underwent an interim response assessment by PET/CT. If interim PET/CT showed good response, TG1 patients received one cycle COPDAC (cyclophosphamide, vincristine, prednisolone, dacarbazine), TG2 patients received two cycles of COPDAC, and TG3 patients received four cycles of COPDAC.[27,29]

Treatment Response Assessment and Indications for Radiotherapy

Indications for radiotherapy were based on response assessment on the interim PET/CT. The patients were classified as "adequate responders" and "inadequate responders." Children with Deauville score ≤3 were classified as adequate responders and they received only chemotherapy as mentioned earlier. Those with a Deauville score ≥4 were classified as inadequate responders and were given radiotherapy to the involved fields (in the interim PET) at the end of COPDAC cycles as per TG.[27,29]

Outcome

The children were followed up till July 2024 for OS and EFS. OS was calculated from diagnosis till last follow-up or death, and event was defined as disease progression, recurrence, second malignancy, or death due to any cause.

Statistical Analysis

For continuous variables, mean value with standard deviation was calculated. The mean SUVmax and MTV in the baseline PET/CT were compared for correlation. The study parameters (SUVmax and MTV) were compared and analyzed based on the PHL stage, TGs, response groups, and outcome. A comparison of the median baseline PET/CT MTV was done among the four stages at diagnosis and three TGs using paired *t*-tests. Based on the interim PET/CT response, the patients were divided into "adequate" and "inadequate" responders. The Median SUVmax and MTV in the baseline

PET were compared between the two groups using paired *t*-test. Percentage reduction of MTV from baseline to interim PET was assessed and compared with stage, TGs, and response groups using the rank-sum nonparametric test. The study patients were further classified into two outcome groups with or without an event. The median SUVmax and MTV in the baseline PET/CT were compared between the two outcome groups using paired *t*-test. Receiver operating characteristic (ROC) analysis was used to identify sensitivity and specificity of PET/CT for predicting EFS. Using the ROC curve, the 85th percentile MTV value cutoff was determined to identify the subjects with high MTV. A *p*-valve of less than 0.05 was considered statistically significant.

Ethical Approval

All procedures performed in the study subjects were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments/comparable ethical standards. This retrospective study was approved by the Institutional Review Board (IRB) of the institution (IRB Min No. 14743/6.7.22).

Results

Sixty-one children with PHL were included in the study; their baseline characteristics with subjects classified by age, gender, stages, TGs, and response assessment are shown in ►**Table 1**. The mean age at diagnosis was 10.10 years, with a male-to-female ratio of 1:0.6. The majority were in stage III (21/61) at diagnosis, and designated to TG3 (34/61).

MTV and SUVmax were assessed for all patients in the baseline as well as interim PET/CT scan. The mean SUVmax in the baseline PET/CT was 11.1 (± 3.5) and that in the interim PET/CT was 1.9 (± 3.4). Similarly, the mean MTV in diagnostic and interim PET/CT was 232.73 (± 206) and 72.7 (± 192.4) mL, respectively. Percentage change in MTV and SUVmax between the two PET/CT scans were compared, and both showed significant reduction in the values ($p = 0.001$). The positive correlation between SUVmax and MTV ($r = 0.41$, $p = 0.002$) is shown in ►**Fig. 1**.

►**Table 2** shows the median SUVmax and MTV of various stages of disease and TGs. The median SUVmax and MTV were found to increase with advanced stages of the disease. The difference in the median MTV was statistically significant for stage II versus stage III ($p = 0.004$), but not for the median SUVmax ($p = 0.13$). The difference in the median MTV was statistically significant for TG2 versus TG3 ($p = 0.001$) but not for the median SUVmax ($p = 0.06$)

PET/CT Parameters and Treatment Response

Further analysis was done to compare the SUVmax and MTV on the diagnostic PET/CT scan between "adequate" and "inadequate" responders as well as those who had or did not have an event. Among the 61 patients, 47 patients were classified as "adequate responders" according to the interim PET/CT and 14 as "inadequate responders" (►**Table 1**). Twelve

Table 1 Baseline characteristics of subjects

Baseline characteristics	Number
Age (y)	Mean: 10.10 (4–17)
0–5	5
6–10	30
11–15	22
16–18	4
Gender	Male:female = 1:0.6
Male	37
Female	24
Stage	
IA	11
IIA	10
IIB	5
IIIA	7
IIIB	14
IVA	7
IVB	7
Treatment levels	
Level 1	16
Level 2	11
Level 3	34
SUVmax	
Baseline PET	11.1 (± 3.5)
Interim PET	1.9 (± 3.4)
Metabolic tumor volume	
Baseline PET	232.73 (± 206)
Interim PET	72.7 (± 192.4)
Response assessment	
Adequate response	47
Inadequate response	14

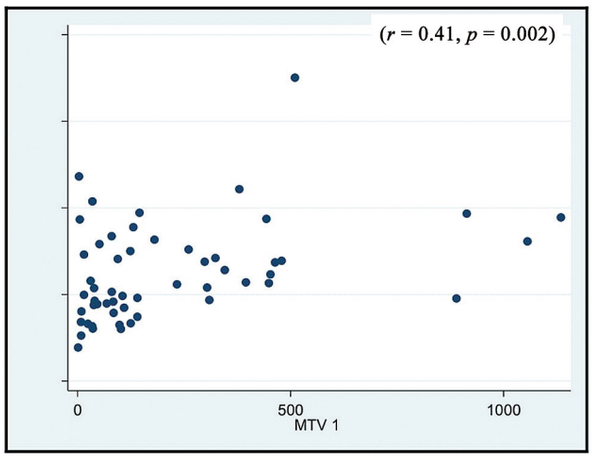

Fig. 1 Correlation between maximum standardized uptake value (SUVmax) and metabolic tumor volume (MTV) in the baseline PET scan.

Table 2 Median maximum standardized uptake value (SUVmax) and metabolic tumor volume (MTV) versus stage of disease and treatment groups

Median SUVmax and MTV vs. stage of disease				
Stage	**Median SUVmax (interquartile range [IQR])**	***p*-value between**	**Median MTV (IQR), mL**	***p*-value between**
I	8.04 (6.06–10.7)	I and IV: 0.009	36.8 (7.9–46.1)	I and IV: 0.0001
II	9.95 (7.4–16.7)	I and II: 0.2	39.9 (14.8–105.3)	I and II: 0.8
III	10.3 (9.12–15.01)	II and III: 0.13	140 (83.6–309.2)	II and III: 0.004
IV	14 (11.4–16.3)	III and IV: 0.5	362.4 (180–478.8)	III and IV: 0.1
Median SUVmax and MTV vs. treatment groups				
Treatment groups (TG)	**Median SUVmax (IQR)**	***p*-value between**	**Median MTV (IQR), mL**	***p*-value between**
1	8.2 (6.5–12.7)	1 and 3: 0.005	36.8 (8.8–51.1)	1 and 3: 0.001
2	9.6 (8.54–11.57)	1 and 2: 0.9	39.9 (14.8–105.3)	1 and 2: 0.6
3	13.7 (9.5–16.3)	2 and 3: 0.06	304 (123.7–452.7)	2 and 3: 0.001

out of these 14 inadequate responders attained complete remission (CR) with chemotherapy and radiotherapy. The remaining two had disease progression and were treated with salvage chemotherapy followed by radiotherapy; one attained remission; however, the second child succumbed to the disease.

For each patient, MTV and SUVmax were calculated in the baseline and interim PET/CT. ►**Table 3** presents median MTV and SUVmax in "adequate" and "inadequate" responders. Both the median MTV and the SUVmax from diagnostic PET/CT were higher in the "inadequate" responders compared to "adequate" responders, but were not significantly different. The median change in MTV between the response groups were analyzed. The group of children with "inadequate" response showed significantly wider variation in reduction of MTV ($p = 0.001$) compared to those with "adequate" response. The MTV range among "inadequate responders" varied from good response of greater than 90% volume reduction to a paradoxical increase in volume.

As further subgroup analysis, the median MTV reduction was compared between initial stage and treatment levels against response groups. However, there was no significant association between stage at presentation, TGs, and median percentage reduction in MTV.

Further analysis was done, based on the Milgrom et al study on the prognostic value of baseline MTV in pediatric and adolescent HL anticipating relapse/refractory disease in about 15% of patients. They opined that a subgroup analysis using the 85th percentile PET/CT MTV value may aid in identifying those who are likely to have refractory disease/disease relapse.[19] In our cohort, the 85th percentile was determined using the ROC curve area under the curve (AUC), and the cutoff was found to be 449. Based on the cutoff value, the study subjects were divided into two groups, high MTV ($n = 9$) and low MTV ($n = 52$) at presentation. The nine patients who had baseline MTV more than the 85th centile included five (55.6%) with stage IV disease, three (33.3%) with stage III disease, and one patient (11.1%) with stage IIBE disease; hence, all belonged to TG3. Three of nine (33%) children had "inadequate response" during interim assessment. Of the nine children with high baseline MTV, one had disease progression and another had disease relapse (both

Table 3 Comparison of median metabolic tumor volume (MTV) and median maximum standardized uptake value (SUVmax) in diagnostic PET/CT with Deauville score–based treatment response groups

Comparison of median MTV and median SUVmax in diagnostic PET/CT with Deauville score–based treatment response groups		
Outcome	**Median MTV (interquartile range [IQR]), mL**	**Median SUVmax (IQR)**
Responders	98.35 (37.93–298.2)	10.73 (8.47–14.61)
Inadequate responders	145 (84.43–463.5)	12.25 (8.54–18.66)
p-value	0.31	0.37
Comparison between occurrence of event with median MTV and median SUVmax in the diagnostic PET/CT		
Outcome	**Median MTV (IQR), mL**	**Median SUVmax (IQR)**
Event	214.3 (32.45–420.7)	11.1 (7.6–13.49)
No event	101.6 (37.9–298.2)	10.3 (8.5–15.3)
p-value	0.63	0.68

were stage 4 at presentation); however, both attained CR postsalvage therapy.

The 52 patients with low MTV belonged to the following stages: stage I = 11, stage II = 14, stage III = 18, and stage IV = 9. Sixteen of 52 children were in TG1, 11 of 52 were in TG2, and 25 of 52 were part of TG3. In all, 43 of 52 children had adequate response in the interim PET. Nine of 52 children, however, had inadequate response in the interim PET. One had progressive disease requiring salvage chemotherapy; however, the remaining eight attained CR at the end of chemotherapy with radiotherapy.

PET/CT Parameters and Follow-Up Outcome

At a median follow-up period of 54 months (5–97 months), the OS was 96.7% (59/61) and the EFS was 85.2% (52/61). Among the study subjects who had an event ($n = 9$), eight of nine children had stage IV disease at initial diagnosis (TG3). All but one child who had a relapse had adequate response in the iPET. Two of nine children had primary progressive disease: one attained remission with salvage chemotherapy and radiotherapy, whereas the other child did not respond to therapy and expired. Seven of nine children had disease relapse: one had an early relapse (3 months after completion of initial treatment) and six had late relapse (after 1 year of initial treatment). Six of seven children who relapsed are in complete remission now, following salvage treatment for first relapse, and one succumbed to the illness.

Children who had an event were studied according to their initial SUVmax, initial MTV, percentage reduction in MTV, and response to treatment. As depicted in ►**Table 3**, the median MTV and SUVmax values in the children with an event were found to be higher, but the difference when compared to those who remained event free was not statistically significant. In addition, the median reduction of MTV between the above two groups also showed no significant difference.

The ROC curve was constructed to assess sensitivity and specificity of diagnostic PET/CT MTV as a prognostic parameter in assessing EFS; the AUC was 0.52 (95% confidence interval [CI]: 0.243–0.815); hence, further analysis was not feasible.

Discussion

In our PHL cohort of 61 subjects, the ^{18}F-FDG PET/CT parameters (MTV and SUVmax) were measured in baseline and interim PET/CT. The median values of both parameters in the baseline PET/CT scan were found to increase as the stage advanced and there was a significant positive correlation. The percentage reduction in MTV and SUVmax between baseline and interim PET/CT was statistically significant. MTV in baseline PET/CT distinguished stages II and III and TG2 and TG3 significantly better than SUVmax, similar to the German study on 50 subjects with PHL by Rogasch et al who were treated according to the EuroNet-PHL-C1 or EuroNet-PHL-C2 treatment protocol.[25] In their study, the mean MTV values were 386.2 mL (137.9–537.8 mL) in stage III and 350.6 mL (207.4–555.9 mL) in stage IV, in comparison to our study values of 140 (83.6–309.2) and 362.4 (180–478.8) mL for the corresponding stages, alluding to a more significant positive correlation with advancing stage.[25] Our study had almost equal distribution of patients across all stages, unlike the Rogasch et al study, which had only one subject in stage I; thus, we were able to demonstrate more convincingly that there was a positive correlation of MTV with advancing stage.[25]

The MTV and SUVmax values in the baseline PET/CT were evaluated as treatment response prognosticators in this study. Although these were noted to be higher among "inadequate" responders as compared to "adequate" responders, the difference was not statistically significant. Nevertheless, the median change in MTV values between the response groups showed wider variation in reduction for the group with "inadequate" response compared to the group with "adequate" response, which was statistically significant. Our study results contrast the Rogasch et al observation, where log-linear analysis showed a significant correlation between a high MTV and response to induction therapy ($p < 0.001$).[25] Similarly, the Reed et al study on 69 subjects in South Africa showed that only MTV on baseline PET/CT, not SUVmax or TLG, was predictive of treatment response.[20] The subjects across stages in our study were more uniformly represented compared to the studies by Rogasch et al and Reed et al, in which stage 1 had only one subject each.

At a median follow-up of 54 months, the OS and EFS in this study were 96.7 and 85.2%, respectively. Baseline PET/CT parameters (MTV and SUVmax median values), although higher in the group with an event, were not significantly different from the group without an event, even on univariate analysis. This is similar to that observed by Reed et al, where none of the metabolic parameters (SUVmax, MTV, or TLG) in the baseline PET/CT were independent predictors of neither EFS nor OS in PHL.[20] The above two contrasts the findings of Milgrom et al, who assessed the prognostic value of baseline MTV in intermediate-risk PHL patients treated with chemoradiation therapy as per the children's oncology group (COG) AHOD0031 trial. This group was able to demonstrate that total-body MTV based on four thresholds (MTV20% SUVmax, MTV1.5Lv, MTV1.5Lv + 2SD, and MTV2BP) and TLG based on two thresholds (TLG60% SUVmax and TLG2BP) were significantly associated with EFS on univariate Cox regression analysis. MTV2BP could distinguish high from low tumor burden and showed the highest sensitivity (91%) and specificity (60%) in identifying the 5-year EFS. This significance was retained on multivariate analysis ($p = 0.012$) after controlling for other prognostically influential covariates, such as disease bulk and response to chemotherapy.[19] A study from China by Zhou et al on 47 PHL subjects, with a median follow-up of 36 months, showed that unlike SUVmax, MTV and TLG in the baseline PET/CT of patients with disease progression were significantly higher than those without disease progression ($p = 0.036$ for MTV and $p = 0.015$ for TLG). However, on multivariate analysis, only TLG was found to be an independent prognostic factor for PFS ($p = 0.021$).[30] Lopci and Mascarin analyzed the usefulness of MTV and TLG in the prediction of outcomes in 150

children with high-risk PHL (stage III/IV disease) who were treated on the EuroNet-PHL-C2 protocol.[31] They were able to show that a high baseline tumor burden (defined as TLG >1,841) correlated with EFS. There was a statistically significant difference for all baseline parameters and treatment evaluation at early response assessment PET/CT (interim PET) between adequate and inadequate responders ($p < 0.05$), with logistic regression confirming significant association for MTV ($p = 0.008$) and TLG ($p = 0.009$).[31]

The difference between varying study groups may be related to sample size and subject characteristics. MTV in children, unlike in adults, could be significantly influenced by the proportion of the tumor burden in relation to the body weight. This has not been factored in by most pediatric studies, despite being an obvious confounder.[20] Studies have also shown that liver SUVmean and liver SUVmax may show variation with age, which may result in variation in MTV calculation in different age groups as well.[32] The other confounders that have been studied are blood glucose levels during the PET/CT scan, differential uptake time, contrast decay, and lack of standardization/consensus in tumor boundary assessment.[12,16,17] The differences in ethnicity, coexisting comorbidities in the recruited subjects, differences in definitions of treatment responders, and median follow-up times postinterim PET/CT[12,19,20,25,33] could also contribute to interstudy variations. However, as most studies, like ours, were single-center retrospective studies on small sample cohorts, we expect them to have lower intra and intercenter variability in technique as well as interpretation of PET/CT as compared to large multicenter trials.[31]

In our study, diagnostic ^{18}F-FDG PET/CT MTV and SUVmax increased with advanced stages of the disease. Diagnostic PET/CT MTV was a better correlate than SUVmax in delineating stage and TGs. However, we found that diagnostic PET/CT MTV or SUVmax alone was not sensitive or specific enough in prognosticating treatment response or EFS (relapse or death).

We note as a limitation that in our study diagnostic and interim PET/CT TLG was not performed. Interim PET/CT MTV and SUVmax were also not analyzed against outcomes. However, given that treatment is modified ("response adapted") based on the interim PET/CT results, the true predictive/prognostic value derived from the interim PET/CT MTV and SUVmax values in HL is difficult to predict.[34]

Conclusion and Future Directions

In our PHL cohort of 61 subjects, we used diagnostic PET/CT MTV and SUVmax values to assess their discriminating ability among staging and TG allocation, as well as their ability in predicting treatment response, EFS, and OS. Baseline PET/CT MTV was a better correlate than SUVmax in delineating stage and TGs as the difference in the value of baseline MTV showed a significant difference between each group. However, MTV in isolation, in our setting, was not sensitive or specific enough in prognosticating treatment response, EFS, or OS. Future studies should involve larger sample sizes with validated clinico-biochemical parameters such as Childhood Hodgkin International Prognostic Score[35] in addition to PET/CT parameters to more accurately prognosticate outcomes in PHL.

