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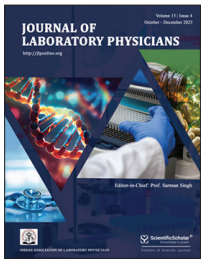

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O-1

Microbiome profiling in tobacco consumers and normal healthy individuals: A comparative analysis

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Aims and Objectives: The aim of this study was to investigate and comparison of bacterial diversity in tobacco consumers, and healthy individual to understand potential differences, providing insights into the impact of tobacco consumption on oral microbiome health.

Methods: In this investigation, a total of 24 buccal swab samples and 24 gum swab samples were collected from different groups of tobacco chewers, tobacco smokers, and normal healthy persons. All samples were processed in duplicate. Qiagen deoxyribonucleic acid (DNA) Mini Kit procedure was modified to isolate DNA. DNA samples were amplified using a primer targeting the bacterial 16S ribosomal RNA gene's V3-V4 region. Raw data were processed with Perl script and Prinseq lite. Metagenomic analysis was done with Quantitative Insights Into Microbial Ecology (QIIME 2–2022.2) and default command line settings. Reads were denoised and demultiplexed using (DADA2) pipelines. Use of SILVA database for taxonomic analysis.

Results: There are 92% of the sequences in good quality for downstream analysis,which is 15 gbp. The predominance of the phyla *Bacteroidota*, *Firmicutes*, *Fusobacteriota*, and *Proteobacteria* was shown to be significantly higher in those who consume tobacco. *Alloprevotella*, *Prevotella*, *Prevotella* 7, and *Veillonella* showed significantly higher levels of abundance in those who chew and smoke tobacco products, as compared to those who do not use tobacco. *Streptococcus* is the most common genus found in each and every sample.

Conclusion: The study reveals significant variations in the diversity of alpha and beta microorganisms in tobacco users compared to healthy individuals. This suggests a distinct oral cavity microbiota, potentially increasing the risk of oral diseases like oral cancer. The study highlights the importance of understanding the oral microbiome's role in health and disease, and how behavioral decisions like tobacco use can influence microbial communities and disease risk. This metagenomics investigation contributes to understanding the complex interplay between lifestyle elements and microbial communities.

Keywords: Metagenomics, Oral bacterial diversity, Tobacco chewers and tobacco smokers.

O-2

"JAK" of many trades? V617F: Beyond blood – in the solid tumor terrain

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Introduction: Janus Kinase (JAK)/signal transducer and activator of transcription pathway hyperactivation are common in hematological malignancies, acting as predictive biomarkers for JAK-targeted therapy. In solid tumors, this phenomenon is rare, and the implications of targeted therapy are not fully elucidated.[1]

Aim: The aim of this study was to investigate the frequency of Janus kinase 2 (*JAK2*) mutation (V617F) in solid tumors and conduct a comparative analysis with the cBioPortal database.

Objectives: The objectives of this study were as follows: (1) Determine JAK2 V617F frequency in solid tumors subjected to next-generation sequencing (NGS) analysis from June to November 2023 and analyze distribution for potential clinical associations. (2) Mine the cBioPortal database and characterize the genomic landscape of V617F in solid tumors.

Methods: Two hundred and sixty tumor samples (June 1st, to November 30th, 2023) underwent NGS sequencing (Ion GeneStudio S5 plus) and were analyzed using Ion Reporter™. The assay covered 52 genes relevant to solid tumors; reported variants had a minimum variant allele frequency (VAF) of 5% and 500× depth of coverage. The Cancer Genome Atlas (TCGA) PanCancer Atlas studies and other PanCancer studies were explored in the open-access database www.cbioportal.org,[2] querying 87,050 samples from 42 studies for JAK2 V617F mutation in solid tumors.

Results: In our cohort, JAK2 V617F mutation occurred in five cases (1.9%) surpassing cBioPortal database's reported rate of 0.09% (75/87050), with VAF) 5–16%. Our cases included tracheal adenoid cystic carcinoma, hepatic neuroendocrine tumor, retroperitoneal myofibroblastic sarcoma, ovarian clear cell carcinoma, and poorly differentiated adenocarcinoma of gallbladder, in patients aged 32–54 years (M: F = 1:4). All cases exhibited one or more concomitant pathogenic variants in kirsten rat sarcoma virus (KRAS) (n = 3), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) (n = 2) and catenin Beta 1 (CTNNB1) (n = 1). cBioPortal shows V617F in 19 tumor types, notably in atypical lung carcinoid (33%, 25/75) and in one case of ovarian clear cell carcinoma. Concomitant mutations were seen in 49 cases. No overt signs of superimposed hematologic malignancy were evident in both cohorts.

Conclusion: JAK2 mutation frequency (1.9%) exceeds prior reports, frequently co-occurring with other driver mutations. Given its targetability, integrating JAK2 analysis into future NGS) panels is crucial.

Keywords: Janus kinase 2 V617F, Solid tumors, cBioPortal.

O-3

To identify potential pathways involved in chemotherapy resistance in locally advance hormone receptor-positive breast cancer

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Introduction: Locally advanced hormone receptor-positive breast cancer (LABC) is treated with neoadjuvant chemotherapy (NACT), but the risk of recurrence is significantly higher, if a pathological complete response (pCR) is not achieved. It is essential to identify resistance mechanisms to select and improve treatment therapies with minimal toxicity and maximal efficacy.

Aim: The aim of this study was to explore transcriptomic biomarkers for predicting response to neo-adjuvant chemotherapy in locally advanced hormone receptor-positive breast cancer (BC).

Objectives: The objectives of this study were as follows: (1) Evaluation of residual cancer burden (RCB) in post- NACT specimens of locally advanced hormone receptor-positive breast cancers (BCs). (2) Transcriptome profiling of pre-NACT and post-NACT hormone receptor-positive breast cancers.

Method: Hormone receptor (HR+) BC patients (diagnostic biopsies) having locally advance disease who underwent NACT (4 × Anthracycline followed by 4 × Docetaxel) were enrolled (n = 62). Based on the treatment response, RCB class was assigned. Transcriptomic profiling was done on pre- and post-NACT (n = 10 paired) and four diagnostic biopsies of pCR to identify the resistance mechanism to NACT.

Results: Patients were stratified into four distinct RCB classes according to their response to treatment. Out of 62, 28 (45.1%) show pCR, 3 (4.8%) belong to RCB I, 17 (27.4%) belong to RCB II, and 14 (22.5%) belong to RCBIII. Patients were, further, grouped into good responder (RCB0 and RCBI) and poor responder groups (RCBII and RCBIII). Thirty-one patients (50%) good responder,and 31 patients (50%) poor responder toward NACT. Transcriptomic profiling between pre- and post-NACT specimens of the poor responder group reveals higher expression levels of the PI3K-AKT-mTOR pathway, estrogen response late and epidermal growth factor (EGF)-epidermal growth factor receptor (EGFR) pathway genes, and lower expression of immune response in the majority of post-NACT surgical specimens compared to diagnostic biopsies. Furthermore, the gene expression comparison in diagnostic biopsies of the good and poor responder group reveals downregulation of ABCA8, ABCC2, BRCC3, TMCC3, and upregulation of SMAD6 in the diagnostic biopsies of the poor responders group compared with good responder group.

Conclusion: Our data reveal that 50% of HR+ LABC patients receiving NACT show poor response to treatment. Transcriptomic differences between pre- and post-chemotherapy samples show wide range of distinct, but related mechanisms and genes shortlisted may act as a novel biomarker in treating locally advanced breast cancer (LABC). Our data may reveal potential mechanisms of therapy resistance.

O-4

Frequency of additional cytogenetic abnormalities in chronic myeloid leukemia CP and its impact on treatment response

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Aim and Objective: The aim of this study was to determine the frequency of additional cytogenetic abnormalities (ACAs) in chronic myelod leukemia in chronic phase (CML-CP) and its impact on tyrosine kinase inhibitor (TKI) and major molecular response (MMR).

Method: We screened 482 chronic myelod leukemia (CML) cases over two years with available karyotype data referred to the Department of Cancer Cytogenetics, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC) |, for determining the frequency of ACAs. Clinical data was obtained from the institute's Electronic Medical Records.

Results: Of the 482 CML-CP cases, 30 (6.2%) patients harbored ACAs at the time of diagnosis, including 18 (60%) males and 12 (40%) females. Of the 30 cases, 3 (10%) cases progressed to chronic myeloid leukemia- blastic phase-accelerated phase (CML-BC/AP. We identified major route abnormalities: +8, +19, der22 in 12 (40%) cases, while minor route abnormalities: 3q26, −Y, +Y were present in 6/30 (20%) cases, and other balanced translocations in 12 (40%) cases {derivative chromosome (6, 7, 9, 10, 17) and t(9; 10), t(5;13), t(9;9), t(4;9), t(7;9), t(9;14), t(6;9)}. Of 30 patients, up to 2 years follow-up data was available in 25 (83.33%) cases. These cases were treated with first-line imatinib therapy of which 10 (40%) patients showed optimal response while 15 (60%) showed failed imatinib response and were started with second - and third -line Tyrosine kinase inhibitors (TKI) treatment. Major molecular response (MMR was achieved in 8 (32%) cases. While the molecular response was not achieved in 17 (68%) cases, which included five cases showing major route abnormality (der22, +8, +19), five cases showing minor route abnormality (−Y, +Y, inv3q26), and seven cases had other balanced translocations {t(9;10), t(9;9), t(4;9), t(7;9), t(9;14), der6 t(1;6), t(6;9)}.

Conclusion: Additional cytogenetic abnormalities (ACAs) provide insight into the disease progression in CML. This study examines the impact of ACAs on TKI treatment and molecular response in CML patients, which may have useful implications for clinical management and prognosis

assessment. Larger cohort studies would be of considerable interest.

Keywords: Additional cytogenetic abnormalities, Frequency, Imatinib therapy, Tyrosine kinase inhibitor, Major molecular response.

O-5

Serum proteomic profiling in autoimmune hepatitis: Unraveling molecular signatures for diagnostic and therapeutic insights

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Background: Autoimmune hepatitis (AIH) is a complex inflammatory liver disease characterized by immune-mediated hepatocyte destruction leading to liver damage. Given its varied clinical presentation and diverse immunological mechanisms leading to its delayed diagnosis, advanced molecular tools are needed to enhance our understanding of pathogenic mechanisms of AIH. Serum proteomics has emerged as a potent approach to unravel the intricate molecular landscape associated with AIH-1, offering insights for diagnostic applications and targeted therapeutic development.

Objectives: This study aims to elucidate molecular signatures underlying AIH, focusing on diagnostic applications and insights into disease pathogenesis for developing targeted therapeutics through serum proteomics.

Methods: Five AIH-1 patients fulfilling simplified diagnostic criteria for AIH and four healthy controls (HCs) were included in the study. Serum samples were processed for untargeted label-free quantitative proteomics using Liquid Chromatography-Mass Spectrometry (LC-MS/MS). Data were analyzed using proteome discoverer (v 2.5) against the Universal Protein Resource (UniProt) Human database. Gene ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis utilized the Database for Annotation, Visualization and Integrated Discovery (DAVID) database.

Results: Proteomic profiling identified 118 significantly differentially expressed proteins between patients with autoimmune hepatitis (AIH) and HC. Among them, 64 proteins were upregulated, and 54 were downregulated. Gene ontology analysis revealed involvement in innate and adaptive immunity, complement pathway activation, cell adhesion, fibrinolysis, extracellular matrix modulation, and enzymatic processes. KEGG pathway analysis indicated enrichment in complement and coagulation cascades, extracellular matrix (ECM)-receptor interaction, and metabolic pathways.

Conclusion: The dysregulation of proteins in complement pathways, ECM-receptor interaction, and metabolic pathways presents potential targets for future therapeutics. Proteins involved in fibrinolysis, ECM modulation, and cell adhesion may serve as promising biomarkers. Nevertheless, the diagnostic and therapeutic utility of individual proteins requires validation in subsequent studies. This study provides new avenues for targeted therapeutic interventions and biomarker discovery.

O-6

Comprehensive profiling of molecular cytogenetics, gene mutations, and their impact on overall survival in large cohort of Indian myelodysplastic syndrome

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Introduction: Myelodysplastic syndromes (MDSs) are a heterogenous disease with a risk of developing acute myeloid leukemia (AML).[1,2] The World Health Organization (WHO) 2022 classification[3] and molecular IPSS (IPSS-M) of MDS emphasize on genetic covariates.[4]

Aim: The aim of this study was to describe molecular mutations and their impact on prognosis as per the WHO 2022 classification and IPSS-M.

Objective: The objective of this study was to identify genetic changes including Copy number variation CNVss, loss of heterozygosity(LOH), gene mutations, and their correlation with the overall survival of patients.

Methods: The study included 200 MDS patients. The cytogenetic, mutational profiling, and single-nucleotide polymorphism (SNP) array were carried out in patients and were clinically followed up. Statistical analysis was performed using GraphPad Prism and Statistical Package for the Social Sciences software.

Results: The chromosomal aberrations were identified in 35% of patients. The next-generation sequencing (NGS) identified gene mutations in 70% of cohort. The most frequent (n = 30) gene mutation was Splicing Factor 3B Subunit 1A (SF3B1); however, only few were MDS – RS (n = 6), and it showed independent prognostic value. The survival analysis showed that the mutations in TP53 (n = 15) were more significantly (P < 0.0001) associated with poor survival (5 months) than other gene mutations. Single nucleotide polymorphism (SNP) array (n = 77/200) in combination with NGS confirmed the biallelic loss of function of the TP53 gene (5/15), which is a new genetic-based MDS entity, that is, MDN-biTP53. Eight MDS patients with NPM1 mutations showed rapid progression than other mutations. The re-classification of MDS patients as per WHO 2022 revealed that MDN-LB (37%) was frequent, followed by MDN-SF3B1 (15.9%) and MDN-IB1 (15.9%). Survival analysis showed shorter survival of patients re-classified as MDN-biTP53 (5 months) and MDN–IB (10 months) compared to other re-classified subsets.

Conclusion: Molecular cytogenetics along with gene mutations helps in accurate diagnosis and prognosis of disease. For better management of MDS, patients with SF3B1 and tumor protein p53 (TP53) mutation should be classified as distinct group regardless of marrow morphology and also patients with Nucleophosmin 1(NPM1) mutation should be classified as Acute myeloid leukemia (AML)irrespective of blast percent.

Keywords: World Health Organization 2022 classification, Gene mutations, Molecular cytogenetics, Overall survival.

O-7

Frequency and prognostic impact of PAX5 gene alterations in B-other acute lymphoblastic leukemia

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Aims and Objectives: The aim of this study was to determine the frequency of paired box 5 (PAX5) alterations including gene rearrangements/fusions, internal tandem duplications (ITDs), other PAX5 mutations, and P80R mutation in newly diagnosed B-other-ALL and evaluate its impact on clinical outcome (post-induction minimal residual assessment and overall survival).

Methods: We screened 803 B-Other ALL patients (negative for recurrent abnormalities in B-ALL) from the year 2021 to 2023 and recruited 135 PAX5 altered patients for the study. Fluorescence *in situ* hybridization (FISH) was carried out using LSI dual color break apart PAX5 probe (Empire Genomics, Williamsville, NY) for detecting PAX5 gene rearrangements. Targeted RNA sequencing was carried out to detect PAX5 fusion partners, PAX5 P80R or other mutations, and PAX5-ITD and other known fusions or mutations. Clinical data including post-induction Minimal Residual Disease (MRD) and 2-year overall survival data were correlated with genomic data and analyzed using the Statistical Package for the Social Sciences.