Authors' Contributions

S.R. and S.S.S. contributed to the design of the study, literature search, clinical studies, data acquisition and analysis, and manuscript preparation. H.N.S. contributed to the concepts, literature search, clinical studies, data analysis, and manuscript editing. R.R.J. contributed to the concepts, design, literature search, clinical studies, data analysis, manuscript editing, and manuscript review. R.Z.K. contributed to the design, literature search, clinical studies, data analysis, and manuscript editing. M.G. contributed to the design, data analysis, statistical analysis, and manuscript editing. L.L.J. contributed to the concepts, design, literature search, clinical studies, data analysis, statistical analysis, and manuscript review. J.H. contributed to the concepts, design, literature search, clinical studies, statistical analysis, and manuscript review. L.G.M. contributed to the concepts, design, definition of intellectual content, literature search, clinical studies, statistical analysis, and manuscript review, and served as a guarantor.

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

Patient Consent

Individual patient consent was not required for this retrospective study, as approved by the Institutional Review Board (IRB) of the institution (IRB Min No. 14743/6.7.22).

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Conflict of Interest

None declared.

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Mutation Analysis of Advanced Non-Small Cell Lung Cancer: A Retrospective Observational Study from South India

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Abstract

Introduction Lung cancer is a leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) being the predominant type. Comprehensive genomic profiling plays a crucial role in identifying actionable mutations to guide personalized treatment strategies, especially in regions like India where late-stage diagnoses are common.

Objective This retrospective observational study aimed to determine the prevalence of actionable mutations in patients with advanced NSCLC from a tertiary cancer care center in South India.

Materials and Methods A retrospective observational study was conducted at the Department of Medical Oncology, Government Medical College, Kozhikode, South India. The study included 50 histologically confirmed patients with advanced NSCLC who underwent molecular testing between November 2022 and July 2024.

Results Among the 50 patients, adenocarcinoma was the predominant histological subtype (70%), with *TP53* and *EGFR* mutations found in 42 and 34%, respectively. Co-mutations, including *TP53* + *EGFR* exon 19 deletions, were observed in 4% of cases. Low PD-L1 expression (<1%) was identified in 78% of patients, suggesting limited eligibility for single agent immunotherapy.

Conclusion Comprehensive genomic profiling is largely inaccessible to most patients in India due to high costs, but targeted next-generation sequencing (NGS) panels offer a cost-effective way to optimize treatment. This study highlights the heterogeneity of mutations in NSCLC in South Indian patients and showcases the importance of targeted NGS panels in optimizing therapeutic strategies.

Keywords
- non-small cell lung cancer
- mutations
- personalized treatment
- immunotherapy
- EGFR mutations

Introduction

According to GLOBOCAN 2022, lung cancer was the most frequently diagnosed cancer in 2022, responsible for almost 2.5 million new cases, with an estimated 1.8 million deaths.[1] Non-small cell lung cancer (NSCLC) is the most frequently diagnosed type of lung cancer, with adenocarcinoma identified as the predominant histological subtype.[2] In India, an alarming 75% of patients with lung cancer are diagnosed at advanced stages. Around 66% patients are diagnosed at stage

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IV.[3,4] Approximately only 3.5 to 7.2% patients are diagnosed at an early stage, which considerably limits treatment options and survival chances. In India, patients with lung cancer have a median survival period of 8.8 months, improving to 12.6 months with specific treatments. The 5-year survival rates range from 92% at stage I to as low as 3 to 8% at stage IV.[3,5]

International guidelines published by National Comprehensive Cancer Network and European Society for Medical Oncology recommend molecular testing for clinically relevant biomarkers in advanced NSCLC.[6,7] International Consensus Guidelines also emphasize the critical role of molecular testing in the management of NSCLC, particularly in identifying actionable genetic alterations such as EGFR (epidermal growth factor receptor) mutations, ALK and ROS-1 rearrangements, and PD-L1 overexpression.[8] EGFR mutations are typically analyzed using real-time polymerase chain reaction (qPCR) or Sanger sequencing, focusing on hotspot regions such as exons 19 and 21. For ALK rearrangements, immunohistochemistry (IHC) serves as a rapid and cost-effective primary diagnostic tool, while fluorescence in situ hybridization (FISH) remains the gold standard due to its high specificity. Similarly, ROS1 rearrangements are detected via FISH, ensuring precise identification of chromosomal alterations. The implementation of these methodologies enables the identification of targetable mutations and optimizes therapeutic strategies for patients with NSCLC.[9]

In India, considerable efforts have been made to characterize the clinical and molecular profile of patients with NSCLC to enhance treatment outcomes. A study conducted at All India Institute of Medical Sciences, New Delhi analyzed 1,862 lung cancer cases (2008–2018), identifying adenocarcinoma (34%) as the most common subtype, followed by squamous cell carcinoma (28.6%).[3] Over the 10-year period, there was a marked increase in the incidence of adenocarcinoma from 9.5% in 2008 to 35.9% in 2018, indicating a shift in the histopathological landscape of lung cancer in India. The systematic review and meta-analysis by Raman et al found that approximately 40% of patients with adenocarcinoma and 30% of patients with NSCLC in India harbor actionable mutations in EGFR or ALK. The pooled prevalence rates for EGFR mutations were 28.7% in adenocarcinoma and 24.2% in NSCLC, while ALK rearrangements were found in 8.3% of adenocarcinoma cases.[10] The prevalence of EGFR mutations in India mirrors global trends, showing higher rates in Asian populations (30–40%) compared with Caucasians (15–20%).[11]

Recent advancements in immunotherapy and precision medicine, including development of first- and second-line targeted therapies for metastatic NSCLC, have significantly enhanced patient survival outcomes compared with traditional chemotherapy. This highlights the critical need for timely identification of actionable mutations to facilitate biomarker-guided treatments and improve overall survival (OS) rates.[12,13]

In this study, we conducted a comprehensive analysis of the mutational landscape in advanced NSCLC patients, focusing on identifying key actionable genetic alterations. With these findings, we aimed to provide insights into the molecular mechanisms of NSCLC and emphasize the importance of integrating molecular testing into routine clinical practice for improved patient outcomes.

Materials and Methods

Study Design and Setting

In this retrospective observational study, conducted at a single tertiary cancer care center in South India, the prevalence of actionable mutations in patients with advanced NSCLC was analyzed. The study was performed in the Department of Medical Oncology at the Government Medical College, Kozhikode, South India, between November 2022 and July 2024.

Inclusion and Exclusion Criteria

The study population included patients with histologically confirmed advanced NSCLC (stage III unresectable/IV) who had undergone molecular testing for driver mutations using next-generation sequencing (NGS). Patients who had not undergone molecular testing were excluded from the study. Within the study period, we had 50 patients satisfying the inclusion criteria and included them.

Variables

Relevant data were extracted from medical records, including demographic information (age, sex, and smoking history), clinical characteristics (stage, laterality, tumor location, lobe, metastatic sites, histopathology, and IHC), and results of mutation analysis. Mutations analyzed included *ALK, BRAF, EGFR, ERBB2, FGFR3, KEAP1, KRAS, MAP2K1, MET, NRAS, NTRK1, NTRK2, NTRK3, PIK3CA, TP53, RET, ROS1,* and *STK11* by NGS. NGS assay was done from two laboratories by custom hybrid capture technique to a minimum depth of 250X on Illumina NovaSeq X plus (Illumina Inc., San Diego, California, United States) and AmpliSeq technology using the Ion S5 system (Thermo Fisher Scientific Inc. Waltham, Massachusetts, United States) to a minimum depth of 500X. Sequences are processed using a customized and validated analysis pipeline designed to accurately detect all classes of genomic alterations (single nucleotide variants, insertions, deletions, copy number variations, and fusions). IHC for PD-L1 was done using VENTANA SP263 Assay (Roche Holdings AG, Basel, Switzerland).

Outcomes

Primary outcome is to find out the prevalence of actionable genomic alteration and PD-L1 status in advanced NSCLC in our population. Secondary outcome of the study is to find out the prevalence of actionable genomic alteration in different NSCLC histologic types.

Statistical Analysis

All statistical analyses were performed using the SPSS version 20 (IBM, Armonk, New York, United States). Descriptive statistics were used to summarize demographic and clinical data. Frequency analysis was conducted to determine the distribution of driver mutations.

Ethical Approval

The study protocol was approved by the Institutional Ethics Committee of the Government Medical College, Kozhikode (Ref. No. GMCKKD/RP 2024/IEC/307). All procedures performed in the study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Results

Out of 72 advanced biopsy-proven NSCLC cases registered during the study period, testing was recommended for all patients. A total of 50 patients, who had available test results, were included in the study. Six patients could not be tested due to insufficient tumor content in the biopsy block and 16 due to various other reasons like patient death, financial constraints, lost to follow-up, etc. Majority of the patients were in the age group of 61 to 70 years with a male predominance of 66%. Most patients were nonsmokers, and the majority of the cohort presented with stage IV disease. Adenocarcinoma was the most common histological subtype. Further details on baseline demographic and clinical characteristics are summarized in ►**Table 1**.

Table 1 Baseline demographic details

Variables	Frequency, *N* (%)
Age	
<40	3 (6%)
41–50	5 (10%)
51–60	11 (22%)
61–70	23 (46%)
71–80	7 (14%)
>80	1 (2%)
Gender	
Male	33 (66%)
Female	17 (34%)
Smoking	
Yes	22 (44%)
No	28 (56%)
Stage	
III	9 (18%)
IV	41 (82%)
Laterality	
Right lung	32 (64%)
Left lung	15 (30%)
Unknown	3 (6%)
Lobe	
Upper	21 (42%)
Middle	1 (2%)
Lower	24 (48%)
Unknown	4 (8%)
Location	
Peripheral	24 (48%)
Central	16 (32%)
Not specified	10 (20%)
Histopathology (HPR)	
Adenocarcinoma	35 (70%)
Squamous cell carcinoma	12 (24%)
NSCLC-NOS	2 (4%)
Poorly differentiated	1 (2%)
IHC	
TTF1 or napsin A positive	25 (50%)
P40 or P63 positive	5 (10%)

Abbreviations: IHC, immunohistochemistry; NSCLC-NOS, non-small cell lung cancer-not otherwise specified; TTF1, thyroid transcription factor 1.

Mutational Analysis

Among the mutations identified in patients with advanced NSCLC, the most frequently altered genes comprised TP53 (42%), EGFR (34%), ALK (10%), PIK3CA (10%), MET (4%), KRAS (4%), RET (4%), ROS1 (2%), and BRAF (2%) (►**Fig. 1**).

Mutation Distribution by Histological Subtype

Mutation Analysis in Adenocarcinoma

In the study of 35 patients with adenocarcinoma, genomic alterations were observed as both single-gene mutations and combinations of mutations. The most common alterations included EGFR exon 19 deletions and TP53 mutations, in 16 and 8% of patients, respectively. Additionally, complex mutational profiles were observed, with combinations such as TP53 + EGFR exon 19 del + PIK3CA and TP53 + ERBB2 exon 20, each present in 4% of cases (►**Table 2**).

Mutation Analysis in Squamous Cell Carcinoma

In squamous cell carcinoma patients, TP53 mutations were the most frequently observed, occurring in 12% of cases. A small number of patients exhibited mutations in PIK3CA, ERBB2, and KRAS, each identified in 2% of cases. Additionally, a single patient (2%) presented with a combination of TP53 + EGFR (L858R) mutations (►**Table 3**).

Mutation in Other Subtypes

In the study, one patient (2%) with poorly differentiated carcinoma had an EGFR exon 19 deletion. Two patients (4%) were diagnosed with Not Otherwise Specified subtype of NSCLC, and no mutation was detected in their samples.

PD-L1 Expression Profile

PD-L1 expression testing was conducted in all 50 patients and 39 patients (78%) had low PD-L1 expression (<1%; ►**Fig. 2**).

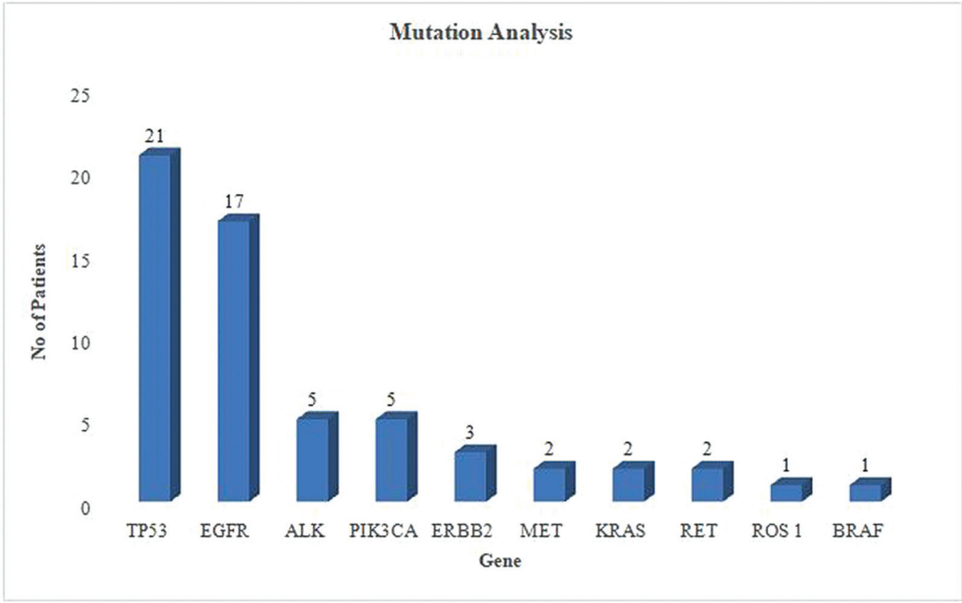

Fig. 1 Distribution of gene mutations.

Table 2 Mutations in adenocarcinoma patients ($N = 35$)

Genomic alteration	Frequency, *n* (%)
No mutation	4 (8%)
EGFR exon 19 del	8 (16%)
TP53	4 (8%)
ALK	3 (6%)
RET	2 (4%)
MET	1 (2%)
BRAF	1 (2%)
TP53 + EGFR exon 19 del + PIK3CA	2 (4%)
TP53 + EGFR L858R	1 (2%)
TP53 + ERBB2 exon 20	2 (4%)
TP53 + MET	1 (2%)
TP53 + ALK + PIK3CA	1 (2%)
ROS1 + PIK3CA	1 (2%)
TP53 + ALK	1 (2%)
KRAS + PIK3CA	1 (2%)
TP53 + EGFR exon 19 del	1 (2%)
TP53 + EGFR S768I, G719A	1 (2%)

Table 3 Mutation in squamous cell carcinoma patients ($N = 12$)

Genomic alteration	Frequency, *n* (%)
No mutation	2 (4%)
TP53	6 (12%)
PIK3CA	1 (2%)
ERBB2	1 (2%)
KRAS	1 (2%)
TP53 + EGFR L858R	1 (2%)

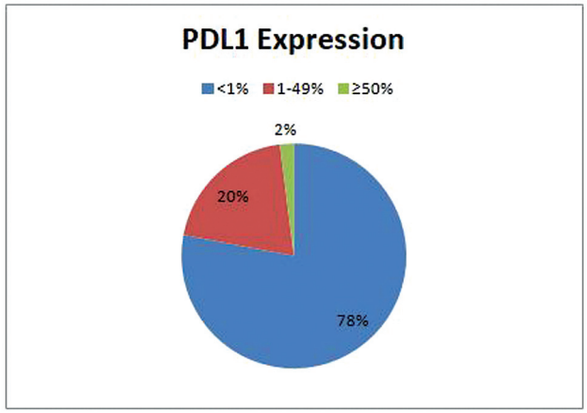

Fig. 2 PDL1 expression levels.

Discussion

Genomic profiling has emerged as a cornerstone in the management of NSCLC, enabling personalized treatment strategies tailored to specific molecular alterations. The average age of our patients was 60 years, which is similar to that reported in previous Indian studies.[14,15] Similarly, the male predominance in our study is similar to other Indian reports.[16,17] This study revealed significant heterogeneity in the mutational profiles of advanced NSCLC among South Indian patients, with TP53 and EGFR mutations emerging as the most common.

TP53 mutations are widely recognized as tumor suppressor gene alterations associated with poor prognosis in

various cancers, including NSCLC. These mutations are particularly prevalent in smokers, reflecting the mutagenic effects of tobacco carcinogens.[18] TP53 mutations are known to influence treatment response, with shorter progression-free survival (PFS) and OS, necessitating novel therapeutic strategies. EGFR mutations, on the other hand, are pivotal in the pathogenesis of adenocarcinoma, particularly in nonsmoking Asian populations. These mutations, including exon 19 deletions and L858R substitutions, drive tumorigenesis and serve as primary targets for tyrosine kinase inhibitors (TKIs).[19]

The predominance of TP53 (42%) and EGFR (34%) mutations observed in our study is consistent with findings from other studies. For instance, Lai et al reported a similar predominance of adenocarcinoma (91.8%) and high frequencies of EGFR (63%) and TP53 (50.7%) mutations in a Taiwanese cohort.[20] Similarly, Kaler et al detected EGFR mutations in 38.67% of patients with NSCLC, while Rajadurai et al identified EGFR mutations in 46.5% of cases, with adenocarcinoma comprising 84% of their cohort.[21,22] These studies emphasize the pivotal role of EGFR mutations in adenocarcinoma tumorigenesis, particularly in nonsmoking populations, suggesting a potential genetic predisposition unique to Asian populations.

In an Indian context, Sharma et al analyzed 53 patients with lung adenocarcinoma and identified TP53 and EGFR mutations as the most frequent, with prevalence rates of 52.8 and 50.9%, respectively.[23] Their findings also highlighted exon 19 deletions and L858R as the most common EGFR mutations, collectively accounting for 20.7% of cases. Demographic differences, such as a higher proportion of female patients (47.3%) compared with our cohort (34%), reflect potential regional and sample-specific variations. Roy et al further highlighted differences in molecular profiles between Indian and U.S.-based South Asian cohorts, with EGFR mutation prevalence closely aligning with our findings.[24]

The findings of this study highlight the critical role of comprehensive genomic profiling in NSCLC management. Identifying mutations in the EGFR gene allows for the selection of first-line TKIs, such as gefitinib, erlotinib, and osimertinib, which have demonstrated substantial improvement in PFS compared with chemotherapy.[25,26] Additionally, addressing resistance mechanisms, including EGFR amplification and MET activation, through tailored therapeutic strategies can further optimize outcomes. The high prevalence of TP53 mutations underscores the need for innovative treatment approaches. In patients with EGFR and TP53 co-mutations, combining EGFR-TKIs with chemotherapy has shown improved response rates and survival outcomes.[27,28] Furthermore, the low PD-L1 expression observed in most of our cohort limits eligibility for immune checkpoint inhibitors, emphasizing the importance of genomic-driven treatment selection.