Results: In the study, 38.3% (n = 803) patients were classified as B-other ALL. Of these, 15.3% patients (n = 123) harbored sole PAX5 abnormalities, while PAX5 aberrations were present as secondary changes along with Ph-like aberrations [Janus kinase (JAK2), cytokine receptor-like factor 2 gene (CRLF2), erythropoietin receptor (EPOR) and Platelet-derived growth factor receptor beta (PDGFRB) rearrangements], B-other defined abnormalities (MEF2D), and KMT2A rearrangements in 1.5% (n = 12) cases. The predominant age groups in the study cohort (n = 123) included: <11 years; n = 59 (43.7%), 11–15 years; n = 27 (20%) followed by 16–39 years; n = 40 (29.6%), 40–59 years; n = 8 (5.9%); and >59 years; n = 1 (0.7%). The frequency of PAX5 altered group was 94% including 82.3% PAX5 gene rearrangement/fusions, 9.6% PAX5 sequence mutations, and 2.2% PAX5-ITD, while 5.9% cases harbored PAX5 P80R mutation. PAX5 gene rearrangement/fusions were majorly identified in 67% pediatric patients of age group 0–11 years while P80R was commonly identified in adolescents and adults (10.6%). The most frequent fusion partners of PAX5 identified were AUTS2 (14.5%), ETV6 (14.5%), CBFA2T3 (10.9%), and MBNL1 (7.2%), while novel fusion partners included ANKRD12, GEMIN8, NLRC5, NFIC, and CA10. IKZF1 and/or CDKN2A codeletions were identified in 61.4% PAX5 alt cases. Of 103 cases with available MRD data, 35% were positive for post-induction/consolidation MRD and majorly consist of PAX5 gene rearrangement/fusions (61%), while 65% were negative. Overall survival data of patients on standard treatment care were 78% patients and 22% patients succumbed.

Conclusion: The prospective study provides overall incidence, clinical and genetic characteristics of PAX5 altered subgroup in Indian cohort for the first time with several novel rearrangements. The overall findings provide rationale for clinical testing of therapies with PAX5 alterations to improve treatment outcomes in MRD-positive cases.

Keywords: B-cell precursor acute lymphoblastic leukemia, PAX5-fusions, ELN, AUTS2, Novel, B-ALL subtype, PAX5alt, PAX5 P80R, IKZF1, CDKN2A.

O-8

The spectrum of clonal disorders in patients with hypereosinophilia

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Objectives: Hypereosinophilic syndrome may result from a variety of reactive and clonal disorders, while the cause remains unknown in a subset of patients. In patients with clonal disorders, eosinophils may be clonal, as in myeloid neoplasms, or reactive, as in lymphoid disorders. We aimed to identify the spectrum of clonal disorders in patients with hypereosinophilic (HE), who were referred for bone marrow examination.

Methods: A retrospective analysis of HE cases (absolute eosinophil count [>1.5 × 10^9/L or ≥10% eosinophils in peripheral blood) referred for bone marrow examination over 6 years from 2018 to 2023 was performed. A comprehensive laboratory evaluation was performed in all cases using fluorescence *in situ* hybridization for PDGFRA, PDGFRB, FGFR1, Janus kinase 2 (JAK2), ABL1, and ETV6 (using break-apart probes), BCR: ABL1, RUNX1:RUNX1T1, CBFB: MYH11, JAK2 V617F, CALR mutations (by RT PCR), and flow cytometry for abnormal T-cell clones were performed. Patients with BCR: ABL1 were excluded from further analysis. Next-generation sequencing for the myeloid mutation panel (44 genes) was performed in a few cases.

Results: During the study period, a HE was associated with a clonal disorder in 48 cases. Their age ranged from 1 to 75 years (median 38 years). There were 40 males and eight females. The hemoglobin (g/dL), total leukocyte count (×10^9/L), platelet count (×10^9/L), eosinophil percentage (%), and absolute eosinophil count (×10^9/L) ranged (median) from 3.2 to 17.8 (10.3), 9–1704 (106), 3.5–307 (28.3), 1–92 (25), and 0.95–134.2 (7.8), respectively. Bone marrow eosinophil % ranged from 2 to 83% (median 21%). 25 (52%) had myelofibrosis (1+ in 14; 2+ in 8 and 3+ in 3 cases). Eosinophilia was clonal in 40 cases (83%), and it was reactive to a clonal disorder in the remaining cases. The cause of clonal eosinophilia includes (1) FIP1L1:PDGFRA (n = 16), JAK2+ myeloid neoplasm (n = 6), PDGFRB-r myeloid neoplasm (n = 3), FGFR1-r myeloid neoplasm (n = 2), STAT5bN642H+ chronic eosinophil leukemia (n = 2), CALR mutated myeloid neoplasm (n = 2), ABL1-r myeloid neoplasm (n = 1), CSF3R mutated myeloid neoplasm (n = 1), myelodysplastic syndrome with 5q- (n = 1), mixed phenotypic acute leukemia (n = 1), and acute myeloid neoplasm with eosinophilia (n = 4). Reactive HE was associated with T-lymphoblastic lymphoma/leukemia (n = 2), Hodgkin lymphoma (n = 2), angioimmunoblastic T cell lymphoma (n = 2), B-acute lymphoblastic leukemia (n = 1), diffuse large B-cell lymphoma (n = 1), and the lymphocytic variant of hypereosinophilia (n = 1).

Conclusions: FIP1L1:PDGFRA was the most common cause of clonal eosinophilia in our study, followed by JAK2 mutations and PDGFRB rearrangement. Nearly half of our patients had some degree of myelofibrosis.

O-9

Comparison of IDH mutation, 1p/19q codeletion, epidermal growth factor receptor amplification, MGMT methylation, and TERT promoter mutation in 200 glioma patients (Astrocytoma, Oligodendroglioma, and Glioblastoma) and their prognostic significance

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Introduction: There has been rapid change in understanding of gliomas in adult. Isocitrate Dehydrogenase (IDH) mutation, 1p/19q co-deletion, O6-methylguanine-DNA methyltransferase (MGMT) methylation, epidermal growth factor receptor (EGFR) amplification, and Telomerase reverse transcriptase (TERT) promoter mutation have significant prognostic value with overall survival.

Materials and Methods: We analyzed 200 glioma patients of combined astrocytoma grade 2,3, 4 oligodendroglioma grade 2,3 and glioblastoma grade 4 of all the parameters using immunohistochemistry, fluorescence *in situ* hybridization, and pyrosequencing.

Results: About 98% oligodendroglioma patients had TERT mutation while 94% had MGMT methylation. About 11% astrocytoma patients had TERT mutation and 80% had MGMT methylation. About 65% glioblastoma patients had TERT mutation and 35% had MGMT methylation. Five diffuse midline glioma patients showed neither IDH, 1p/19q, EGFR, MGMT methylation, or TERT mutation. About 40% IDH wild type grade 2 gliomas had shown MGMT methylation but no TERT mutation. About 41% of glioblastoma cases had shown EGFR amplification. Single IDH mutant astrocytoma had shown EGFR amplification. IDH mutation with 1p/19q codeletion and TERT promoter mutation has significant good overall survival compared to IDH mutation without 1p/19q codeletion and TERT promoter methylation. Majority of IDH-mutant oligodendrogliomas were MGMT methylated and showed TERT promoter mutation. IDH wild type glioblastoma with TERT promoter mutation had shown worse prognosis while IDH wild type glioblastoma with MGMT methylation had shown better overall survival than unmethylated glioblastoma.

Conclusion: IDH-mutation combined with 1p/19q codeletion and TERT promoter mutation and MGMT methylation was associated with good overall survival and IDH-wild type with TERT promoter mutation or EGFR amplification was associated with poor prognosis, while IDH wild type glioblastoma with MGMT methylation had shown better overall survival than unmethylated glioblastoma.