This pioneering study from South India underscores the critical role of genomic profiling in shaping personalized treatments for NSCLC, especially given the substantial prevalence of TP53 and EGFR mutations in the region.

While the findings offer valuable insights, the study's limitations, including small sample size (50 patients) and single-center design, may affect generalizability and the ability to assess prognostic outcomes. We could not do NGS for all patients due to financial constraints and low tumor content. Future multicenter studies with larger cohorts and extended follow-up are essential to validate these findings and further refine mutation-based therapeutic strategies.

Conclusion

In 2025, testing for EGFR, ALK, ROS, and PD-L1 is insufficient for making treatment decisions in metastatic NSCLC due to the presence of multiple genomic alterations. Ideally, all patients should undergo comprehensive genomic profiling; however, in India, financial constraints limit access for many patients. In this context, targeted gene panels using the latest NGS technology with hybrid capture and PD-L1 by IHC can help identify relevant genomic alterations at a reduced cost, enabling effective personalized treatment decisions.

Patients' Consent

Written informed consents of patients were not taken as it is a retrospective observational study, and data were collected from hospital records.

Funding

None.

Conflict of Interest

None declared.

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Fear of Cancer Recurrence and Coping among Breast Cancer Survivors among Indian Females: A Longitudinal Study

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Abstract

Introduction Breast cancer survivors (BCSs) may experience a cascade of negative reactions during the entire treatment process. Post-treatment, the most common challenge among all fears is cancer recurrence. The fear brings many forms of psychological morbidity during follow-up. However, coping throughout and beyond cancer helps survivors hold a strong pillar of support as part of backup.

Objective This study aims to assess the interplay of the fear of cancer recurrence (FCR) and how it impacts the distress and relation of coping with recurrence.

Materials and Methods A longitudinal study was conducted in a tertiary care center in North India: T1 (January 2021–April 2021) and T2 (May 2023–September 2023). A total of 700 BCSs were approached initially, and after 2 years, they were again screened using the purposive sampling technique. Statistical Package for Social Sciences (SPSS) 23 was used to analyze the data.

Results The patients' mean age (standard deviation [SD]) was 48.14 (8.53) years. The mean (SD) FCR score was 15.26 (4.45). There was an inverse association between the FCR score and the coping score. The higher the age, the lesser the FCR score and the better religious coping.

Conclusion Psychological FCR is a detachable part of patients' worry once a person is diagnosed with any threatening illness. The coping mechanism should be strengthened by providing psychosocial interventions, family therapy, and other individual-centered therapies.

Keywords
- breast cancer survivor
- fear of cancer recurrence
- coping
- psychological distress
- depression
- women
- India

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Introduction

Fear of cancer recurrence (FCR) among breast cancer survivors (BCSs) is a significant and often enduring psychological challenge that profoundly impacts their quality of life. This phenomenon encompasses a broad spectrum of emotional responses, ranging from mild anxiety to debilitating distress. Understanding the predictors, magnitude, and coping strategies associated with fear of recurrence is vital for enhancing this unique population's well-being and long-term survivorship. This study embarks on an exploration of these facets to shed light on the complex landscape of FCR among BCSs.

Fear of recurrence has garnered increasing attention within the oncology community due to its pervasive influence on survivors' psychological and emotional well-being. As breast cancer (BC) is one of the most common malignancies affecting women, its survivors represent a substantial proportion of the cancer survivor population. For these individuals, the specter of cancer recurrence looms as an ever-present concern, casting a shadow over their lives long after treatment has ended.[1]

This study's primary objective is to find the predictors that contribute to the magnitude of fear of recurrence. Various factors, such as demographic characteristics, disease-related variables, and psychosocial factors, have been identified as potential contributors. For instance, age, time since diagnosis, and treatment type are among the predictors that may influence the intensity of fear of recurrence.[1]

Furthermore, BCSs' coping strategies to manage their fear of recurrence will be scrutinized in this study. These strategies can profoundly impact survivors' overall well-being and quality of life. Gaining insight into these strategies, which may encompass emotional regulation, information-seeking, and social support utilization, will provide a more comprehensive understanding of how individuals navigate the ongoing psychological challenges associated with BC survivorship.

This research endeavors to contribute to a deeper understanding of FCR among BCSs by examining its magnitude, coping strategies, and intricate relations with different demographic and clinical variables. By doing so, we hope to provide first-of-its-kind findings per our literature search till September 2023; no Indian data are available in this area. This can inform the development of tailored support and interventions for this resilient and unique population, ultimately enhancing their overall well-being and survivorship.

Materials and Methods

We conducted a longitudinal study: T1 (January 2021–April 2021) and T2 (May 2023–September 2023). We have approached (matching the study population) 700 BCSs for the study who have taken treatment from our institution between January 2016 and June 2020. We conducted detailed review of the patients' medical records to verify their BC diagnosis, the timing of diagnosis, the treatment methods utilized, and the cancer stage. The study sample in T1 was 165 and in the follow-up study (T2) the study sample was 142, which were meticulously selected following specific criteria for inclusion and exclusion. Patients were actively recruited using a purposive sampling technique during their follow-up appointments, while some individuals were contacted via telephone for participation. We clearly explained the study's objectives in the participants' native language.

In phase 1, we have included women aged between 18 and 60 years who had been diagnosed with and treated for BC and had completed their active treatment regimen, encompassing surgery, chemotherapy, and radiotherapy, for a minimum of 3 months, and patients with metastatic cancer, cancer recurrence, or those currently undergoing treatment for any psychiatric comorbidities were excluded from the study. During follow-up, the inclusion criteria were those who participated in previous study and willing to participate while patients having any severe ailment, and metastasis and recurrent cancer were excluded. To safeguard the anonymity of participants, no names or other personally identifying information were included in the questionnaires or the database.

Measure

A semi-structured proforma was used to examine patients' socio-demographic and clinical characteristics. Psychological distress was assessed using the Depression, Anxiety, and Stress Scales-21 (DASS-21), the FCR Scale, and Brief COPE. All of the tools were administered by the interviewer (mental health professional). Patients confirmed positive for any psychiatric illness were sent to the Department of Psychiatry for further evaluation.

Fear of Cancer Recurrence

The fear of cancer recurrence inventory (FCRC) SF was developed for assessing fear of recuurence among cancer patients.[1] The FCRI-SF measures FCR through a 9-item Likert scale, where each item assesses a distinct aspect of FCR. The response options range from 0 (not at all or never) to 4 (a great deal or all the time), capturing the intensity and frequency of fears related to cancer recurrence. Summing the items yields a total score for each subscale as well as the total scale. A higher score reflects higher levels of FCR.

Depression, Anxiety, and Stress Scale

The DASS-21 is a self-report questionnaire that assesses three dimensions of negative emotional states: depression, anxiety, and stress.[2] Each dimension is measured by seven items, each of which is computed on a 4-point Likert scale ranging from 0 ("did not apply to me at all") to 3 ("applied to me very much, or most of the time"). Scores of at least 10 for depression (DASS-D), 8 for anxiety (DASS-A), and 15 for stress (DASS-S) indicate clinically significant distress for each subscale. We utilized the Hindi version of the tool.[3]

Brief COPE

The "Brief COPE" is a widely used and well-established psychological assessment tool designed to measure how individuals cope with stress and challenging life events. It was developed by *Carver, Scheier, and Weintraub in 1989* and has since become a

valuable instrument in psychology and health research. The Brief COPE assesses people's coping strategies when faced with stressors, providing insights into their adaptive or maladaptive responses.[4] The Brief COPE comprises 28 items divided into 14 subscales, each reflecting different coping strategies. These subscales cover a mix of adaptive and maladaptive coping methods, ensuring a comprehensive assessment of how individuals handle stress. Examples of these subscales include:

- Adaptive strategies:
  - Active coping.
  - Planning.
  - Positive reframing.
  - Acceptance.
  - Humor.
  - Use of emotional support.
  - Use of instrumental support.
- Maladaptive strategies:
  - Denial.
  - Substance use.
  - Behavioral disengagement.
  - Self-blame.

Statistical Analysis

Statistical analysis was performed using SPSS-23 software. The statistical tests were used after checking the data for normal distribution.

Ethical Approval

The procedures followed were according to the ethical norms of the responsible "*Committee on human experimentation and with the Helsinki Declaration of 1964, as revised in 2013*." The Institutional Ethics Committee "King George's Medical University Lucknow, Uttar Pradesh, India" approved the study (*Ref code: 104th ECM IIA/P14*). Data were collected once. Patients provided informed consent.

Results

Socio-demographic and Clinical Details of the Study Sample

During phase 1 (T1), the mean age of patients was 48.14 (8.53) years. Homemakers comprised 158 (95.8%) of the study's patients, and 56 were illiterate (30.8%). Among the BCSs, 163 (98.8%) were married, 142 (78.9%) were of the Hindu religion, 129 (78.2%) were living in joint families, 115 (69.7%) were from urban or semi-urban backgrounds, and their monthly family income ranged from 5,000 to 10,000 INR 119 (72.1%).

Treatment was completed in a mean (standard deviation [SD]) of 13.05 (10.54) months, with a median of 12 months from 4 to 64 months. In the subjects, family history was negative for cancer—159 (96.4%). Most patients received a diagnosis of cancer at stage three—89 (53.94%). More than half of the women, 72 (43.6%), were in premenopausal status. The majority, 152 (92.10), of the patients expressed "fear of recurrence of BC." There was little variation in phase two (T2); an almost similar trend was observed (►**Table 1**).

Prevalence of Distress

The prevalence of DAS (*depression, anxiety, and stress*) in our study was 29.11, 33.5, and 25.25%, respectively. The majority of the patients were mildly ill (►**Table 1**). In phase 2, patients still had depression but comparatively at less frequency; the majority had mild scores (24.20, 23.5, and 26.25%).

Coping

The most commonly used coping strategy was acceptance, followed by religion and positive reframing, and the least used was humor (►**Fig. 1**). One of most used maladaptive strategies was self-blame, and the least used was substance use (►**Fig. 2**). The mean (SD) coping score was 13 (8.2). The mean subdomain score of various coping strategies is given in ►**Fig. 2**. Coping was inversely associated with FCR and it is found to be inversely correlated with anxiety, depression, and stress.

Discussion

Demographic and Clinical Variable

The mean age of the participants was 43.25 (8.53) years. The mean (SD) of "*duration of the completion of treatment*" in this study group was T1: 13.64 (10.45) and T2: 28 (10.46) months. The mean age of the participants was increased as we conducted this study after 2 years. Another factor is the higher incidence of BC in this age group.[1] The demographics of our study are similar to other studies conducted in this area. This tertiary care center provides free-of-cost care and serves people from low socio-economic backgrounds and rural backgrounds, making the population sample not only limited to one area but an entire region, making the findings' implications more generalizable. Most patients were treated for third stage of malignancy—(54.04%) (T1) while 85 (59.85%) in T2. Lack of awareness, poor financial status, and lack of resources in nearby treatment facilities can be the plausible reasons for the majority of the patients in third stage of malignancy.[5,6]

FCR, Coping, and Distress

Most patients expressed a "fear of recurrence" in both time T1 and T2. The finding itself is significant with respect to percentage of patients having FCR, which shows that survivors have many underlying concerns, resulting in psychological disturbances and decreased quality of life. Evidence suggests FCR as one of the most frequent unmet supportive care needs among BC patients. The study suggested that FCR will be present among all patients irrespective of its high and low percentage, which can go as high as 40 to 60%.[7–9]

Since the initial assessment, there have been no significant changes in the FCR level, as indicated by a meta-analysis conducted by Luigjes-Huizer et al in 2022. The findings suggest a consistent score over time, with no discernible variations or significant differences observed since the completion of cancer treatment. This lack of change could be attributed to repetitive testing and inadequate coping mechanisms, resulting in heightened fear. Furthermore, patients may experience psychological distress when anticipating

Table 1 Socio-demographic and clinical details of the study sample of Time 1 and Time 2 (follow-up)

Variable	Categories	T1 frequency and percentage ($n = 165$)	T2 frequency and percentage ($n = 142$)
Age (in years)	Mean	42	48.14 (8.53)
Occupation	Nonworking (homemaker)	158 (95.8%)	135 (95.07%)
	Working	7 (4.2%)	7 (4.9%)
Education	Not literate	53 (32.1)	50 (35.21)
	Primary	15 (9.1)	14 (9.8)
	High school	40 (24.2%)	34 (23.94%)
	Intermediate	15 (9.1)	16 (11.26)
	Graduation and above	42 (25.5)	30 (21.12)
Marital status	Married	163 (98.8%)	139 (98.8%)
	Single/widowed/divorced/separated	2 (1.2%)	2 (1.2%)
Religion	Hindu	129 (78.2%)	121 (85.21%)
	Muslim	36 (21.8%)	21 (14.78%)
Family monthly income (Rs)	Up to 2,500	13 (7.9%)	12 (8.4%)
	2,501–5,000	9 (5.5)	8 (5.63)
	5,001–10,000	119 (72.1%)	105 (73.94%)
	Above 10,000	24 (14.5)	17 (11.97%)
Type of family	Nuclear	50 (30.3%)	41 (28.87%)
	Joint	115 (69.7%)	101 (71.12%)
Domicile	Rural	57 (34.4%)	44 (30.98%)
	Urban	108 (65.5%)	98 (69.01%)
Family history of BC	Yes	6 (3.6%)	5 (3.52)
	No	159 (96.4%)	137 (96.47%)
Stage of cancer	Stage 1	5 (3.03%)	5 (3.52%)
	Stage 2	71 (43.03%)	62 (43.66%)
	Stage 3	89 (53.94%)	85 (59.85%)
Time of completion of treatment (months)	Mean (SD); **T1**: 13.64 (10.45); **T2**: 28 (10.46)		
Menopausal status	Premenopausal	93 (56.4%)	62 (43.66%)
	Postmenopausal	72 (43.6%)	80 (56.33%)
Fear of recurrence	Yes	152 (92.10%)	132 (92.95%)
	No	13 (7.9)	10 (7.04)

Abbreviations: BC, breast cancer. SD, standard deviation.

test results, contributing to a sustained level of fear that may not diminish, especially when health care professionals also cannot provide unrealistic assurances. Our study also suggested a correlation between distress scores and fear of recurrence, which might be due to a high fear of recurrence that leads to distress among patients; hence, it can be indicated that although distress decreases over a period of time, factors like FCR can be responsible for its maintenance. Age was associated negatively, which is similar to multiple kinds of literature as young people have more depression; hence, they tend to worry more about the future, directly or indirectly contributing to the FCR.[7,9–13]

Our findings suggest that more or almost equal growth in the adaptive coping subdomain occurs among survivors. The ability to cope with stressful situations tends to develop confidence in oneself.[14] A possible explanation is the high score in the domain of *acceptance.* The reason for a higher mean score in this domain can be associated with acceptance of illness as it starts or comes in many ways and warning to patients. First, the general symptoms bring the patient to the doctor. Second, all the assessment and diagnostic tests performed by the hospital make patients aware that something is wrong with them. Third, delivery of the diagnosis/breaking bad news and planning of the treatment. Although

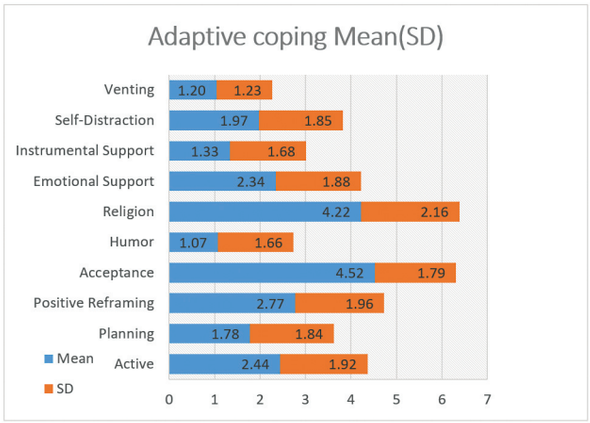

Fig. 1 Depicting mean (SD) of adaptive coping strategies. SD, standard deviation.

treatment comes in the form of shocks, the process, if seen backward, has an entire process of acceptance, making patients cope with the illness easily, at least by accepting and doing whatever possible to reduce its further growth and cure.[15,16]

When individuals fall ill, they often turn to prayer or embrace a path of spirituality. In many cases, people find solace in religious beliefs, especially among cancer patients, where such beliefs contribute positively to coping with adversity. Previous literature has indicated that individuals may perceive illness as a divine test or a consequence of their past life's karma, leading them to turn to belief in a higher power as a final solution to their problems.[13,15–18]

Contrary to this, some individuals may experience a loss of trust in the face of illness.[19,20] The limited growth reported in these areas in past research studies may be attributed to demographic variations and cultural values, especially within the Indian subcontinent. In this region, spiritual beliefs and family support form integral parts of the value system, fostering a more positive trajectory in dealing with challenges.[16]

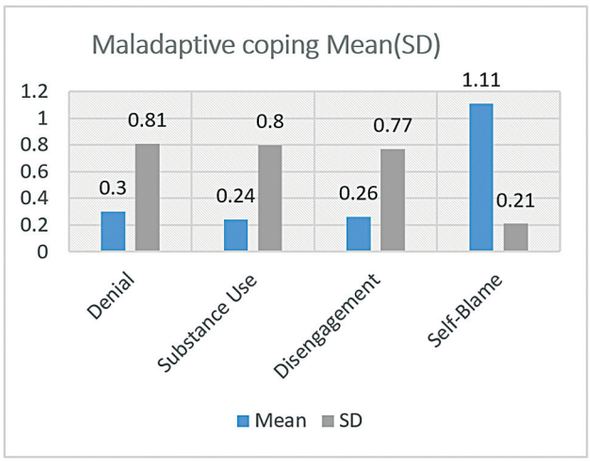

Fig. 2 Depicting mean (SD) of maladaptive coping strategies. SD, standard deviation.