Keywords: IDH mutant, IDH-wild type, MGMT, TERT.

O-10

Exploring pyruvate kinase deficiency in Indian patients: Enhanced genetic testing reveals a heterogenous clinical and molecular spectrum

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Aims and Objectives: Pyruvate kinase deficiency (PKD) is the most common disorder of the glycolytic pathway leading to chronic non-spherocytic hemolytic anemia. Clinical spectrum ranges from mild hemolysis to severe transfusion-dependent anemia, with pallor, hepatosplenomegaly, and/or iron overload. Diagnosis involves enzyme assay, but false negatives may arise from increased reticulocytes or recent transfusions. The PK enzyme activity is difficult to standardize and involves removing leukocytes, which hinders the diagnostic assay; therefore, next-generation sequencing (NGS)-based genetic testing is the gold standard for diagnosing PKD.

Methods: We encountered 25 cases from 23 families following detailed laboratory investigations and hemolytic workups. Causes, such as hemoglobinopathies, hereditary spherocytosis, glucose-6-phosphate dehydrogenase deficiency, and autoimmune hemolytic anemia, were excluded from the study. Targeted NGS was performed using custom gene panels and sequenced on a MiSeq (Illumina).

Results: A total of 21 variants were identified, including 16 missense (76.1%), 3 splice sites (14.2%), and two nonsense variants (9.5%). Among these, 4 (19%) variants were novel. The most recurrent variant found in our cohort was c.1456C>T, (p.R486W) (9/25, 36%). Nine patients had homozygous variants (36%), 12 had compound heterozygosity (48%), and in 4 (16%), only one heterozygous variant was identified. Patients with homozygous variants had severe transfusion-dependent phenotypes. Whereas, patients with compound heterozygous variants exhibited mild to severe phenotypes depending on the type of variants inherited. Four out of the 25 patients were initially suspected to have congenital dyserythropoietic anemia based on bone marrow morphology and reticulocyte count, and the remaining were classified as unexplained hemolytic anemias. After correct diagnosis, eight patients underwent splenectomy, achieving transfusion-free status, except one with significantly reduced transfusion requirements.

Conclusion: Molecular diagnosis using NGS proved crucial for unexplained cases of hemolytic anemia. This data shows the marked genetic and clinical heterogeneity and was used for genetic counseling, predictive testing, and disease management.

Keywords: Hemolytic anemia, Pyruvate kinase deficiency, Next-generation sequencing, Molecular diagnosis.

O-11

Genetic spectrum and molecular heterogeneity in patients of von Willebrand disease: A targeted resequencing-based approach

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Aims and Objectives: Von Willebrand disease (VWD) is the most common inherited bleeding disorder. The deficiency of von Willebrand factor (VWF) causes both quantitative (type 1 and 3) and qualitative (type 2 subgroups 2A, 2B, 2M, and 2N) defects. Detailed coagulation screening and molecular analysis are required for subcategorization and confirmatory diagnosis of patients. The aim of this study was to determine the genetic spectrum in VWD cases.

Methods: Forty (thirty-one index cases and nine affected family members) patients with bleeding history, decreased levels of VWF antigen, activity, and/or an abnormal ristocetin-induced platelet aggregation test were enrolled along with a complete history. Thirty-one index cases were subjected to in-house targeted resequencing. The output files were analyzed using Local Run Manager (Illumina). Pathogenicity of variants was predicted using various *in silico* tools. Sanger validation of pathogenic variants was done in the index cases and family members.

Results: Based on clinical and molecular findings, the type 3 subtype was the most common (57%) followed by type 2 (32%). Consanguinity was noted in four families of which three were type 3. Family history was positive in 14 cases. Probably pathogenic variants were found in 28 cases (90%). Total 29 variants were found in VWF gene that include 13 missense (44.8%), 7 nonsense (24.1%), 5 splice site (17.2%), and 4 frameshift (13.8%) variants. Twelve variants (41.4%) were novel. Four variants (13.7%) were recurrent. The genetic analysis helped to confirm the diagnosis in three clinically miscategorized VWD subtypes. The majority of the cases were homozygous (46%). Exon 28 was the hotspot (31%) for variants, and the rest of the variants were distributed throughout the gene.

Conclusion: Targeted next-generation sequencing yielded probably pathogenic variants in 90% of the cases. The obtained results have helped for the accurate categorization of VWD subtypes. Predictive testing and prenatal diagnosis can be provided to the affected families using this data in future.

Keywords: Inherited bleeding disorder, von Willebrand disease, Next-generation sequencing, Genetic spectrum.

O-12

Retinoblastoma transcriptional corepressor 1 in the dynamic cellular plasticity of epidermal growth factor receptor mutant lung adenocarcinomas with small cell histological transformation post-tyrosine kinase inhibitor therapy

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Background: Small cell transformation (SCT) is an uncommon mechanism of tyrosine receptors kinase inhibitor (TKI) resistance in epidermal growth factor receptor (EGFR) mutant lung adenocarcinomas (LUAD). The contributory role of RB1/TP53 gene alterations in this phenomenon is not well documented.

Aims: The aim of this study was to study the clinicopathological features of EGFR-mutant LUADs diagnosed with SCT in post-TKI setting.

Methods: Ambispective study (2019–2023) including all biopsies of patients with SCT subject to Rb1 immunohistochemistry and 72-gene targeted panel next-generation sequencing (NGS) was performed.

Results: Nine patients were identified with 19 samples available: baseline (n = 9) and post-TKI (n = 10) including two post-TKI samples in patient 9. Baseline biopsies showed adenocarcinoma with TKI-sensitive EGFR mutations in all and exhibited loss of Rb1 in six of seven samples tested. Baseline biopsy of patient 9 was Rb1-proficient. Median time to progression on TKI was 10 months. Post-TKI biopsies in patients 1–8 showed small cell transformation (SCT), harbored only founder EGFR mutations (7/8), and showed Rb1 loss (7/7). In patient 9, first post-TKI sample was Rb1-proficient adenocarcinoma with EGFR T790M mutation while the second showed SCT with Rb1 loss and undetectable T790M. Among 11 samples subject to NGS, only EGFR (11/11), TP53 (11/11), RB1 (4/11), and PTEN (2/11) mutations were detected. RB1 mutations showed poor concordance (20%, 2/10) with Rb1 loss.

Conclusion: Baseline Rb1 loss and tumor protein p53 (TP53) mutations were detected at high frequency among EGFR-mutant LUADs undergoing SCT suggesting baseline Rb1 loss as a potential marker for predicting SCT. Specific molecular events that drive phenotypic switch from adenocarcinoma to small cells remain unclear.

Keywords: Small cell lung cancer, Epidermal growth factor receptor, TP53, TKI, Transformation, RB1, Mutation, Immunohistochemistry.

O-13

Genetic landscaping in colorectal cancer cohort using whole exome sequencing to identify novel variants

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Introduction: Colorectal cancer (CRC) is the third most frequent cancer causing a high number of cancer-associated deaths globally every year.

Aims and Objectives: The aim of this study was to explore the genetic landscape in a cohort of CRC patients.

Methods: Next-generation sequencing was performed in formalin-fixed, paraffin-embedded tissue from 50 CRC patients. A total of 46 CRC-related genes were selected for analysis. The raw data for 50 FFPE samples were trimmed for adapter sequences and filtered for quality (Q ≥ 30). The high quality data were aligned onto the human reference genome (hg19) using bwamem software. The variant calling was done in the tumor in comparison to thehg19 genome sequence using the Mutect2 plugin of Genome Analysis Toolkit (GATK).