Coping was not correlated with age, suggesting a lack of relationship between age and coping, which is similar to other data. However, some findings contradict our current findings, where results differ in the relationship between age and coping, revealing that the older generation has better and more mature coping mechanisms.[16] Since most of the women in our study belong to rural demography, most have no employment, and coping might differ completely among urban women with active jobs and challenges to meeting financial expenditures and loss of job or work adjustment.

Clinical Implications

The clinical implications of this study can guide clinicians in tailoring psychosocial support for patients by identifying specific coping strategies that survivors employ, allowing for personalized interventions that address individual needs. Assessing the impact of fear of recurrence on functional impairment informs intervention planning, allowing for collaborative efforts to address specific areas of life affected by fear, such as family, work, and relationships. Moreover, recognizing time since diagnosis as a variable of fear informs long-term survivorship planning, emphasizing the need for ongoing support beyond the active treatment phase. Additionally, understanding the correlation between the severity of fear and psychological distress enables clinicians to stratify patients based on their risk for emotional challenges, leading to more targeted monitoring and mental health support for those at higher risk. The study underscores the need for proactive mental health screening, early identification of high-risk individuals, and support. Furthermore, the study emphasizes on explaining the research purpose. The use of tools in the local language underscores the significance of cultural sensitivity in patient communication, ensuring effective understanding and engagement in psychosocial care. Overall, the clinical implications derived from such research contribute to enhanced, patient-centered care, promoting improved quality of life and well-being for BCS.

Limitations

Since the study was conducted in a single facility with outpatients, using a small sample was one of the limiting factors for generalizing the results. Also, some treatment variables like chemotherapy, hormonal therapy, radiation therapy, may impact treatment, may impact the outcome of distress, impact the FCR, and distress might be the result of poor coping. Further longitudinal research should be focused on results predicting the protective factor for FCR and strengthening coping mechanisms. Future studies should also consider including women from urban backgrounds and employed women, which might provide a different understanding of this issue. To our knowledge, no data on

this topic are available among Indian patients, which makes these results very relevant for future studies.

Conclusion

In conclusion, recognizing the intricate interplay among FCR, coping strategies, distress levels, and clinical variables is imperative for a comprehensive understanding of the patient experience. The dynamic relationships between these factors may act as a complex web, each influencing and potentially serving as a protective mechanism for the others. A nuanced understanding of this nexus is crucial for health care professionals as it allows for a holistic approach to patient care. This knowledge fosters a more empathetic and patient-centered care model and underscores the importance of a multidisciplinary approach in addressing the diverse dimensions of the cancer survivor experience.

Data Availability Statement

The Authors elect to not share data.

Patient's Consent

Informed consent was obtained from the patient before participating in the study.

Funding

None.

Conflict of Interest

None declared.

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Gilteritinib

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Abstract

Introduction Acute myeloid leukemia (AML) is a heterogeneous and aggressive form of blood cancer that affects the myeloid lineage of the cells. Among various genetic mutations associated with AML, the *FLT-3* mutation is one of the most common and associated with poor prognosis. Gilteritinib (previously known as ASP2215) is the first tyrosine kinase inhibitor approved as monotherapy for treatment of relapsed/refractory AML.

Areas Covered We review gilteritinib in detail, including its mechanism of action, pharmacology, efficacy, toxicity profile, and key clinical trials.

Keywords
- FLT3 mutation
- relapsed/refractory AML
- TKI
- gilteritinib

Introduction

Acute myeloid leukemia (AML) has an annual incidence of approximately 2 to 3 per 100,000 in adults in India. Fms-related tyrosine kinase 3 (*FLT3*) gene, which encodes type III receptor tyrosine kinase protein, is the most common gene that is mutated in AML.

FLT3-mutated AML is characterized by a younger age of onset, high white blood cell count, and high blast percentage at presentation, along with an elevated lactate dehydrogenase. It does achieve remission with conventional therapy, but has a pronounced tendency to relapse, relapse quickly, and die sooner.

Based on the interim results of the ADMIRAL study, gilteritinib was approved by the U.S. Food and Drug Administration (FDA) in November 2018 for the treatment of adults with relapsed and/or refractory (R/R) AML with a *FLT3* mutation.[1] It has been available in India since April 2024. ►**Table 1** summarizes the drugs available with *FLT3* activity.

Gilteritinib

Mechanism of Action

Gilteritinib is a highly selective FLT3 inhibitor, which has activity against both *FLT3-ITD* and *FLT-TKD* mutations. In comparison, midostaurin has slightly lower efficacy against *FLT3-TKD*. Gilteritinib also inhibits *ALK* and *AXL*, which is overexpressed in AML and has shown potential role in chemoresistance.[2]

Pharmacokinetics

Maximum plasma concentration achieved: 4 to 6 hours. High-fat meal delays it by 2 hours.

It is metabolized via CYP3A4, and it is excreted mainly via feces. It has a long elimination half-life of 113 hours, permitting once daily dosing.[2]

Drug Interactions

Strong CYP3A inhibitors (voriconazole, posaconazole) increase gilteritinib levels by 34.24%. Hence, dose reduction to 80 mg is advised. However, effect on efficacy is not known. It is pertinent to note that the ADMIRAL trial did not utilize these azoles for prophylaxis.[3]

Safety[1,4]

Gilteritinib is generally well-tolerated compared with traditional chemotherapy. Its nonhematological toxicity includes:

1. Aspartate and alanine aminotransferase elevation was the most commonly reported adverse effect (AE).

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Table 1 FDA-approved drugs for *FLT3*-mutated AML

Drug	Type	*FLT3* activity	Generation	Selectivity	Upfront therapy	Relapsed therapy
Sorafenib	2	Only ITD	1	Multikinase inhibitor (*FLT3*, *VEGFR*, *PDGFR*)		
Midostaurin	1	ITD and TKD	1	Multikinase inhibitor	As combination	
Quizartinib	2	Only ITD	2	*FLT3*	As combination	
Gilteritinib	1	ITD and TKD	2	*FLT3*, *ALK*, *AXL*		Monotherapy

Abbreviations: AML, acute myeloid leukemia; FDA, Food and Drug Administration; ITD, internal tandem duplication; TKD, tyrosine kinase domain.

2. QT segment prolongation
   Recommendation is for electrocardiogram monitoring on days 1, 8, and 15 of cycle 1 and day 1 of subsequent cycles. It is advised to withhold the drug if QTc > 500 ms, and restart at a lower dose once the QTc < 480 ms. Periodic potassium and magnesium monitoring is mandated.
3. Differentiation syndrome (DS)[5]
   DS is a unique side effect initially reported with the use of all-trans retinoic acid in acute promyelocytic leukemia (APML). In the therapy of AML, gilteritinib shares this unique AE with the isocitrate dehydrogenase (IDH) inhibitors. Unlike DS seen in APML, DS occurs less frequently, has a later onset, and is associated with prominent skin involvement, in the form of Sweet's syndrome.
4. Gastrointestinal (GI) disturbances, muscle pain, fatigue, dizziness, and peripheral edema have also been reported.
5. Rare but serious toxicities include posterior reversible encephalopathy syndrome and Pancreatitis.

Clinical Efficacy

Approval for gilteritinib was obtained based on the results of CHRYSALIS and ADMIRAL trials.

CHRYSALIS Trial[5]

This is the first-in-human, open-label, phase I/II dose escalation, dose expansion trial in R/R AML.

Dose ranged from 20 to 450 mg, with maximum tolerated dose identified as 300 mg.

ADMIRAL Trial[1]

This global, open-label, phase III trial enrolled 371 adult patients with R/R *FLT3*-mutated AML. Assigned 2:1 to either gilteritinib or salvage chemotherapy (MEC/FLAG-Ida/azacitidine [Aza]/low dose cytarabine). The median overall survival was 9.3 months in the gilteritinib group compared with 5.6 months in the chemo group. The objective response rate was considerably higher in the gilteritinib group (67.6% vs. 25.8%). Similarly, gilteritinib demonstrated a longer duration of response (11 vs. 1.8 months).

MORPHO Trial[6]

This phase 3 trial by the BMT CTN group evaluated gilteritinib as maintenance therapy posttransplant. The strongest benefit was seen in patients who were MRD (measurable residual disease) positive in the peritransplant period, whereas no benefit was observed in patients who were MRD-negative.

The posttransplant relapse kinetics demonstrated that those who did not receive maintenance relapsed early (64.3% relapsed within 8 weeks and 93.9% by 16 weeks). Whereas clonal eradication due to graft versus leukemia occurred only by 3 to 6 months. Based on these kinetics in MRD-positive cases, it is advised that gilteritinib should be started soon after engraftment.

Current Uses

As Monotherapy

It is the only *FLT3* inhibitor approved as monotherapy by the FDA in the relapsed setting.

As Combination Therapy

Combination therapy is preferred in the front-line setting. The Lacewing study initially included a monotherapy arm upfront, however, it was dropped midway through the trial, due to changing treatment paradigm. ►**Table 2** summarizes the trials with gilteritinib combination therapy.

1. Gilteritinib with venetoclax[7]
   It is hypothesized that targeting both *FLT3* and *BCL-2* pathways may enhance cell death in *FLT3*-mutated AML cells. Pharmacological modeling also suggests higher synergism between gilteritinib and venetoclax in comparison to midostaurin.[8]
2. Gilteritinib with Aza[9]
   Aza is believed to enhance *FLT3* inhibition by reducing cellular proliferation. The combination has shown manageable toxicity in elderly AML. However, the Lacewing study failed to demonstrate survival benefit for the addition of gilteritinib to Aza.[9] GI toxicity, especially, GI hemorrhage, was higher with gilteritinib + Aza.
3. Triple therapy: Gilteritinib with Aza and venetoclax[10]
   It is the preferred combination and has proven to have the best efficacy. However, it is associated with higher toxicity with a 62% incidence of infection and 38% febrile neutropenia, More so with R/R AML.
4. Gilteritinib and chemotherapy[3,11]
   Adding gilteritinib to standard chemotherapeutic regimens can induce a deeper molecular response by targeting residual disease more effectively than chemotherapy. However, concern exists regarding the efficacy of gilteritinib in this setting, as there is rebound increase in wild-type *FLT3*, induced by the recovering marrow post-chemotherapy.

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Table 2 Trials of combination therapy with gilteritinib

Trial / Phase	*n*	Patient profile	ORR	CR	OS	PFS	Key message
Venetoclax plus gilteritinib[7] phase IB	61	Failed > 1 line of AML therapy	-	mCRc- 75%[a]	10 months	–	Ven + Gilt had high mCRc, regardless of prior FLT3 inhibitor exposure
Triple therapy with venetoclax, FLT3 inhibitor, and decitabine for FLT3-mutated AML[9]– Phase II trial	25	Newly diagnosed AML and R/R AML Excl. favorable cytogenetics and prior venetoclax exposure	Newly diagnosed 67–92% R/R AML 26–83%	Newly diagnosed CRc- 92% R/R AML CRc- 62%	Newly diagnosed OS not reached (2-year OS – 80%) R/R AML 6.8 months	Newly diagnosed 18-month PFS-59% R/R AML- 58%	Triplet therapy safe and effective
Lacewing trial[10] Phase III trial	123	Newly diagnosed AML patients ineligible for intensive chemotherapy		Gilt + AZA- CRc- 58% CR- 16.2% AZA CRc–26.5% CR-14.3%	Gilt + AZA -9.8 months AZA- 8.8 months	EFS Gilt + AZA- 4.5 months AZA-0.3 months	Negative study
Gilteritinib + 3 + 7 chemotherapy[3] phase I B trial	80	Newly diagnosed, fit for intensive chemotherapy Gilt in induction, consolidation, and maintenance	–	FLT-3 WT -CRc- 50% FLT3–mutated CRc-89%	mOS- 46.1 months	–	Gilteritinib can be safely combined with conventional 3 + 7 therapy. It induced prolonged myelosuppression. Gilteritinib maintenance well tolerated
Gilt plus CLIA chemotherapy (cladribine, idarubicin, cytarabine) ± venetoclax[11] Phase II trial	18	Newly diagnosed, fit for intensive chemotherapy	CLIA + gilteritinib 80% Vs. CLIA+ gilteritinib + venetoclax 88%		21. 9 months For entire cohort (not reached for CLIA + Gil arm and 22.4 months in CLIA + Gil + Ven arm		Addition of venetoclax produced similar results but prolonged the count recovery

Abbreviations: Aza, Azacitidine; AML, acute myeloid leukemia; CRh, complete remission; Cri, complete remission with incomplete hematological recovery; CRc, composite CR (CR + Cri + CRh); Gilt, Gilteritinib; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; R/R, relapsed and/or refractory; Ven, Venetoclax.

Use of Gilteritinib Post-Failure of Other FLT3i[12]

There is a concern that prior use of FLT3i can drive the expansion of clones with additional on-target mutations, which may confer resistance to gilteritinib. However, data from the Admiral trial and real-world data clearly demonstrated its efficacy in this population (composite complete remission [CR] rates of ~50%).

Maintenance Therapy for Nontransplant patients[13]

Gilteritinib maintenance is more effective in preventing early relapse at 0.5 to 1 year. However, its utility is limited if the MRD is negative.

Myeloid Sarcoma[14]

Shatilova et al have reported superior efficacy of gilteritinib over other *FLT3*i for the therapy of extramedullary disease.

Dosing[1,7,9–11]

The dose of gilteritinib is 120 mg for monotherapy (only FDA-approved dose).

Dose escalation up to 200 mg was permitted if the patient is not in CR at 28 days of treatment.

Gilteritinib is used at a dose of 120 mg for doublet combination therapy (either Aza or venetoclax) and 80 mg for triplet combination therapy (Aza + venetoclax + gilteritinib).

Cost: Monotherapy with 120 mg of gilteritinib for 1 month will cost INR 6.3 lakh.

Ongoing Phase 3 Trials

Gilteritinib versus midostaurin in combination with intensive chemotherapy for upfront AML and MDS (HOVON 156 AML trial).[15]

Important Points about Gilteritinib[15]

1. Inhibition of *FLT3* needs to be near complete and sustained for days, not hours. Gilteritinib has a long half-life—hence, once daily dosing. It is important to minimize drug interruptions.
2. Lower myelosuppression
   KIT and *FLT3* are structurally similar. Gilteritinib selectively inhibits mutated *FLT3*, it has fivefold less activity against wild-type *FLT3* and nil against KIT—hence, lesser myelosuppression compared with other FLT3i.
3. *FLT3-ITD* AML evolves from diagnosis to relapse
   Leukemic cells are dependent on *FLT3-ITD* signaling for survival. This constitutes a small proportion of the total leukemic cell population at the time of diagnosis. However, it is of a large proportion at the time of relapse. Hence, monotherapy works well at relapse, whereas a combination regimen with synergistic cytotoxicity is a better option for therapy upfront.
4. Gilteritinib acts by a combination of apoptosis and terminal myeloid differentiation
   Apoptosis of peripheral blood blasts results in rapid clearance. However, bone marrow (BM) blasts undergo differentiation. This tends to be a slower process. Hence, the attainment of CR is later than conventional chemotherapy and recovery of normal count is also delayed.
5. Clinical endpoint: BM aplasia is avoided with gilteritinib monotherapy; however, count recovery is delayed. Cri + CRh (CR with incomplete hematological recovery + CR with partial hematological recovery) were included as a valid endpoint in clinical trials. This trend is seen with monotherapy with other targeted therapies like the IDH1 and 2 inhibitors as well. CRh has been associated with transfusion independence and lower risk of infections. Thus, it is a clinically relevant endpoint.
6. MRD analysis: Polymerase chain reaction (PCR) is insufficient to analyze MRD in *FLT3*-mutated AML due to the template bias issue. Hence, a high sensitivity PCR-next-generation sequencing combination method is utilized to test MRD. This is currently not available in India.

Molecular Spectrum of Action[16]

Analysis of the molecular makeup of good responders to gilteritinib demonstrates that:

- Good response was seen with those with concomitant DNMT3A, IDH1/2, and WT-1 mutations.
- While the best response was seen with dual-mutated DNMT3A and NPM1.

The impact of FLT3 mutations on response was also assessed:

- Presence of multiple *FLT3-ITD* mutations had no impact on response.
- Longer *FLT3 ITD* length responded better than shorter ITD length.
- High allele ratio *FLT3* (↑allele burden) is known to have poorer prognosis—gilteritinib was superior to chemotherapy. However, it was not able to fully abrogate the poor risk.

Treatment resistance to gilteritinib:

- Failure of gilteritinib therapy can be caused by primary and secondary mechanisms.
- F691L gatekeeper mutations and *RAS/MAPK* pathway gene mutations constitute the most common resistance pathway. They both are mutually exclusive.
- On-target point mutations: *D835*, *F691* (gatekeeper mutation), *N676*, and *FLT-3* juxtamembrane (JMD) *E598D* mutations.
- Off-target mutations in *Ras/MAPK* pathway: *NRAS*, *PTPN11*, and *KRAS*.
- Nongenetic mechanisms like *FGF2*-activated *FGF1* receptor 1 can also contribute.

Conclusion

Gilteritinib is the first oral tyrosine kinase inhibitor approved for the management of AML in the R/R setting. Its tolerance and lack of marrow aplasia makes it an ideal agent for

managing AML patients in an outpatient setting. Ongoing clinical trials in combination therapy and in upfront setting will open broader avenues in the management of *FLT-3*-mutated AML.

Patient Consent
Patient consent is not required.

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Conflict of Interest
None declared.