Results: Total 222 variants were identified in 46 CRC-related genes. Variants were classified as pathogenic (19.37%), likely Pathogenic (32.88%), benign (0.45%), likely Benign (2.25%), variant of unknown significance (VUS) (8.56%), and VUS/weak pathogenic (36.48%). Pathogenic variants were identified in KRAS (12%), TP53 (10%), MSH6 (10%), MLH1 (10%), APC (8%), MSH2 (6%), CDKN2 (4%), ATM (6%), SMAD4 (4%), and NRAS, PTEN, PIKCA, and BRAF (all 2%) genes. Two or more pathogenic variants were found in 11 (22%) samples. We also discovered 32 novel variants in ATM (6), APC (4), BRAF (2), CDH1 (1), CDX2 (1), EGFR (1), HNF4A (1), KRAS (1), MAPK3 (1), MLH1 (1), MSH2 (2), MSH6 (1), PIK3CA (1), PIK3CG (2), AKT1 (2), SMAD4 (3), and ZEB1 (1) genes which were not previously reported in COSMIC, ClinVar, Mastermind Genome and dbSNP databases.

Conclusion: This pilot study showed many novel variants in our cohort of Indian CRC patients. The ATM gene had most of such variants identified. Functional analyses of such genes and variants may prove beneficial in Indian patients for disease understanding and therapy.

Keywords: Colorectal Cancer, Whole exome sequencing, Novel variants, Next-generation sequencing, ATM gene

O-14

Comparison of deutsches krebsforschungszentrum/ methylation classifier with histopathology and molecular profile in correlation with prognostication of pleomorphic xanthoastrocytoma

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Introduction: Deoxyribonucleic acid (DNA) methylation profiling is a powerful objective technique for classifying central nervous system (CNS) tumors with capacity to distinguish >80 CNS tumor groups and subgroups. Capper *et al*. pioneered methylome analysis and showed that distinct tumor types could be described by applying a random forest algorithm with multinomial logistic regression to methylation data derived from tumors with comprehensive prior histologic and molecular characterizations. The platform was developed and prospectively tested at Deutsches Krebsforschungszentrum (DKFZ) (DKFZ; German Cancer Research Centre, Heidelberg, Germany) in a large series of pediatric and adult CNS tumors, culminating in the development of an online tumor classification tool that is available freely. Tumor classifications are derived by uploading raw DNA methylation data from Illumina (San Diego, CA) EPIC methylation arrays to the online platform. DNA methylation array profiling for classifying CNS tumors is a valuable adjunct to histopathology. However, unbiased prospective and interlaboratory validation studies have been lacking. Pleomorphic Xanthoastrocytoma (PXA) is a rare CNS malignant neoplasm. Histopathological analyses of tumor usually show pleomorphism and may show mixture of spindle cells, monocyte like cells, multinucleated giant cells, and often with numerous eosinophilic granular bodies and reticulin deposition. They harbor BRAF p.V600E mutation (or other MAPK pathway gene alteration) and homozygous CDKN2A and/or CDKN2B deletion. While there is significant overlap between various other tumors and PXA, the patient prognosis differs significantly with PXA having a more favorable one. Herein, we discuss three cases, two of which were diagnosed as glioblastoma and one as high-grade glioma, all of them diagnosed as PXA on DNA methylation Profiling. Later, on follow-up, it was found that all the patients died within 1 year, which is not in line with diagnosis of PXA posing significant challenge to diagnosis based on DNA methylation profiling indicating, methylation profiling should be carefully examined in correlation with histopathology.

Aims and Objectives: The aim of this study was to compare DKFZ/methylation classifier with histopathology and molecular profile in correlation with prognostication of PXA.

Methods: Three diagnostically challenging ambispective cases of pediatric high-grade glioma H3 wild type, IDH wild type World Health Organization grade 4, and NOS were selected that DNA was extracted from FFPE tissue with more than 70% tumor content. Whole genome methylation profiling was performed on all the cases and the raw. Idat files were uploaded in the DKFZ classifier to obtain a suitable methylation class.

Results: Three diagnostically challenging ambispective cases of pediatric high grade glioma H3 wild type, IDH wild type, WHO grade-4, NOS with a mean age of 12.6 years and male to female ratio of 1:2 were included in the study. The DKFZ classifier rendered the classification as Pleomorphic Xanthoastrocytoma for all cases. The CNV profile was balanced except for a case showing CDKN2A/2B deletion. The mean overall survival was found to be 6.33 months. Methylation profiling does not give grade of tumor. Despite harboring BRAF, patients died within one year, none of the patients were diagnosed as Pleomorphic Xanthoastrocytoma on histopathology. DKFZ Classifier Should be redefined to give the risk stratification of Tumors with *BRAF: 600* E mutation and *CDKN2A* codeletion. Multidisciplinary approach should be used including: Histopathological evaluation , IHC Molecular profiling, Methylation profiling, Clinical and Radiological details. Pediatric High grade gliomas with BRAFV600 E mutation should be included in next WHO classification because it leads to Proper prognostication and Targeted therapies are available.

Conclusion: Methylation profiling does not give grade of tumor. Despite harboring BRAF, patients died within 1 year, none of the patients were diagnosed as PXA on histopathology.

O-15

Analysis of MET exon 14 skipping mutation in non-small cell lung carcinoma by next generation sequencing: A single-center experience from Eastern India

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Aims and Objectives: The aim of this study was to evaluate MET exon 14 skipping mutations in non-selected non-small cell lung carcinoma (NSCLC) detected by next-generation sequencing (NGS) assay and to correlate it with clinical, histopathological features and clinical outcomes.

Methods: We performed retrospective evaluation of 394 NSCLC cases for whom NGS was performed at our institute over an 18 month period from June 2022 to November 2023. The NGS Assay was performed using a targeted Oncomine Focus Assay® panel from Thermo Fischer Scientific, followed by sequencing in Ion Torrent gene studio S5. Cases with reported MET exon 14 skipping mutations were identified. Electronic medical records of these cases were searched to learn about the patient demographics, histopathological features, and treatment profile.

Results: Out of 394 non-selected NSCLC cases, 12 (3%) were found to have MET exon 14 skipping mutation. The patients were predominantly male (M: F - 5:1), with a mean age of 67 years. The positive cases (n = 12) had a wide variety of histomorphology, including adenocarcinoma (n = 7), squamous cell carcinoma (n = 2), poorly differentiated or sarcomatoid carcinoma (n = 2), and adenosquamous carcinoma (n = 1). Programmed Cell Death Ligand 1 (PDL1) immunostaining was done for nine of these cases, of which 5 cases (56%) showed Tumour Proportion Score (TPS) >50%. NGS-based RNA sequencing detected exon 14 skipping mutation (fusion of exons 13 and 15 in RNA transcript) in all 12 cases (100%); however, NGS-based deoxyribonucleic acid (DNA) sequencing was able to detect the variant in only 5 cases (42%). Five patients received targeted therapy with capmatinib (n = 3) and tapotinib (n = 2), one patient was treated with immunotherapy. Four patients received standard chemotherapy, due to unaffordability of targeted drugs. Two patients succumbed to the disease before initiation of treatment.

Conclusion: RNA-based detection of fusion of exons 13 and 15 of MET gene is a superior approach than DNA-based sequencing to detect MET exon 14 skipping mutations. The frequency of MET exon skipping mutation in our study was 3%, which is similar to published literature. NSCLC with MET exon 14 skipping mutation do not have specific clinical and histological characteristics. Patients with MET exon 14 skipping mutations show higher TPS scores and can be candidate for immunotherapy.

O-16

High-density copy number variation profiling in multiple myeloma with next-generation sequencing-based digital MLPA

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Introduction: Copy number variations (CNVs) found in multiple myeloma (MM) genome encompass several functional genes spread across multiple chromosomes sites and impact disease progression/response to treatment. The recently introduced digital MLPA (dMLPA) assays (MRC Holland) allow a relatively denser mapping of CNVs than the conventional MLPA and offer an opportunity to dissect out fine details across extended regions.