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Broncho-alveolar Lavage Cytology Evidence of Pulmonary Metastasis by Neuroblastoma

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Introduction

Neuroblastoma (NB) is the most frequent pediatric extracranial solid tumor. It exhibits a high metastatic rate, with approximately 70% of patients presenting with metastasis at diagnosis. While bone and liver are the most common sites, lung involvement occurs in 1.3 to 6.6% of cases.[1,2] To our knowledge, this report describes the first instance of NB pulmonary metastasis diagnosed using broncho-alveolar lavage (BAL) samples.

Case History

A 1-year-old female pediatric patient previously diagnosed with abdominal *MYC-N*-amplified NB presented 8 months later with dyspnea and fever. Chest scan by computed tomography revealed multiple bilateral parenchymal thickenings, suggestive of infection. A BAL was performed. The recovered fluid was processed using the cell-block (CB) method, involving formalin fixation, centrifugation, agar pre-embedding, and paraffin embedding.[3] Three-micron sections were stained with hematoxylin and eosin.

Microscopic examination revealed a highly cellular specimen composed predominantly of macrophages. However, rare smaller cells with a high nuclear/cytoplasmic ratio, hyperchromatic nuclei, occasional prominent nucleoli, cell–cell adhesion, and instances of cell "cannibalism" were also observed. Immunocytochemistry revealed these cells to be CD56+, Tyrosine-hydroxylase+, and Synaptophysin+ (►**Figs. 1** and **2**). Based on these findings, a diagnosis of pulmonary metastasis from NB was made.

Discussion

The peculiarity of this case lies in diagnosing NB pulmonary metastasis via a minimally invasive BAL procedure. BAL, introduced in 1974, allows the collection of cells and secretions from the distal airways, providing valuable diagnostic information.[4]

The patient's prior NB diagnosis included *MYC-N* amplification, a marker of poor prognosis. Microscopically, *MYC-N*-amplified NBs often show enlarged and prominent nucleoli (►**Fig. 3**).[5] The BAL sample maintained these microscopic features.

We also noted cell "cannibalism," a survival mechanism activated under stress, where neoplastic cells engulf others.[6] While not specific to NB, its presence raised suspicion. Since only a few cells displayed these features amidst numerous macrophages, immunohistochemistry via the CB technique was essential for confirmation. This is particularly important in cases with limited cellularity, where traditional histologic

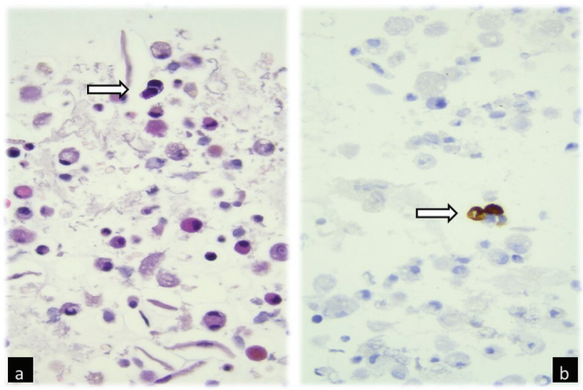

Fig. 1 Broncho-alveolar lavage cell-block photomicrograph showing (**a**) two neoplastic cells with altered nuclear–cytoplasmic ratio and nuclear hyperchromasia (arrow) among macrophages and exfoliation cells (H-E, 400×); (**b**) neoplastic cells (arrow) positive for tyrosine-hydroxylase immunohistochemistry, characteristic of neuroblastoma (400×).

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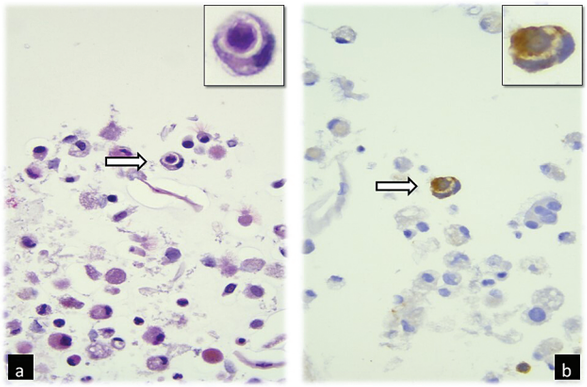

Fig. 2 Broncho-alveolar lavage cell-block photomicrograph showing (**a**) a cannibalistic neoplastic cell with semilunar nucleus and cytoplasm containing the engulfed cell (arrow and insert, H-E, 400×); (**b**) the correspondent positivity for Synaptophysin immunohistochemistry, characteristic of neuroblastoma (arrow and insert, 400×).

analysis might be challenging. The differential diagnosis of small blue round cell tumors in cytology can be broad, necessitating the use of immunohistochemistry to accurately classify the tumor.

The main strength of this report is its demonstration that BAL combined with immunocytochemistry can enable a rapid and minimally invasive diagnosis of NB pulmonary metastasis, even in samples with limited cellularity. However, because it is based on a single case, the report's findings are limited in scope and may not be generalizable to all patients or settings. Further studies are needed to assess the sensitivity and specificity of BAL for this purpose, as well as to define its role compared with other diagnostic methods. Our approach may be restricted to specialized centers with cytopathology expertise. There are still uncertainties regarding the optimal indications for BAL and its diagnostic reliability in cases with very few tumor cells. This highlights areas for future research.

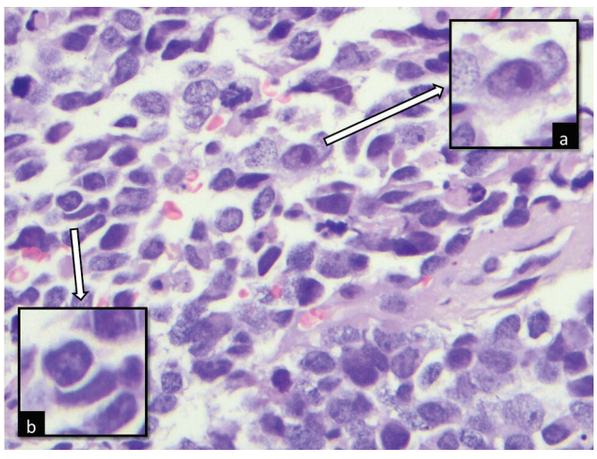

Fig. 3 Histologic photomicrograph showing a neoplastic cell with an enlarged and prominent nucleolus typical of *MYCN*-amplified neuroblastoma (insert a) and a "cannibalistic" neoplastic cell (insert b) (H-E, 400×).

Conclusion

In conclusion, we emphasize the importance of the BAL technique, microscopic characteristics, and immunohistochemical confirmation in diagnosing NB lung metastasis. This case represents the first reported instance of NB lung metastasis diagnosed using BAL, enabling a faster diagnosis and tailored therapy while avoiding more invasive procedures.

Authors' Contributions

S.B.: concept, data acquisition, manuscript preparation, manuscript editing, and manuscript review.
V.G.V.: design, definition of intellectual content, data analysis, statistical analysis, manuscript editing, and manuscript review.
B.C.: clinical studies, experimental studies, data acquisition, and manuscript review.
G.G.: literature search, definition of intellectual content, manuscript preparation, manuscript editing, and manuscript review.
All authors have read and approved the manuscript, meet the requirements for authorship, and believe the manuscript represents honest work.

Patient's Consent

Patient's consent is not required.

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Conflict of Interest

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Burnout among Oncologists in India: Challenges in Outpatient Department Practice and the Way Forward

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Running a cancer outpatient department (OPD) in India poses significant challenges for oncologists. The rising incidence of cancer, coupled with an inadequate oncologist-to-patient ratio and the concentration of oncology centers in urban areas, results in long working hours for oncologists. This situation is particularly prevalent in government or trust-run cancer hospitals, where a large proportion of cancer patients seek treatment due to financial constraints, since many Indians lack insurance coverage. It is not uncommon for a single oncologist to attend upwards of 50 patients in a single OPD session, extending working hours well beyond the standard 8-hour day, sometimes late into the evening. Considering these challenges, it is crucial to address the emotional toll and decision-making fatigue experienced by oncologists in India as they navigate the complexities of managing cancer patients.

Understanding Burnout in Oncology

Burnout is defined by the World Health Organization as a syndrome resulting from chronic workplace stress that has not been successfully managed. It is characterized by three dimensions: feelings of energy depletion or exhaustion, increased mental distance from one's job, and reduced professional efficacy. Oncologists in high-volume OPD settings, particularly in India, are especially vulnerable.[1,2] The demanding workload, emotional toll of cancer care, and systemic constraints often contribute to persistent stress, placing these professionals at significant risk. These issues, along with the way forward to address them, are discussed below.

The oncologist-to-patient ratio in India is 1:2,000. Each oncologist is consulted by 475 new patients per year.[3,4] This statistic shows the extent of scarcity of oncologists. Approximately 95% of referral cancer centers in India are located in urban areas, creating a significant geographic disparity in access to oncology services. This urban-centric distribution forces patients from rural and semi-urban regions, where nearly 70% of the population resides, to travel long distances for cancer diagnosis and treatment.[5] As a result, the OPD of specialized cancer centers in urban areas experience a disproportionately high caseload, often exceeding their operational capacity. This leads to overcrowding, extended waiting times, and increased pressure on oncologists, contributing to professional burnout and potentially compromising the quality of care delivered.

Tales That Take a Toll on Mental Health

As I type this article sitting inside my consultation room, I remember the story of a triple-negative breast cancer patient who developed a distant recurrence within 3 months post completion of adjuvant therapy. Her husband told me that their wedding happened just a year before she was diagnosed with cancer, as they were planning pregnancy and that they had to drop the plans because of the diagnosis. Coincidentally, his wedding happened in the same city and on the same day as mine, which I came to know from him through our conversation in one of the consultations. Neither of us were aware then that destiny would bring us together in my consultation room. His mother was taking treatment for advanced esophageal cancer at the same time. Neither his wife nor his mother had health insurance coverage. Listening to his story, my eyes turned teary, which I could somehow hold back. Another woman in mid-30s, who lost her husband to a road traffic accident just 1 year before, came to me with a

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diagnosis of advanced pancreatic cancer. She had already lost both her parents and has two school-going children. Her office colleagues who were helping with treatment expenditure accompanied her into my consultation room. They felt she is emotionally fragile and would give up if made aware of the adverse prognosis and signaled to me not to reveal the stage. Being kept in the dark regarding stage and prognosis, she innocently enquired about the diet she must take besides treatment to get cured of the disease. That moment I felt a lump in my throat. A series of such patients encountered in a day can take a toll on the mental health of an oncologist.

Managing Elderly Cancer Patients

Another challenge that many oncologists find is discussing treatment options with an elderly cancer patient. It becomes important to consider their physical fitness, comorbidities, logistics, and their goals of treatment. In India, most of the elderly cancer patients are dependent on their children for health care, which makes the job even more complicated because often the treatment goals of children do not match with those of the patient. Many elderly patients do not cope with their dependency on children for treatment, either financial or physical, fearing they might become an additional burden. Whereas, children feel it is their responsibility to take care of them no matter what. Such complex dynamics arise from the strong emotional attachments present in most Indian families. In some cases, their children are the sole bread earners of the family, who cannot accompany the patient leaving his/her job frequently. Time toxicity factor forces the physician to avoid weekly chemotherapy regimens or the regimens warranting admission for multiple days. Other factors like the patient's residence being situated at a far-off rural place without proper access to a hospital in case of need for supportive care or travel expenses also impact the type of treatment offered to the patient. Multiple such considerations in planning the treatment of every elderly cancer patient result in emotional drain. Comprehensive geriatric assessment (CGA), though recommended in various guidelines, is often challenging due to manpower and financial constraints in India. Additionally, delayed presentation to an oncologist warrants quick initiation of treatment precluding further assessment. This places the entire onus of evaluating an elderly cancer patient for treatment fitness on the treating consultant, contributing to burnout. It becomes all the more challenging when a majority of the patients encountered at OPD are of geriatric age group.

Pressure of Not Being Able to Offer the Standard of Care

As pointed out earlier in this discussion, the financial status of the patient plays a very key role in decision making in most of the patients in India. Drugs like immune checkpoint inhibitors, antibody drug conjugates, and some of the targeted oral drugs are not accessible to most of the cancer patients. This is true even if generic or biosimilar forms of such drugs are available. The best example is biosimilar of Trastuzumab emtansine, which continues to remain beyond the reach of many patients. No matter how practical an oncologist tries to be, at some stage, he/she would feel the pressure of not being able to offer the standard of care to most patients encountered in practice. The vast amount of detailed documentation and paperwork, given the expenditure and toxicities associated with anticancer drugs, adds to this burden. This eventually leads to burn out and mental health issues in oncologists, affecting their own personal and social life.[6,7]

How Can We Improve upon This?

Improving the workflow and well-being of individual oncologists is crucial for providing optimal care to cancer patients. Here are some practical solutions that can enhance the efficiency and mental health of oncologists:

- Utilizing trained physician assistants (PAs): implementing the concept of having trained PAs in every oncology OPD can significantly reduce the administrative burden on oncologists. While common in the Western world, this practice is under-utilized in India due to the lack of a structured training system for PAs. Developing a training program and providing adequate financial incentives can help bridge this gap effectively.
- Establishing a medical social worker (MSW) department: having a dedicated MSW department attached to each oncology OPD can assist in addressing the financial concerns of cancer patients. By collaborating with the oncologist to devise optimal financial solutions after treatment planning, the burden on the oncologist can be lessened, allowing them to focus primarily on medical and scientific aspects of patient care.
- Leveraging technology for medical record keeping: incorporating user-friendly applications and software specifically designed for oncology record documentation can streamline the documentation process for oncologists. It also helps in easy retrieval of data for clinical audits and research. By adopting technology that is easy to use and cost-effective, even government and trust-run hospitals can benefit from improved efficiency in record-keeping tasks.
- Short geriatric assessment in elderly: using online screening tools for frailty, prediction of toxicity, noncancer life expectancy, timed up and go test, etc., to guide treatment decisions in elderly cancer patients, if CGA is not feasible.
- Prioritizing self-care and mental health: recognizing the importance of self-care, oncologists should incorporate regular breaks into their schedules to prevent burnout. Taking a lunch break with colleagues, enjoying a brief coffee time, and listening to good music help recharging oneself to resume patient care and to maintain mental well-being.

To conclude, mental fatigue and burnout among oncologists in India are not just prevalent but deeply entrenched, particularly in OPD settings. Addressing this requires systemic reforms and personal self-care practices. Streamlining

OPD workflow, leveraging technology, involving trained personnel, and prioritizing oncologist mental health are essential steps toward sustainable oncology practice. Ultimately, caring for oneself is central to caring for the patient.

Ethical Approval

The manuscript has been written in accordance with the Declaration of Helsinki.

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Conflict of Interest

None declared.

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First Report of a Novel Unbalanced Complex Variant Translocation t(3;9;22) with Ph Duplication and Evolving Triple Trisomy (8,12,21) in Newly Diagnosed Chronic Myeloid Leukemia without Blast Crisis: Resistance to Frontline Therapy

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Abstract

In chronic myeloid leukemia (CML), tyrosine kinase inhibitors (TKIs) targeting the *BCR/ABL1* fusion gene have transformed CML into a manageable condition. While most cases involve the standard t(9;22) translocation, 5 to 10% exhibit variant translocations and/or additional chromosomal abnormalities (ACAs), often linked to disease progression. Hence, cytogenetic analysis is crucial for monitoring clonal evolution and guiding treatment.

This report presents a unique case of chronic-phase CML with a three-way unbalanced variant translocation involving chromosomes 3, 9, and 22, alongside an interstitial deletion of the reciprocal *ASS1/ABL1/BCR* region. ACAs with Ph duplication and trisomy of chromosomes 8, 12, and 21 in the present case complicate the genomic landscape, with specific breakpoint involvement and additional abnormalities indicating a poorer outcome, highlighting the need for further investigation and tailored treatment strategies.

Given the rarity and poor prognostic association of this finding, our study highlights the importance of identifying complex chromosomal variations to enable modified targeted therapy regimens as first-line treatment. This emphasizes the need for individualized prognostic assessment and continuous monitoring to optimize therapeutic outcomes.

Keywords

- chronic myeloid leukemia
- variant translocation
- karyotype
- fluorescence in situ hybridization
- imatinib
- ponatinib

Introduction

Chronic myeloid leukemia (CML) is a hematopoietic disorder characterized by a hallmark reciprocal translocation between chromosomes 9 and 22; t(9;22)(q34;q11.2), which forms the Philadelphia (Ph) chromosome.[1] In over 90% of CML cases, t(9;22) is the sole abnormality; however, 5 to 10% exhibit variant Ph translocations (vPh) involving three or more chromosomes in addition to 9 and 22. Although vPh are genetically diverse, available data suggest that patients with complex vPh do not have worse outcomes compared to those with conventional Ph-positive CML. Beyond vPh, the emergence of additional chromosomal abnormalities (ACAs) is a hallmark of clonal evolution and disease progression, particularly in CML cases progressing to blast crisis.

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Variant Ph translocations combined with ACAs add complexity to CML, contributing to disease progression.[2] Consequently, cytogenetic analysis by karyotyping and fluorescence *in situ* hybridization (FISH) remains the gold standard for detecting vPh and ACAs, particularly in assessing clonal evolution, which critically impacts treatment outcomes.

To our knowledge, this is the first reported case of newly diagnosed chronic phase CML involving a variant Ph with an unbalanced atypical three-way translocation between chromosomes 3, 9, and 22, accompanied by an interstitial deletion adjacent to the translocation breakpoint on the derivative chromosome 9. Additionally, ACAs, including Ph duplication and the clonal evolution of trisomy 8, 12, and 21, were observed. This case underscores the genetic heterogeneity present in a subset of CML patients, with initial resistance to standard frontline therapies successfully overcome by third-line treatment with ponatinib, which stabilized the disease.

Case Presentation

Our patient is a 42-year-old female patient who presented with fever and weight loss of 2 months' duration. She was admitted with COVID-19 (coronavirus disease 2019) infection and was incidentally found to have leukocytosis and hepatosplenomegaly. At presentation, her white blood cell (WBC) count was 362×10^9/L, hemoglobin (Hb) was 64 g/L, and platelets were substantially reduced to 17×10^9/L. Her peripheral smear was suggestive of CML and bone marrow biopsy was consistent with chronic phase myeloid/myelomonocytic leukemia.

Bone marrow evaluation at diagnosis revealed a hypercellular marrow with considerable decreased megakaryopoiesis and erythropoiesis, accompanied by a substantial myelopoiesis with M:E ratio of 1.5:3.5 and blast percentage of 2.3%. Reticulin staining demonstrated a normal pattern (World Health Organization grade 0–1MF). Immunostaining for CD34 and CD117 identified approximately 2 to 3% scattered interstitial myeloblasts, consistent with chronic leukemia in the chronic phase (CML-CP).

Karyotyping, FISH, and RT-PCR Analysis

The karyotype analysis at diagnosis revealed 20 metaphases with a three-way variant translocation involving chromosomes 3, 9, and 22, along with high-risk additional numerical chromosomal abnormalities, including duplication of the Ph chromosome and trisomy of chromosomes 8, 12, and 21, indicative of clonal evolution.

As per ISCN (International System for Human Cytogenomic Nomenclature) 2020 guidelines, the karyotype was

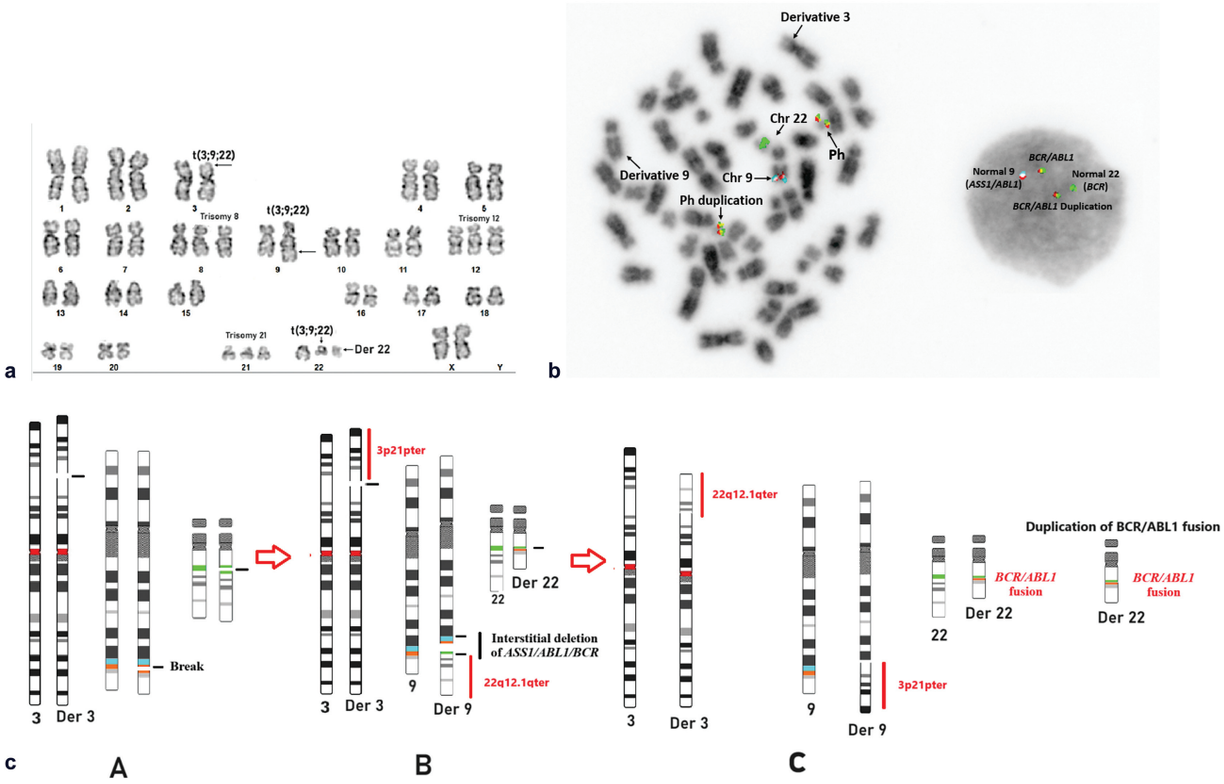

Fig. 1 (**a**) G banded karyotype showing translocation t(3;9;22) along with duplication of Ph, trisomy of chromosome 8,12, and 21. (**b**) Inverted DAPI interphase and metaphase FISH showing intact *ASS1/ABL1* (aqua/orange) on normal chromosome 9, *BCR* (green) on normal chromosome 22, *BCR/ABL1* fusion (orange/green) on derivative 22 (Ph chromosome), and an additional *BCR/ABL1* fusion (orange/green) on the duplicated derivative 22 (Ph duplication). (**c**) Schematic illustration of the three-way complex karyotype involving chromosomes 3, 9, and 22.

described with stemline and sideline clones as sl and sdl1 designated as:

47,XX,t(3;9;22)(p21;q34;q11.2),+der(22)t(9;22)[2]/49, sl,+8,+12[16]/50,sdl1,+21[2] (►**Fig. 1a**).

Subsequently, FISH and metaphase chromosome analysis using LSI Dual Color Dual Fusion probes for *BCR/ABL1* (Abbott Molecular/Vysis, Des Plaines, Illinois, United States) confirmed the presence of *BCR/ABL1* fusion in all 200 interphase cells (100%), which displayed a standard 2 fusion, 1 orange, 1 green pattern. However, metaphase FISH analysis identified an unbalanced atypical variant fusion pattern, with a deletion of the reciprocal *BCR/ABL1* and *ASS1* regions on the derivative chromosome 9 with a three-way unbalanced translocation between chromosomes 3, 9, and 22 (►**Fig. 1b**).

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis confirmed the presence of the major (p210) *BCR/ABL1* fusion mRNA transcript.

Treatment Course

The patient was initially started on tyrosine kinase inhibitor (TKI) therapy with imatinib. After 3 months of treatment, she exhibited labile counts due to severe thrombocytopenia and leukopenia, followed by a rapid increase in WBC counts while on imatinib.

She was subsequently switched to bosutinib, a second generation TKI, which initially yielded a good hematological response. However, after 2 months, she experienced disease progression with a WBC count increasing to 103×10^9/L and platelets dropping to 15×10^9/L. She was then treated with ponatinib, with stabilization of counts.

Due to an inadequate initial response, the patient continued to exhibit persistent leukocytosis with an increasing trend. A TKI resistance study was performed to detect *ABL1* exon 4–10 tyrosine kinase domain mutations, but no mutations were identified.

The patient had a fully human leukocyte antigen (HLA)-matched sibling donor and considering the aggressive nature of her disease, she proceeded for allogenic bone marrow transplant. Two months post-transplant, she had evidence of decrease in donor chimerism and hematological relapse and was restarted on ponatinib and is planned for donor lymphocyte infusion (DLI).

Discussion

Comprehensive karyotype and metaphase FISH analysis in the present case revealed multiple chromosomal breaks leading to a complex translocation. The initial break at the 9p34 and 22q11.2 regions resulted in the fusion of *BCR/ABL1* on the derivative chromosome 22. Subsequent reciprocal fusion of 22q11.2 to 9q34 occurred through two simultaneous breaks, causing an interstitial deletion involving the *ASS1/ABL1/BCR* region. The normal chromosome 9 retained the intact *ASSI/ABL1* (Aqua/Orange) which are present at spatial proximity. The region from 22q12.1 to the telomere (22qter) on the derivative chromosome 9 was replaced with a segment from 3p21pter, as illustrated in the schematic representation (►**Fig. 1c**).

Our patient represents the first reported case of an unbalanced, atypical t(3;9;22) complex rearrangement. Literature on three-way complex rearrangements typically suggests a two-way mechanism for such variants. However, in the present case, the translocation appears to involve multiple complex breaks, leading to the loss of the reciprocal *BCR/ABL1* fusion segment along with *ASS1* gene on derivative chromosome 9 through a nonhomologous recombination mechanism. Under normal conditions, the reciprocal *BCR* should fuse with *ABL1* on derivative chromosome 9, but this complex rearrangement resulted in fusion followed by an interstitial deletion of the reciprocal fusion and *ASS1*. This was accompanied by a translocation of the 22q12.1qter region to 3p21 and an exchange of the 3p21pter region onto 9q34.

Metaphase FISH confirmed that the fusion signal was retained solely on the derivative chromosome 22, with the reciprocal fusion signal absent on derivative chromosome 9. Notably, two copies of the fusion signal were observed on each derivative chromosome 22, indicating Ph duplication. Clonal evolution was observed, starting with the primary stemline clone involving the t(3;9;22) translocation and Ph duplication. The second evolved clone exhibited trisomy of chromosome 8 and 12, while the third clone showed trisomy of chromosome 21. RT-PCR analysis confirmed the presence of the major (p210) *BCR/ABL1* fusion mRNA transcript.

Historical studies have indicated that vPh chromosomes with 9q deletions are associated with poor outcomes. However, in the era of imatinib, favorable outcomes have been observed regardless of the number of chromosomes involved or the presence of a 9q deletion. Nevertheless, certain cases have been linked to worse prognosis, suggesting that specific chromosomes, breakpoints, translocation complexity, and/or the presence of ACAs may influence patient outcomes. In such cases, second-generation TKIs have demonstrated improved responses. Beyond the presence of variant three-way translocations, additional major-route abnormalities such as Ph duplication, trisomy 8, and iso(17p) have prognostic importance, contributing to disease progression.

A study by Clark et al supports the notion that specific ACAs predict disease progression independently of Sokal or EUTOS long-term survival (ELTS) scores when identified at diagnosis.[3] Moreover, studies suggest that response to TKI therapy may be influenced by both the variant translocation mechanism and novel gene interactions resulting from chromosomal breakpoints. For instance, breakpoints in regions such as 7q22 and Xq22 have been associated with improved responses to second-generation TKIs.[4–6]

The coexistence of isodicentric Ph chromosomes and the three-way Ph variant t(3;9;22)(p21;q34;q11.2) has been reported as a rare finding in CML by Li Q et al. The authors suggested that *BCR/ABL1* amplification may contribute to imatinib resistance, which could be overcome by dasatinib, highlighting an alternative therapeutic strategy for CML involving isodicentric Ph chromosome.[7]

ACAs, such as Ph duplication leading to *BCR/ABL1* overexpression, have been associated with aggressive clinical outcomes and imatinib resistance. While trisomy 8 is often considered a secondary event following therapy, recent studies suggest that ACAs present at diagnosis increase the risk of disease progression. Notably, ACAs are not correlated with Sokal or ELTS scores, indicating their potential as independent prognostic markers for treatment decisions.[8]

The recurrent involvement of 3p21 in variant translocations has been documented as an apparently balanced translocation in approximately 18 cases.[9] Although variant translocations are not classified as a "warning" category in the imatinib era, the complexity of chromosomal abnormalities in CML remains significant due to the wide range of chromosomal involvements and specific breakpoints affecting prognosis, particularly at 3p21.

Our findings of an unbalanced three-way complex translocation, along with ACAs, highlight the importance of identifying precise breakpoints and understanding their impact on disease progression. Given the potential role of these abnormalities in influencing therapeutic response and prognosis, detailed breakpoint analysis in variant Ph translocations may provide critical insights into their genetic and pathogenic significance.

A review of the literature suggests that CML patients with t(3;9;22)(p21;q34;q11.2) tend to have an aggressive course and poor prognosis. Conversely, translocations involving the 3q region have been associated with favorable responses to imatinib, emphasizing the importance of breakpoint location as a prognostic indicator.[10]

The TKI resistance study in the present case showed absence of *ABL1* exon 4–10 tyrosine kinase domain mutations, suggesting that the resistance to first-line therapy in our case is likely due to the complex translocation and the presence of ACAs.

Our findings have shown that an unbalanced three-way complex translocation, along with ACAs, is associated with resistance to first-line therapy. This highlights the potential impact of complex chromosomal rearrangements on treatment response and disease progression, requiring a more tailored therapeutic approach in such cases.

Our case is the first to report t(3;9;22) as an unbalanced variant translocation with additional aneuploidies. This imbalance results from a sub-microscopic deletion on derivative chromosome 9. Large deletions in advanced-stage CML have been associated with poor prognosis, as supported by Chandran et al.[11]

The presence of trisomy 8, 12, and 21 in our case adds further complexity. While trisomy 8 and 21 are common in myeloid malignancies, trisomy 12 is rare, despite its involvement in recurrent translocations. Each of these trisomy has been independently linked to disease progression, genetic instability, and disruption of DNA repair mechanisms.[12,13]

The observed clonal evolution, with trisomy of chromosome 8, 12, and 21 arising alongside t(9;22) translocation and Ph duplication, suggests a complex genetic profile in de novo chronic-phase CML, even in the absence of blasts. Our findings raise the possibility that an apparently balanced translocation may not only generate an oncogenic fusion but also result in the loss of tumor suppressor genes near the breakpoint. Few reports address the need for second-line treatment in complex vPh cases with ACAs. As more evidence emerges, a clearer treatment strategy is required for complex cytogenetic group. Our patient underwent a bone marrow transplant but experienced early disease progression post-transplant. Managing CML patients with complex karyotypes remain challenging necessitating careful monitoring for treatment response and disease progression. This report presents a detailed cytogenetic characterization, emphasizing the prognostic impact of complex karyotypes. A key strength of this study is its focus on early detection, modifying targeted therapy, and continuous monitoring to improve treatment outcomes. The study is based on a single case, limiting its applicability to a broader patient population. Given the poor prognosis in present case, long-term follow-up data are needed to establish definitive treatment guidelines for such cases. Further research with larger cohorts is necessary to optimize therapeutic strategies for patients with complex vPh translocations and ACAs in accordance with documented outcomes and established guidelines.

Conclusion

Our study underscores the importance of integrating karyotyping, FISH, and molecular studies to better understand clonal dynamics in vPh cases. This is the first reported case of a patient with a complex unbalanced three-way vPh translocation and ACAs, highlighting the need for alternative therapeutic strategies. Identifying this subset of patients at high risk is essential to ensure close monitoring and timely intervention for treatment efficacy.

Author's Contributions

S.M.A.A.: concept, design, experimental studies, data analysis, manuscript preparation, manuscript editing, and manuscript review.
S.P.S.: clinical study, data analysis, manuscript preparation, manuscript editing, and manuscript review.
A.P.: concept, literature search, data analysis, manuscript preparation, manuscript editing, and manuscript review.

Patient Consent

Patient consent has been obtained.

Funding

None.

Conflict of Interest

None declared.

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Immune-Related Dermatologic Reactions in Cancer Patients Undergoing Immunotherapy: A Case Series

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Abstract

Immune checkpoint inhibitors (ICIs) have transformed the treatment paradigm across various malignancies by reactivating T cell responses against tumor cells. These agents function by blocking inhibitory checkpoint pathways, thereby preventing immune downregulation and enhancing antitumor immunity. Despite their clinical success and relatively favorable safety profile, ICIs are associated with immune-related adverse events (irAEs), which can impact multiple organ systems. Among these, cutaneous irAEs are some of the most common, owing to the skin's immunologic sensitivity. These manifestations range from mild conditions—such as pruritus, maculopapular rashes, and inflammatory dermatoses including eczema, psoriasis, and lichenoid reactions—to more severe immunobullous disorders (such as bullous pemphigoid, pemphigus vulgaris, and Stevens–Johnson syndrome). While most cutaneous irAEs can be effectively managed without interrupting immunotherapy, certain cases may require prompt, multidisciplinary intervention to prevent serious complications. This case series presents four diverse malignancies complicated by dermatologic irAEs, highlighting atypical presentations, critical warning signs, and evidence-based strategies for management. Through these cases, we aim to raise awareness about less common cutaneous toxicities and offer practical guidance to ensure safe continuation of cancer immunotherapy.

Keywords
- dermatology
- immune checkpoint inhibitors
- immune-related adverse events

Introduction

Immunotherapy has transformed cancer treatment, with immune checkpoint inhibitors (ICIs) at the forefront. These agents—targeting inhibitory receptors such as CTLA-4, LAG-3, PD-1, and PD-L1—restore antitumor immunity by blocking immune checkpoints that suppress T cell activity. This reinvigorates immune surveillance, enabling the recognition and elimination of malignant cells, and has led to durable responses across various cancers. Compared with traditional chemotherapy, ICIs generally have a more favorable toxicity profile. However, by enhancing immune function, they can trigger immune-related adverse events (irAEs).[1,2] Among these, cutaneous irAEs are common and range from mild rashes to severe, potentially life-threatening dermatologic conditions. With the growing use of ICIs across malignancies —including melanoma, hematologic, and gynecologic cancers—clinicians must remain alert to these skin manifestations. Early recognition, prompt dermatologic consultation,

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and coordinated management are essential to reduce morbidity and allow patients to continue life-prolonging therapy.

Case History

Case 1: Severe Immune-Related Exfoliative Dermatitis Mimicking Toxic Epidermal Necrolysis Following Dual Checkpoint Inhibition in Metastatic Non–Small Cell Carcinoma Lung

A 52-year-old female, chronic smoker with no prior autoimmune disease, was diagnosed with metastatic squamous cell carcinoma of the lung, complicated by pleural effusion and bone metastases. Molecular profiling revealed no actionable mutations, and PD-L1 expression was <1%. She was started on a combination regimen of nivolumab (PD-1 inhibitor), ipilimumab (CTLA-4 inhibitor), and paclitaxel–carboplatin chemotherapy. The initial cycles were well tolerated. However, by day 11 posttherapy, the patient developed extensive erythematous and exfoliative skin lesions involving the back, trunk, and abdomen, characterized by diffuse desquamation, scaling, and epidermal detachment, without mucosal involvement initially (►**Fig. 1A–C**). She became systemically unwell, with hypotension and signs of toxicity, prompting urgent intensive care unit admission. The clinical picture was highly suggestive of a severe immune-related cutaneous adverse reaction (grade 4), closely mimicking toxic epidermal necrolysis (TEN). Dermatology consultation confirmed the diagnosis of severe exfoliative dermatitis, likely immune-mediated, possibly compounded by chemotherapy. Laboratory workup (including complete blood count, liver function tests, eosinophil count, and inflammatory markers) was initiated to rule out drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome and assess systemic involvement. Immunotherapy was immediately discontinued, and the patient was started on IV methylprednisolone (1 mg/kg/day) along with topical corticosteroids, skin barrier repair agents, and comprehensive supportive ICU care. Over the following 2 weeks, her skin lesions gradually stabilized, with early signs of re-epithelialization. Due to the severity of the grade 4 toxicity, immunotherapy was permanently discontinued, and alternative systemic treatment options were initiated. At the 6-month follow-up, hypopigmented facial patches and alopecia showed partial repigmentation, although some residual depigmentation remained. No new lesions developed, and the patient remained in oncologic remission, continuing alternative therapy without further irAEs.