Aim: The aim of this study was to evaluate CNVs across MM genome using dMLPA and their clinical relevance.

Methods: Genomic dMLPA libraries were prepared from enriched plasma cells obtained from 125 newly diagnosed MM (NDMM) patients using D006-X2-0717 MM dMLPAprobemix and sequenced on Illumina MiSeq. Coffalyser software was used for data analysis and interpretations as per manufacturer recommendations. Kaplan–Meier survival analysis and Cox proportion hazard regression were performed using Sigmaplot.

Results: Nearly 60% NDMM patients had at least ≥1 CNVs (average 9 CNVs per patient). The patients had a median PFS and OS of 50 and 63 weeks, respectively. The most frequent deletions (up to 10%) were found among 13p, 13q, 1p, 22q, 16q, and 22q followed by 14q, 8p, 4p, and 17p. Biallelic deletions were observed at genes located in 11q and 1p. Likewise, most frequent gains (up to 20%) were observed in 15q, 1q, 11q, 9q, 5q, 3p, 11p, and 7p. BRAF V600E mutation was also identified and validated by NGS in 12 patients. Survival analysis revealed a significant correlation of progression-free survival with deletions at CDKN1B, 17p, NF2. A significant correlation was observed between Del 1p with overall survival. Extended signatures were deduced across multiple genes in 1p, 1q, 6p, 8p, 11p, and 13q.

Conclusion: Digital MLPA allows dense mapping of CNVs across multiple loci which could be useful for molecular classification and risk stratification in MM.

Keywords: Multiple myeloma, Copy number variation, Digital MLPA.

O-17

Tumor-informed circulating cell-free nucleic acid-based biomarkers for risk assessment in acute myeloid leukemia

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Aims and Objectives: Acute myeloid leukemia (AML) is an aggressive hematological malignancy with poor prognosis and relapse. NGS has revealed molecular complexity, improved risk stratification, and personalized therapies in majority of AML patients. With reduced long-term survival, MRD assessment is crucial in predicting prognosis. The aim, here, was to evaluate the diagnostic utility of circulating tumor deoxyribonucleic acid (ctDNA) as compared to BM in detecting genetic alterations in AML. Based on tumor-informed approach, ctDNA was evaluated for longitudinal monitoring as a minimally invasive approach. RNAseq was performed to decipher the immunological phenotypes and an integrated approach of ctDNA with gene expression was explored for risk assessment in AML.

Methods: At the time of initial diagnosis, bone marrow (BM) and peripheral blood (PB) were collected from AML patients. Mutations were detected in ctDNA (PB) and gDNA (BM-MNCs) using targeted NGS panel. Targetable mutations were validated in both ctDNA and gDNA by ddPCR. RNAseq was performed to identify the immune signatures and validated in AML cohort.

Results: Similar mutation profiles were obtained using gDNA and ctDNA with a high concordance of variant allele frequency. Few exceptions included BCOR and KRAS mutations exclusively in cfDNA, whereas tet methylcytosine dioxygenase 2 (TET2), phosphatase and tensin homolog (PTEN), and PMS1 homolog 2, mismatch repair system component (PMS2) were unique in genomic DNA (gDNA). Samples harboring targetable mutations tested positive by circulating tumor DNA (ctDNA) droplet digital polymerase chain reaction (ddPCR) at baseline and follow-up. Differential gene expression (DGE) analysis revealed significant alteration of gene expression wherein validation of the top upregulated genes (GPB4, IRAK3, ITPR1/2, JAG1, OAS3, and VAV3) revealed a relatively higher expression at relapse than baseline.

Conclusion: We demonstrated ctDNA as a reliable sample in AML for mutation detection, disease monitoring, and selection of targeted therapy that could complement BM. Immunological phenotypes at diagnosis might, further, stratify AML patients. Integrated analysis of cell-free nucleic acid-based biomarkers hold immense potential in revealing the diverse biological profiles of AML patients that might facilitate timely intervention and better clinical outcomes.

Keywords: Liquid biopsy, Cell-free nucleic acids, Circulating tumor deoxyribonucleic acid, Coding RNA, Messenger RNA.

O-18

Decoding infantile cholestatic disorders: A comprehensive molecular profiling Investigation

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Introduction: Infantile cholestatic disorders are a leading cause of chronic liver disease in the pediatric age group and comprise numerous entities associated with many mutations. Most of the diseases are progressive with liver transplantation being the only option for cure. Due to the lack of hot spots of mutations, sequencing the entire coding region of suspected genes is the best method to know the definite/molecular diagnosis.

Aim: The aim of this study was to study the histomorphological features and decipher the genetic defect by our customized Targeted 37 gene panel next-generation sequencing (NGS) and Pedigree analysis by Sanger sequencing in family members.

Materials and Methods: A total of 15 cases of clinically characterized infantile cholestasis were enrolled in our study after excluding cases of Biliary Atresia. An informed written consent was taken from their parents/guardians. The histomorphology of liver biopsies was reviewed by two

pathologists. A targeted gene panel for NGS of 37 genes was performed. Pedigree analysis was done in 3 cases using Sanger sequencing.

Results: Out of our 15 cases, a molecular confirmation was made in 12 cases. A total of 17 mutations [Pathogenic/Likely pathogenic by American College of Medical Genetics and Genomics (ACMG) classification] were detected out of which 13 mutations were novel {one in JAG1 gene [NM_000214.3:c.2922del NP_000205.1:p.(Thr975LeufsTer9)], three in CFTR gene [NM_000492.3:c.1367T>C NP_000483.3:p.(Val456Ala); NM_000492.3:c.4241del NP_000483.3:p.(Leu1414TrpfsTer3) and NM_000492.3:c.473G>A NP_000483.3:p.(Leu1414TrpfsTer3)], seven in ABCB11 gene [NM_003742.2:c.3728A>G NP_003733.2:p.(Asp1243Gly); NM_003742.2:c.916A>T NP_003733.2:p.(Lys306Ter); NM_003742.2:c.643G>A NP_003733.2:p.(Asp215Asn); NM_003742.2:c.2824G>A NP_003733.2:p.(Glu942Lys); NM_003742.2:c.2824G>A NP_003733.2:p.(Glu942Lys); NM_003742.2:c.3382C>G NP_003733.2:p.(Arg1128Gly); and NM_003742.2:c.1711A>G NP_003733.2:p.(Arg571Gly)], one in ATP8B1 [NM_005603.5:c.2135A>G NP_005594.1:p.(Asp712Gly)], and one in ATP8B1 [NM_018849.2:c.1031C>G NP_061337.1:p.(Ala344Gly)]}. Highest number of mutations was detected in ATP binding cassette subfamily B member 11 (ABCB11) gene.

Conclusion: Infantile cholestasis is a monogenic disease associated with many genes. As there can be mutations anywhere in the entire coding region of the genes, a panel-based NGS is a helpful tool in the definite diagnosis of infantile cholestasis after exclusion of Biliary Atresia.

Keywords: Infantile cholestasis, Targeted next-generation sequencing, Pedigree analysis.

O-19

Morphological and molecular analysis of nodal t-follicular helper cell lymphoma – Angioimmunoblastic type

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Objectives: Nodal T-follicular helper cell lymphoma, Angioimmunoblastic-type (nTFHL-AI) is a rare and aggressive subtype of peripheral T-cell lymphoma originating from follicular T-helper cells. Histologically, Angioimmunoblastic T-cell lymphoma (AITL) is identified by the presence of expanded high endothelial venules, follicular dendritic cells, immunoblasts, and a polymorphic infiltrate. Molecular studies have implicated various mutations associated with the pathogenesis and progression of AITL. Some key mutations found in AITL include the genes encoding tet methylcytosine dioxygenase 2 (TET2), DNA methyltransferase 3 alpha (DNMT3A), Isocitrate Dehydrogenase 2 (IDH2), Ras homolog gene family, member A (RHOA), and Cluster of Differentiation 28 (CD28). These mutations result in the dysregulation of various cellular processes, such as deoxyribonucleic acid methylation, histone modification, and immune signaling, ultimately leading to the dysfunction of regulatory T-cells. The present study aims to evaluate the morphological spectrum and frequency of somatic hotspot mutations in DNMT3A, IDH2, RHOA, and CD28 genes in our cohort of nTFHL-AI.