Case 2: Maculopapular Rash Following Tremelimumab and Durvalumab in Advanced Hepatocellular Carcinoma

A 60-year-old male with hepatocellular carcinoma involving portal vein thrombosis (Barcelona Clinic Liver Cancer [BCLC] Stage C, Child-Pugh A, Eastern Cooperative Oncology Group 1 [ECOG]) underwent transarterial chemoembolization (TACE) followed by systemic immunotherapy with tremelimumab (CTLA-4 inhibitor) and durvalumab (PD-L1 inhibitor), as per the HIMALAYA regimen. The patient tolerated the initial infusion well; however, on day 7 posttreatment, he developed a diffuse erythematous, pruritic maculopapular rash (MPR) over the trunk and back (►**Fig. 2A, B**), without systemic symptoms or mucosal involvement. The rash involved ~10 to 30% of body surface area (BSA). The dermatologic toxicity was categorized as a grade 2 immune-related MPR according to Common Terminology Criteria for Adverse Events, version 5.0 (CTCAE v5.0). He was managed conservatively with topical corticosteroids (mometasone 0.1%), oral antihistamines, and close clinical monitoring. Immunotherapy was continued without interruption, and systemic corticosteroids were not required. The rash gradually resolved, with complete clearance by 4 weeks. At the 4-month follow-up, there was no recurrence or post-inflammatory pigmentation, and immunotherapy continued without further cutaneous adverse events.

Case 3: Triple-Negative Breast Cancer with Cutaneous Immune-Related Adverse Event

A 40-year-old woman with a history of hypothyroidism presented with a 6-month history of a painless, immobile lump in the upper outer quadrant of her left breast. Over time, the lesion became painful, accompanied by bloody nipple discharge. Positron emission tomography–computed tomography (PET-CT) revealed a 1.5×1.7 cm FDG-avid, irregular, spiculated lesion without skin or chest wall involvement. FDG-avid left axillary and subpectoral lymphadenopathy

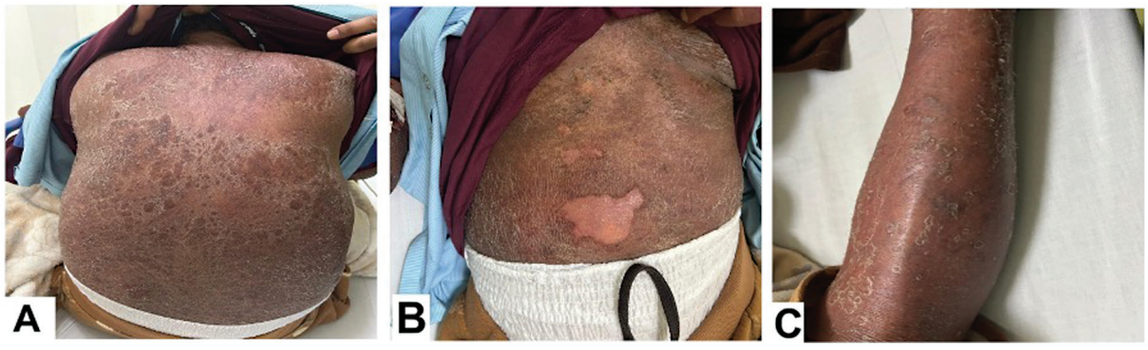

Fig. 1 (**A–C**) Clinical images show extensive erythematous and exfoliative skin lesions involving the back, trunk, and abdomen. Clinical images demonstrate diffuse desquamation, scaling, and areas of epidermal detachment without initial mucosal involvement, consistent with a severe immune-related cutaneous adverse event mimicking toxic epidermal necrolysis.

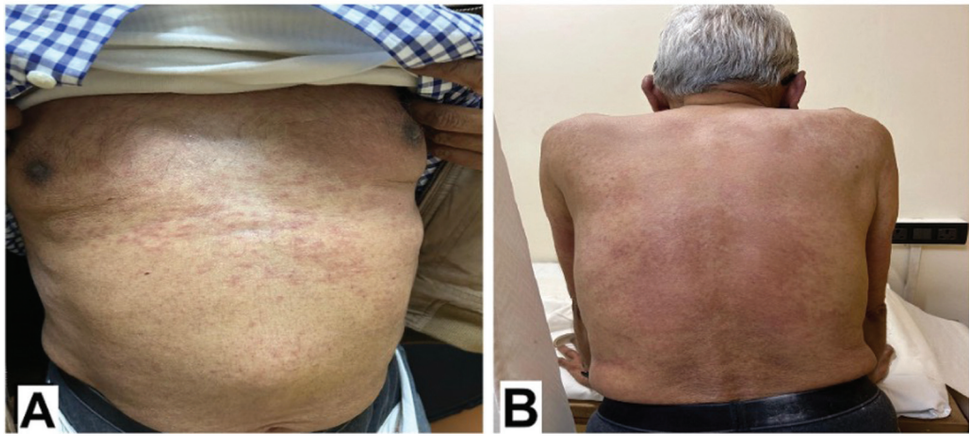

Fig. 2 (**A**, **B**) Clinical images showing diffuse erythematous, pruritic maculopapular rash over the trunk and back, involving 10–30% of BSA.

was noted, with the largest node measuring 2.4 × 2.3 cm (SUVmax: 11.5), along with perinodal fat stranding. Core biopsy confirmed triple-negative breast cancer (ER-, PR-, HER2-). The patient was initiated on neoadjuvant chemo-immunotherapy with pembrolizumab and carboplatin. After three cycles of pembrolizumab, she developed asymptomatic, non-scaly hypopigmented patches over her face and frontal scalp, with associated diffuse alopecia (►**Fig. 3A**). These lesions were consistent with vitiligo-like depigmentation, a recognized cutaneous irAE associated with anti-PD-1 therapy. Based on CTCAE v5.0, this event was categorized as grade 2 vitiligo, given the multifocal involvement and cosmetic impact. The patient was managed conservatively with topical corticosteroids (clobetasol: 0.05%), calcineurin inhibitors (tacrolimus: 0.1%) on facial areas, strict photoprotection, and dermatologic follow-up. No systemic immunosuppression was required, and pembrolizumab therapy was continued without interruption. Following completion of eight cycles of therapy, she underwent a left modified radical mastectomy with axillary clearance. Histopathology revealed a pathological complete response to the neoadjuvant regimen. Postoperative PET-CT showed no evidence of metabolically active residual or recurrent disease. At the 3-month follow-up, the patient had developed post-inflammatory hyperpigmentation over previously affected areas, particularly the back and trunk. No active desquamation was seen, but skin fragility persisted. She remained off immunotherapy and was transitioned to alternative systemic therapy.

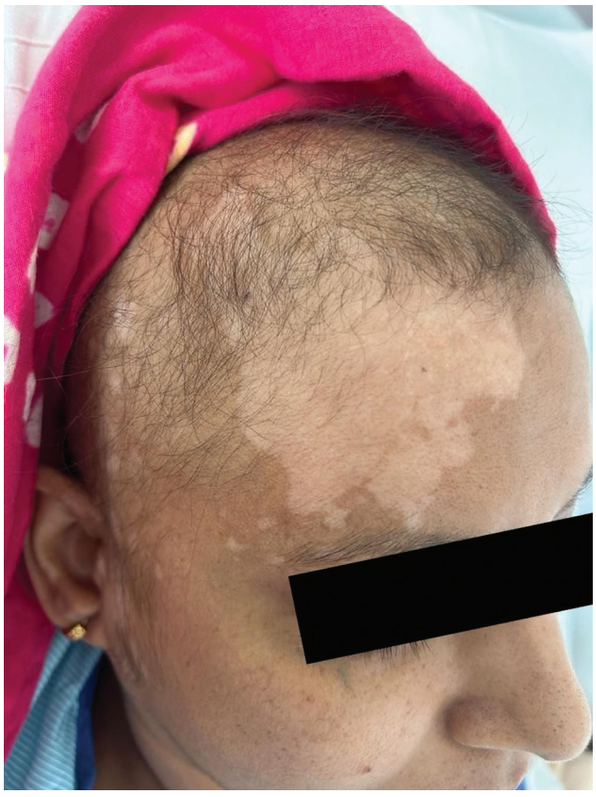

Fig. 3 Clinical image showing asymptomatic hypopigmented facial patches with diffuse frontal scalp alopecia, consistent with vitiligo-like depigmentation.

Case 4: Immune-Related Lichenoid Dermatitis Following Nivolumab-Based Therapy in Recurrent Tongue Carcinoma

A 59-year-old woman with recurrent squamous cell carcinoma of the tongue, previously treated with multiple chemotherapy lines, was started on oral metronomic chemotherapy (methotrexate, erlotinib, and celecoxib) combined with nivolumab (100 mg every 2 weeks). After two cycles, she developed pruritic, hyperpigmented, scaly plaques on her lower limbs, predominantly the shins, with associated linear excoriations, superficial erosions, and post-inflammatory crusting (►**Fig. 4A, B**). The morphology and distribution were consistent with lichenoid dermatitis, an irAE associated with PD-1 inhibition. Initially categorized as grade 1 toxicity, the condition was managed with topical corticosteroids and oral antihistamines. However, following the third cycle, the skin lesions significantly worsened, with more extensive excoriation and superficial ulceration, leading to an upgraded classification of grade 3 dermatitis (CTCAE v5.0). Nivolumab was temporarily withheld, and the patient was started on

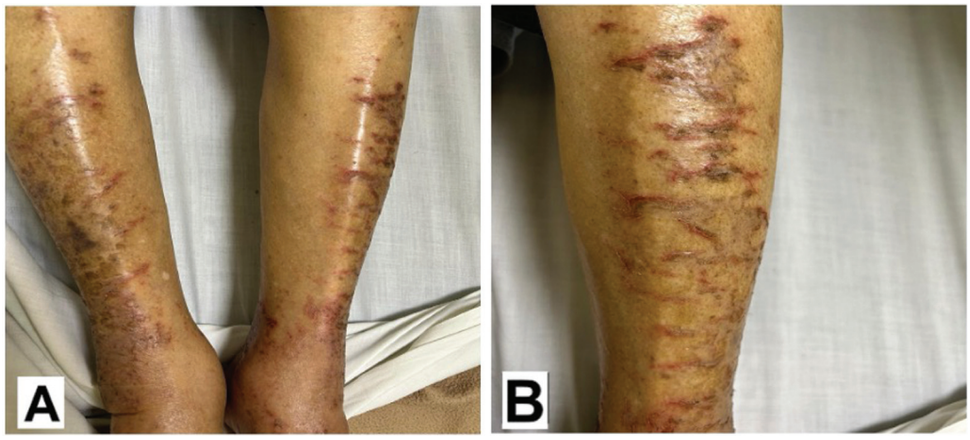

Fig. 4 (A, B) Clinical images showing pruritic, hyperpigmented, scaly plaques with excoriations and superficial erosions on the lower limbs, consistent with lichenoid dermatitis.

systemic corticosteroids (prednisolone 1 mg/kg/day), to which she responded favorably. The patient achieved gradual lesion resolution with systemic steroids, and skin changes continued to improve over 3 weeks during steroid tapering. At the 6-month follow-up, only mild post-inflammatory hyperpigmentation and occasional pruritus persisted. Given the improvement, she was considered for immunotherapy reinitiation and was successfully rechallenged under close dermatologic supervision, with no recurrence to date.

Discussion

Dermatologic irAEs are among the most common and often the earliest manifestations of ICI therapy, typically occurring within the first 2 to 4 weeks after treatment initiation. These cutaneous toxicities can serve as early indicators of systemic immune dysregulation, underscoring the importance of prompt recognition and management. The incidence and severity of immune-related cutaneous adverse events (irCAEs) vary by the type of ICI administered. Skin-related adverse events of any grade occur in ~26 to 43.5% of patients receiving CTLA-4 inhibitors, 7.6 to 34% with PD-1 inhibitor monotherapy, and around 40 to 41% with combined CTLA-4 and PD-1 blockade.[3,4] Some studies report even higher rates, with cutaneous toxicities seen in up to 70% of patients on PD-1/PD-L1 inhibitors and nearly all patients on combination regimens. Most irCAEs are mild to moderate in severity (grades 1–2) and are reversible with timely intervention. Grade 1 involves less than 10% BSA with mild symptoms such as pruritus or burning. Grade 2 includes 10 to 30% BSA involvement, or more than 30% with only mild symptoms not affecting self-care. Grade 3 is defined by more than 30% BSA with moderate to severe symptoms impacting activities of daily living. Grade 4 reactions are life-threatening and require immediate medical attention.[4] Severe (grade 3 or higher) skin toxicities remain relatively uncommon, affecting ~1 to 3% of ICI-treated patients.[5,6] However, when they occur, they may necessitate temporary interruption or even permanent discontinuation of therapy, potentially affecting overall cancer outcomes. Clinically, irCAEs frequently present as nonspecific MPRs, pruritus, or inflammatory dermatoses such as psoriasiform, eczematous, or lichenoid eruptions. Less common manifestations include vitiligo-like depigmentation, alopecia, and autoimmune blistering disorders like bullous pemphigoid. Although most cases are self-limiting, rare but serious dermatologic toxicities—such as Stevens–Johnson syndrome (SJS), TEN, and drug reaction with eosinophilia and systemic symptoms—can arise and require urgent, aggressive management.

In Case 1, a patient receiving a combination of nivolumab, ipilimumab, and chemotherapy developed extensive exfoliative dermatitis with epidermal detachment, suggestive of a TEN-like immune-mediated reaction. This severe and rare event required immediate discontinuation of ICIs, intensive care unit admission, and systemic immunosuppression, emphasizing the importance of early recognition and intervention in rapidly evolving skin toxicities. Case 2 featured a patient with hepatocellular carcinoma treated with tremelimumab and durvalumab who developed a grade 2 MPR—an irAE that typically arises within the first 2 weeks of treatment. Symptoms were effectively managed with topical corticosteroids and antihistamines, allowing immunotherapy to continue without interruption. In Case 3, a woman with triple-negative breast cancer developed vitiligo-like facial depigmentation following pembrolizumab-based immunotherapy. Although this pigmentary change is more commonly observed in melanoma, it is increasingly recognized in non-melanoma malignancies. While not medically harmful, such changes often persist and may carry prognostic significance, potentially reflecting sustained immune activation. Case 4 involved a woman receiving metronomic chemotherapy alongside nivolumab who presented with pruritic, reticulated, and ulcerated plaques on her lower limbs, characteristic of lichenoid dermatitis. Initially mild, the condition progressed to grade 3 toxicity after subsequent treatment cycles, necessitating systemic corticosteroids. This case highlights the importance of regular dermatologic assessment and a stepwise approach to management based on severity.

The management of irCAEs generally involves topical corticosteroids and symptomatic care in mild cases, while more severe or persistent cases may require systemic corticosteroids or additional immunosuppressive therapies such as tumor necrosis factor-α (TNF-α) inhibitors. However, it is important to note that the early use of systemic corticosteroids, particularly around the initiation of ICI therapy, may attenuate the antitumor immune response and should be considered judiciously. The variation in immune-related side effects among different checkpoint inhibitors is believed to arise from how these drugs function within the immune system. CTLA-4 inhibitors mainly influence T cell activation in the lymph nodes and other lymphoid tissues, while PD-1 and PD-L1 inhibitors exert their effects more directly at the tumor site. This difference contributes to the distinct patterns and timing of irAE associated with each class of drug.

Distinguishing between preexisting or unrelated skin conditions and irCAEs can be challenging, especially when symptoms appear long after immunotherapy has begun. A thorough clinical evaluation is essential, focusing on the rash's distribution, appearance, type, estimated BSA involvement, any mucosal lesions, and the presence of lymph node enlargement. Furthermore, these cutaneous toxicities are graded based on severity using the CTCAE. The choice of initial investigations depends on how severe the presentation is and may include vital sign monitoring, urine dipstick testing, swabs from any ulcers or erosions for bacterial or viral cultures, and routine blood work such as complete blood count, kidney and liver function tests, inflammatory markers, thyroid function, blood glucose, and muscle enzyme levels. While most skin reactions can be diagnosed based on clinical appearance, more severe, unusual, or persistent cases may require skin biopsy and histopathological analysis for confirmation. MPRs, pruritus, and depigmentation resembling vitiligo are among the most frequently observed cutaneous immune-related side effects. In contrast, more uncommon but serious manifestations include SJS, TEN, and autoimmune blistering skin disorders.

A pruritic MPR is the most common skin-related adverse event associated with CTLA-4 inhibitors, occurring more frequently than with PD-1/PD-L1 therapies. It affects 49 to 68% of patients on anti–CTLA-4 treatment compared with around 20% on anti-PD-1/PD-L1.[7,8] The rash usually appears within 3 to 6 weeks of treatment initiation and tends to be dose dependent. Commonly affecting the trunk and extensor limbs, it presents as faint erythematous macules and papules that may merge into plaques. Most cases are mild to moderate (grades 1–2), involving less than 30% of the BSA, while ∼4% develop severe (grades 3–4) rashes. Koebner phenomenon may occur due to trauma or scratching. Management of grades 1 to 2 MPR includes mid- or superpotent topical corticosteroids with continued immunotherapy. Grade 3 requires systemic corticosteroids (prednisone: 1–2 mg/kg/day) alongside topical treatment, and immunotherapy should be paused until improvement. For grade 4, therapy must be discontinued and high-dose methylprednisolone (2 mg/kg/day) initiated.

Vitiligo occurs in ∼11% of patients on anti-CTLA-4 therapy and up to 25% with anti-PD-1 treatment.[8,9] In the latter, the immune response involves CD8+ cytotoxic T cells targeting melanoma-associated antigens—such as MART-1/MelanA, gp100, and tyrosinase-related proteins—that are also found in healthy melanocytes. This can result in asymmetrical depigmentation patterns, ranging from large patches to fine, confetti-like spots. When nivolumab is combined with ipilimumab, depigmentation tends to be more widespread and may appear earlier. These pigment changes usually persist after therapy ends and do not require stopping or pausing ICIs. While not medically necessary, interventions like high-SPF sun protection and cosmetic camouflage can help address aesthetic concerns. Topical corticosteroids or calcineurin inhibitors may be used cautiously, though they offer limited clinical benefit.

Although systemic corticosteroids remain the mainstay for managing moderate to severe cutaneous irAEs, a subset of patients may exhibit inadequate response or develop steroid-related toxicity. In such cases, emerging immunomodulatory agents are being explored. Janus kinase (JAK) inhibitors such as tofacitinib and ruxolitinib have shown efficacy in small series and case reports, likely due to their ability to downregulate proinflammatory cytokine signaling. Similarly, targeted biologics including IL-17 and IL-23 inhibitors (e.g., secukinumab, guselkumab)—traditionally used in psoriasis—have demonstrated benefit in selected patients with immune-related psoriasiform or lichenoid eruptions. Other agents such as mycophenolate mofetil, azathioprine, and rituximab may also be considered based on individual presentation and multidisciplinary input. These therapies offer promising steroid-sparing options and may allow reintroduction of immunotherapy in patients with previously severe reactions under careful monitoring.

Among the most serious cutaneous irAEs are SJS and TEN, which are characterized by widespread epidermal necrosis and detachment, often accompanied by mucosal involvement. These conditions are increasingly recognized in association with ICIs such as pembrolizumab, nivolumab, and ipilimumab. Clinically, SJS and TEN may begin with nonspecific targetoid macules that progress to dusky plaques, bullae, widespread epidermal sloughing, and a positive Nikolsky sign. The extent of BSA involvement differentiates the conditions: SJS involves less than 10% BSA, TEN involves more than 30%, and overlap syndromes affect between 10 and 30%. Management requires the permanent discontinuation of ICIs. Treatment involves hospital admission with intensive supportive care to maintain fluid balance and reduce the risk of infection through appropriate wound care. Therapeutic strategies may include administering high-dose corticosteroids, such as methylprednisolone at 2 mg/kg per day, along with the possible use of intravenous immunoglobulin, TNF-α inhibitors, mycophenolate mofetil, or cyclosporine, depending on the severity and clinical judgment.

This case series offers several strengths, including a diverse representation of malignancies and a broad spectrum of dermatologic irAE associated with different ICI regimens. The detailed clinical descriptions, visual documentation, and treatment strategies presented can serve

as a practical reference for oncologists and dermatologists encountering similar presentations in real-world practice. The inclusion of both mild and severe cutaneous reactions enhances clinical relevance and educational value. However, the study is limited by its small sample size, and the absence of long-term follow-up data in some cases. Additionally, the reliance on clinical diagnosis without histopathologic confirmation in all cases may limit diagnostic precision, especially in distinguishing overlapping or atypical presentations. Future research should focus on larger, prospective multicenter studies to better characterize the incidence, risk factors, and treatment responses of cutaneous irAEs across cancer types and ICI combinations. Biomarker-driven approaches to predict susceptibility or severity of irAEs may enable more personalized immunotherapy regimens. The findings, while informative, may not be fully generalizable due to the small number of patients and single-institution setting. However, the diverse clinical contexts represented here do reflect real-world scenarios in oncology practice. Gray areas that warrant further exploration include optimal management strategies for steroid-refractory cutaneous irAEs, long-term dermatologic sequelae post-ICI therapy, and the prognostic significance of skin toxicities in non-melanoma cancers. Additionally, standardized algorithms for rechallenge decisions following high-grade irAEs remain an unmet need.

Conclusion

ICIs have transformed cancer therapy, but their use is often accompanied by irAE, with cutaneous toxicities being among the most common. This case series highlights the clinical diversity of skin-related irAEs, ranging from mild, self-limiting rashes to severe reactions requiring systemic immunosuppression or even treatment discontinuation. Prompt recognition, accurate grading, and individualized management are essential to minimize morbidity and maintain continuity of life-saving cancer treatment. Notably, dermatologic manifestations can serve as early indicators of immune dysregulation and should be carefully evaluated, as they may influence therapeutic decisions and overall outcomes.

Authors' Contributions

Conceptualization was performed by R.C., C.D., S.R., A.V., V.K., and M.A.O.. Data curation was performed by R.C., M.A.O., and V.K. Project administration was managed by C.D., S.R., and A.V. Original draft preparation was undertaken by R.C., C.D., S.R., A.V., V.K., and M.A.O. All authors—R.C., C.D., S.R., A.V., V.K., and M.A.O.—contributed to the review and editing of the manuscript and provided final approval of the version to be submitted. The corresponding author is the guarantor of the submission.

Ethical Approval

For this case series, formal consent from a local ethics committee is not required.

Patients' Consent

The authors certify that they have obtained the appropriate consent from the patients. The patients have given their consent for the images and other clinical information to be reported in the journal. The patients understand that the name and initials will not be published, and due efforts have been made to conceal the same.

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Conflict of Interest

None declared.

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Re: Sasi A. When Less Is More: An Avenue for Academia-Industry Collaboration in Pediatric Cancer

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We read with great interest the insightful article by Sasi et al[1], "When Less Is More: An Avenue for Academia–Industry Collaboration in Pediatric Cancer," which underscores the need for collaborative approaches to address inequities in pediatric cancer care. The authors thoughtfully advocate for academia–industry synergy in developing cost-effective, patient-accessible oncologic therapies, particularly in low- and middle-income countries (LMICs). We wish to expand upon this important discussion by highlighting a few complementary perspectives.

First, while the article emphasizes financial and regulatory barriers, it is equally vital to recognize the role of patient and caregiver engagement in shaping meaningful drug development strategies. In LMICs, where treatment abandonment and sociocultural barriers to care are prevalent, academia–industry collaborations would benefit significantly from integrating patient advocacy groups to ensure that innovation aligns with lived realities and cultural acceptability.

Second, the authors rightly highlight the potential of dose de-escalation trials as cost-saving strategies. However, we propose that dose optimization in pediatric oncology be viewed not merely through an economic lens, but also as a scientific imperative. Children are not small adults, and their distinct pharmacodynamics and pharmacokinetics often support the rationale for tailored dosing strategies. Thus, low-dose regimens should be investigated as a means of improving the therapeutic index—not just reducing costs.

Furthermore, while Sasi et al mention regulatory and financial constraints in LMIC-based academic trials, there is an opportunity to build upon recent reforms. In India, for instance, the 2019 New Drugs and Clinical Trials Rules allow for a more enabling environment for investigator-initiated studies. Aligning such reforms with state-backed funding mechanisms and public–private partnerships could pave the way for globally relevant academic leadership in drug development from LMICs.

We also appreciate the emphasis on real-world data but believe this warrants further elaboration. Academic institutions in LMICs, by virtue of their large patient volumes and diverse case mix, are uniquely positioned to generate real-world evidence. This could complement traditional trial data and support adaptive trial designs, post-marketing surveillance, and drug repurposing efforts.

Finally, the article alludes to the misalignment between cost-effective strategies and pharmaceutical incentives. We propose the exploration of value-based pricing models, preferential procurement for cost-effective innovations, and pharmacoeconomic reward frameworks to align commercial interests with societal benefit. Such approaches could amplify both the reach and impact of pediatric cancer therapies.

In conclusion, we commend the authors for highlighting a crucial and timely theme. We believe that expanding the collaboration framework to include patient voices, regulatory opportunity, scientific rationale for de-escalation, and value-based economic models will help realize the full potential of equitable, effective pediatric oncology care.

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Conflict of Interest
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1 Sasi A, Bakhshi S, Ganguly S. When less is more: an avenue for academia–industry collaboration in pediatric cancer. Indian J Med Paediatr Oncol 2025;46(03):318–320

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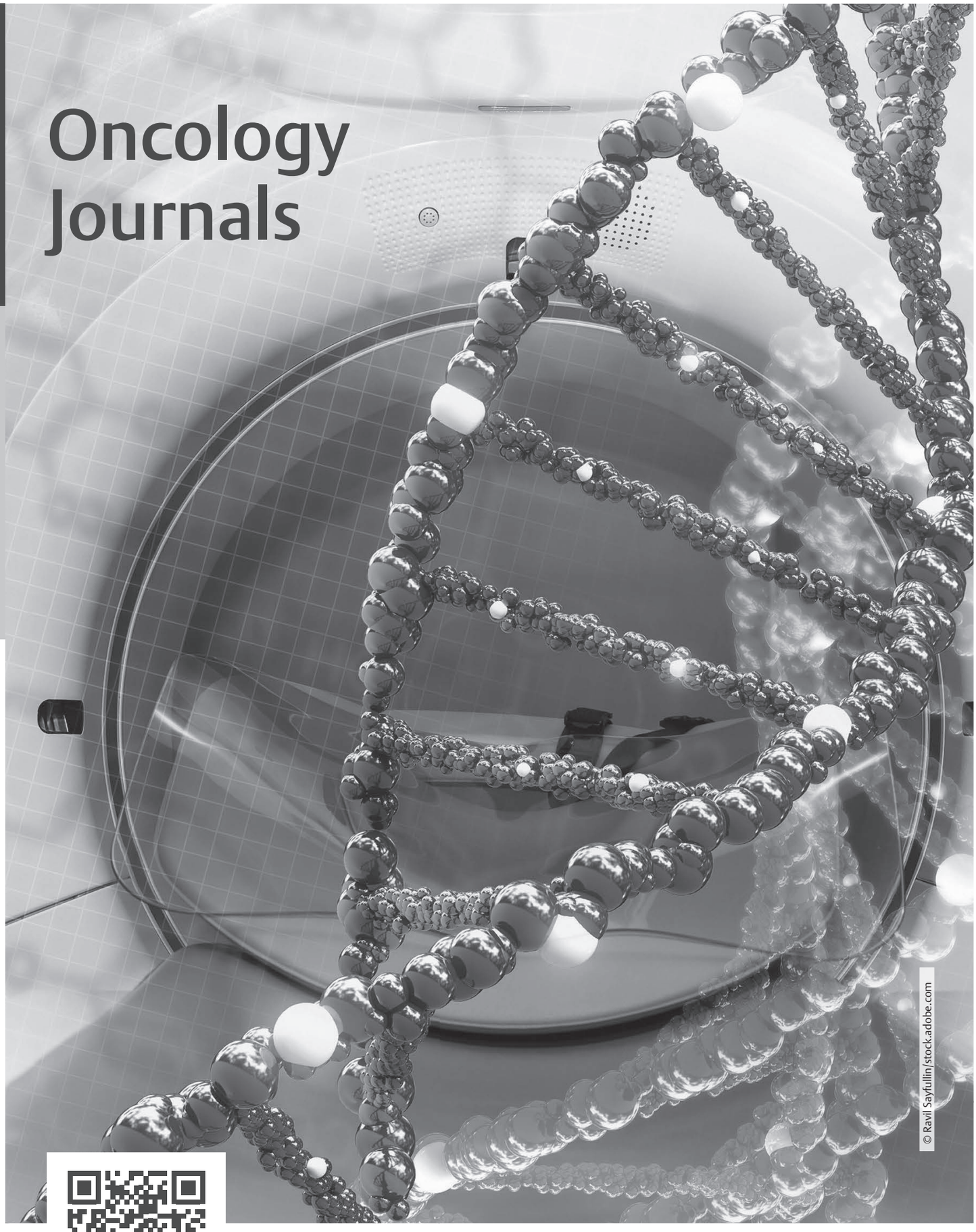

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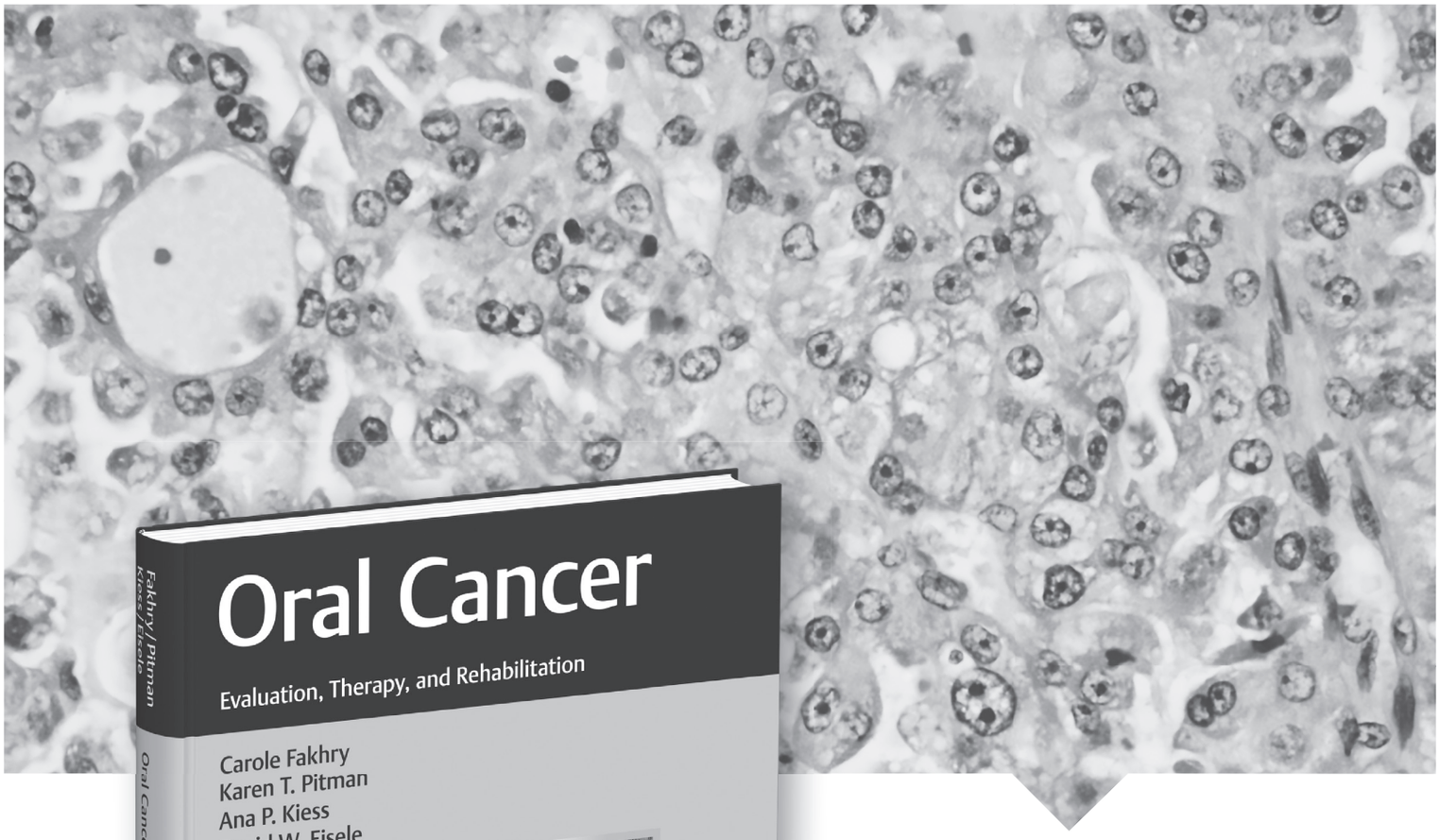

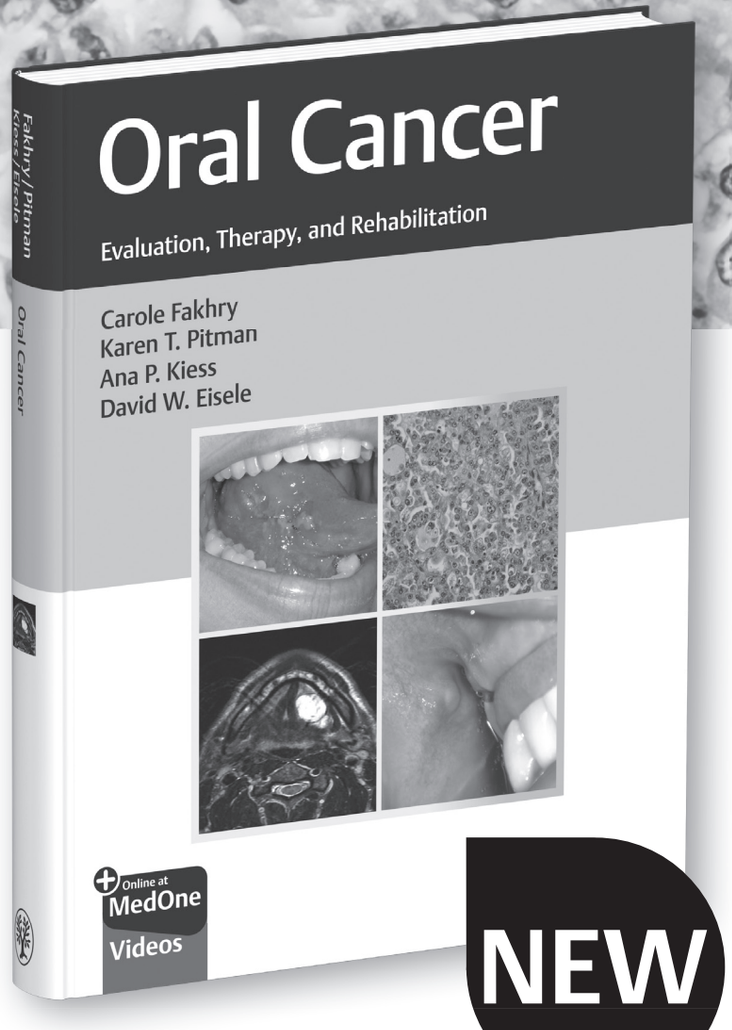

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