Methods: A retrospective analysis of nTFHL-AI cases was performed over the past 9 years, from 2015 to 2023. The slides were retrieved from the archives, and TFH-associated immunohistochemical markers were done to confirm the diagnosis. A set of primers was designed for CD28, DNMT3A, and IDH2, and Sanger sequencing will be carried out for hotspot mutations. RHOAG17V hotspot mutation will be detected using allele-specific real-time polymerase chain reaction method.

Results: A total of 54 cases of nTFHL-AI were retrieved. The median age of presentation was 55 years (range: 26–80 years) with a male-to-female ratio of 3.9:1 (M, 43; F, 11). The cervical lymph node was the common site (50%). Clinically, three (5.45%) relapsed cases were found. Besides characteristic morphological findings, clear cell morphology (16.66%), large CD20-positive B-cells (29.62%), epithelioid cell granulomas (7.4%), and perinodal extension (24.07%) were detected in the background. The findings of TFH markers include PD1 (90.9%), CXCL13 (25.45%), BCL6 (20%), and CD10 (20%). Among the cases showing CD20-positive large B-cells, six cases were positive for EBER-ISH. The hotspot mutation analysis method for the genes IDHR172, RHOAG17V, CD28T195, and DNMT3AR882 are in process, and the results are awaited.

Conclusions: nTFH-Angioimmunoblastic type is a subtype of peripheral T-cell lymphoma that originates from follicular T-helper cells and harbors a variety of mutations, including IDH2, RHOA, DNMT3A, and CD28.

Keywords: nTFHL, Angioimmunoblastic-type, DNMT3A, RHOA, CD28, IDH2, Mutational landscape.

O-20

DQA1*02:01 association with anti-mi2 dermatomyositis: Unveiling the potential risk of phenylalanine at position-22 in DQA1 protein chain

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Introduction: Autoantibodies recognizing anti-nucleosome remodeling deacetylase complex (Mi2) were the first dermatomyositis (DM)-specific serologic marker. These autoantibodies have been associated with the "classic" form of DM exhibiting unique clinical and myopathologic features. The previous studies also demonstrate the unique association of this autoantibody with Human Leukocyte Antigen (HLA) alleles specific to particular ethnic groups.

Aim and Objectives: In this study, we aimed to genotype Class-II Major Histocompatibility Complex (MHC) alleles and to examine specific HLA genetic variants associated with anti-Mi2 positive DMs patients.

Methods: HLA-DRB1, HLA-DQA1, and HLA-DQB1 alleles were directly genotyped in Indian cohort of 25 Mi2 positive DMs patients and 96 ethnically matched controls by next-generation sequencing, sequence-specific primer PCR assay, or multiplex assay using the genomic deoxyribonucleic acid. Allele frequency analysis and amino acid alignments were performed using the Genentech/MiDAS bioinformatics package. Allele frequencies were compared using Fisher's exact test. All analysis was carried out in RStudio version 1.4.1717.

Results: Two alleles DQA1*02:01 (Pa = 1.092 × 10–2, odds ratio [OR] = 4.846 [2.199–11.592]) and DQB1*03:03 (Pa = 3.767 × 10–2, OR = 7.151 [2.349–22.808]) were more frequently detected in the DM patients with Mi2 autoantibody than healthy controls. However, after the stepwise conditioning analysis only HLA-DQA1*02:01 retained its significant positive association with Mi2 autoantibody and, further, attributed the risk

to a phenylalanine residue in position 25 (Pa = 9.484 × 10–5, OR = 4.933 [2.391–11.274]) within the DQA1 protein.

Conclusion: High-resolution HLA sequencing more precisely characterized the allele associated with Mi2 autoantibody. Identification of the critical amino acid residue by advanced biostatistical analysis of immunogenetics data offers mechanistic insights and future directions into deciphering the pathogenic role of adaptive immunity in Mi2-positive DM subgroup.

O-21

Exploring the potential role of liquid biopsy in identifying IDH1 R132H mutation in gliomas

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Background: The World Health Organization (WHO) central nervous system (CNS) 5 classification introduced key molecular markers profoundly impacting glioma diagnosis and prognosis. IDH mutations, occurring early in glioma development, correlate with better survival rates. However, definitive diagnosis requires challenging tumor tissue retrieval through biopsy or surgery, often necessitating risky procedures due to tumor location. Small, non-representative samples often mandate multiple surgeries, causing delays and repeat interventions. To improve diffuse glioma diagnosis and management, a less invasive method is crucial, reducing reliance on complex surgeries. To improve diffuse glioma diagnosis and management, a less invasive method is crucial, reducing reliance on complex surgeries and to assess the clinical utility of plasma cell-free deoxyribonucleic acid (cfDNA) in diffuse gliomas for the detection of Isocitrate dehydrogenase 1 (IDH1) R132H mutation.

Methodology: Seventy prospective cases exhibiting the IDH1 R132H mutation, including CNS WHO Grade 2, 3, and 4, along with 30 controls, were selected. Each case was paired with both FFPE and cfDNA samples. Sanger sequencing and Digital Droplet polymerase chain reaction (DD-PCR) were employed to analyze these cases for the identification of IDH1 R132H mutations.

Results: The study encompassed 70 cases exhibiting the IDH1 R132H mutation across CNS WHO Grade 2, 3, and 4, alongside 30 controls (IDH1 Wildtype) comprising 74 males and 26 females, with a M: F ratio of 2.8:1. The mean age of the patients was 34.05 years. Detection of the IDH R132H mutation in FFPE samples revealed 100% sensitivity and specificity. However, in cfDNA, sensitivity reached 44.29% while specificity remained at 100%. Notably, no substantial correlation was observed between the concentration of cfDNA, mutant allele frequency, and tumor grade or type.

Conclusion: The DD-PCR method's remarkable sensitivity and precision with CNS tumor-derived cfDNA suggest its potential for routine clinical use. This could reduce reliance on complex surgeries, improving diffuse glioma management.

Keyword: Digital droplet PCR, IDH1 R132H PCR, Cell free DNA, Liquid biopsy, Gliomas.

O-22

Deciphering the immunological landscape in supratentorial ependymomas: Prognostic indications and therapeutic considerations

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Introduction: Ependymomas (EPNs) pose clinical challenges due to recurrence and limited chemotherapy efficacy. This study addresses gaps in understanding tumorigenic mechanisms, focusing on the elusive tumor immune microenvironment in supratentorial ZFTA fusion-positive (ST-ZFTA) EPNs, linked to unfavorable prognosis.

Aims and Objectives: Immune checkpoint blockade through the PD-L1/PD-1 axis is a promising cancer treatment. The "ST-ZFTA fusion-positive" EPN subgroup signals poor prognosis. Elevated PD-L1 in tumor cells may evade the immune response, influenced by cancer stem cells. This study aims to explore their association, particularly in ST-EPN patients.

Materials and Methods: A cohort of 130 ST Grade 1/2/3 EPNs, classified by the World Health Organization 2021 criteria, underwent classification based on L1CAM, P65, RELA/YAP1 fusion, and PD-L1 and NFKb mRNA expression profiling. Total RNA sequencing analyzed gene expression patterns in 21 ST-EPNs and 6 controls. Immunohistochemistry (n = 130), chromatin immunoprecipitation-quantitative polymerase chain reaction (PCR) (n = 3), and additional RNA sequencing explored the immunological and molecular landscape, emphasizing the identification of tumor-infiltrating cells (TILs; CD3, CD8, FOXP3) and tumor-associated macrophages (TAMs; CD44, CD68, CD 163) and associated functional phenotypes.

Results: Approximately 20% of ST-EPNs showed ≥1% PD-L1 protein expression, mainly in the ST-ZFTA subgroup, correlating with PDL1 and NFkB mRNA expression and increased cytotoxic T-lymphocyte density. Transcriptomic analysis revealed upregulated CD44, CCL2, immune cell subpopulations, cell adhesion molecules, and MMPs, indicating CD44's role in P13K/Akt activation in tumorigenesis. Positive correlation between CD44 and PD-L1 expression at both mRNA and protein levels establishes CD44 as a crucial therapeutic target. STRING analysis (confidence score 0.9) confirmed CD44's involvement in the ERBB2-EGFR pathway. PrognoScan showed a correlation between CD44 gene expression and various cancers.

Conclusion: This study unveils immune microenvironment dynamics in ependymomas, emphasizing PD-L1 in the ST-ZFTA subgroup and highlighting CD44 as an adverse prognostic factor correlated with PD-L1 expression and immune cell infiltration, emphasizing its therapeutic potential.

Keyword: Ependymoma, ZFTA-fusion, Tumor immune microenvironment, Transcriptomics, Cancer stem cells.

O-23

Molecular profile of triple-negative myeloproliferative neoplasm

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Introduction: With a common biology, myeloproliferative neoplasms (MPNs) are a rare and diverse group of hematological disorders. These can be broadly classified into BCR: ABL1 positive CML and BCR: ABL1 negative MPNs, which classically include polycythemia vera, essential thrombocythemia (ET), and primary myelofibrosis (PMF). Genetically, these diseases are characterized by the presence of somatic driver mutations in CALR, MPL, or JAK2 genes. About 10–15% PMF and ET do not show presence of mutations in any of these genes and are labeled as "triple negative" cases.

Aims and Objectives: We aimed to identify the prevalence and types of somatic mutations in these triple-negative myeloproliferative neoplasms (TNMPNs).

Methods: A total of 35 samples comprising 29 TNMPNs and six driver gene mutation positive MPN patients' samples were included in the study. Whole exome sequencing with analysis targeting 68 genes commonly mutated in myeloid neoplasm, and targeted sequencing of DNA using Oncomine myeloid research assay was used.

Results: Twenty-seven of 29 (93.1%) TN MPNs showed mutation in at least one gene with an average of 3.1 mutated genes per case. Five most frequently mutated genes were ASXL1 (22.2%), SH2B3 (11.1%), TET2 (11.1%), SF3B1 (7.4%), and SETD2 (7.4%). Non-canonical mutations in JAK2, CALR, and MPL genes were also noted in 11.7%, 7.4%, and 11.7% cases, respectively. Five of the 6 (83.6%) driver mutation-positive cases also showed presence of mutation in at least one gene with an average of 1.4 mutated genes per case. Commonly mutated genes were EZH2 (33.3%), TET2 (33.3%), and ASXL1 (16.6%).

Conclusion: Providing evidence of clonality, somatic mutations were noted frequently in TNMPNs. The average number of mutated genes per case was significantly higher than those noted with the driver gene mutated cases. This study also provides a spectrum of somatic genes mutated in TNMPNs.

Keywords: Myeloproliferative neoplasms, Somatic mutations, Triple-negative myeloproliferative neoplasms.

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Discovery of a novel antiviral therapeutic with broad-spectrum antiviral activity

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Aims and Objectives: Hantaviruses, belonging to the Hantaviridae family, are negative-stranded RNA viruses and category A pathogens. Transmitted to humans through aerosolized excreta of infected rodents, they cause severe illnesses such as hemorrhagic fever with renal syndrome and hantavirus cardiopulmonary syndrome with mortality rates of 15% and 50%, respectively. Annually, 150,000–200,000 cases of hantavirus infections are reported globally, and there is currently no treatment available. Rift valley fever virus (Phenuiviridae family) exhibits a mortality rate of up to 60% in certain outbreaks, while the Heartland virus, also from the Phenuiviridae family, has a 30% mortality rate. Crimean Congo hemorrhagic fever virus (CCHFV) from the Nairoviridae family carries a mortality rate of 40%. La Crosse virus, a member of the Peribunyaviridae family, particularly within the California serogroup, induces serious encephalitis, altering the mental status in most infected patients. Several other Bunyaviruses cause severe human illnesses with poor prognoses due to the absence of vaccines and antiviral therapeutics. Consequently, there is an urgent need to develop a broad-spectrum antiviral therapeutic that selectively targets Bunyaviruses, aiming to enhance the prognosis of these deadly diseases.

Methods: Using multifaceted biochemical, biophysical, cell biology, and molecular biology approaches, we identified the interaction between hantavirus nucleocapsid protein and viral mRNA 5' UTR as a novel target for therapeutic intervention of hantaviruses. Using a high throughput screening approach, we identified a lead inhibitor that binds to the nucleocapsid protein, disrupts the N protein-UTR interaction, and inhibits hantavirus replication in cells. Our research program uses a combination of approaches including medicinal chemistry, X-ray crystallography, biochemistry, *in vivo* reporter assays and anti-viral testing to synthesize the derivatives of the lead inhibitor, having high target binding affinity and improved antiviral efficacy.

Results: The identified lead inhibitor shows broad spectrum antiviral activity against multiple bunyaviruses including Hantaviruses, Rift valley fever virus, Heartland virus, LaCrosse virus, and CCHFV. Interestingly, the inhibitor shows antiviral activity against coronaviruses including SARS-CoV-2, Feline coronavirus, and infectious bronchitis virus. Our laboratory is currently focused to use medicinal chemistry approaches for further development of the identified lead inhibitor as a broad-spectrum antiviral targeting RNA viruses.

O-25

Assessment of KIAA1549-BRAF fusions and correlation with clinicopathological features in pilocytic astrocytoma

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Background: Pilocytic astrocytomas (PAs) are the most frequent pediatric low-grade gliomas, accounting for ≈ 20% of all childhood primary brain tumors. They mostly occur in the cerebellum, optic pathway, hypothalamus, brainstem, and few in cerebral hemispheres. The overall prognosis of PAs is good, yet, a substantiable number of cases are deep seated, not amenable to resection and undergo recurrence. The KIAA1549-BRAF fusion is a useful putative diagnostic and therapeutic marker in PAs.

Aims and Objectives: The objectives of this study were to examine the frequency of KIAA1549-BRAF fusions (16–9, 15–9, and 16–11) in different locations of PAs and to assess the KIAA1549-BRAF fusion status on quantitative reverse transcription polymerase chain reaction (qRT-PCR) and fluorescence *in situ* hybridization (FISH) platform.

Methodology: Three different exon rearrangements of KIAA1549-BRAF (16–9, 15–9, and 16–11) fusions were evaluated in 200 cases.

Results: Out of 200 PAs, FISH detected KIAA1549-BRAF fusion in 148/200 (74%) and RT-PCR detected in 126/200 (63%). Of the 126 fusion positive cases, 49% cases were 16–9 fusion, 39% were 15–9 fusion, and 8% had both 15–9 and 16–11 fusion and 4% had both 16–9 and 16–11 fusion. One hundred and twenty-six cases showed positive results on both platforms, while 22 cases were fusion positive on FISH but remained

undetected on qRT-PCR. The overall concordance was 86% between the platforms, using Cohen's kappa (0.69, $P < 0.05$). Correlation of KIAA1549-BRAF fusion with histological and clinicopathological features does not hold statistical significance with overall patient outcomes.

Conclusion: 15–9 and 16–9 KIAA1549-BRAF fusion were the most frequently occurring fusion. RT-PCR is a cost effective and efficient technique but FISH can also be a reliable alternative where RNA quality is compromised.