fields), as well as fibrous tissue that appeared as ropy collagen. Some of the blood vessels were rimmed by a hyalinized cuff. A mild inflammatory component, namely scattered lymphocytes and fewer plasma cells were noted. Immunohistochemistry showed: SMA(faint+), S100(+), CD34(+), CD31(+), FLI1(+), NTRK(+). Negative for ALK1, desmin, SOX10, EMA, keratin AE1/3, CAM5.2, D2-40, myogenin, MUC4, TLE1, STAT6, BCOR, ERG. Both INI1 and H3K27me3 were retained. Proliferative rate by Ki-67 was low, showing <2% positivity.

Next generation sequencing revealed the following: LMNA-NTRK1 fusion; CD36 N53fs*24 and CDKN2A/B CDKN2A loss exon 1. Thus, the histologic, immunophenotypic, and molecular findings together supported a diagnosis of NTRK-rearranged spindle cell tumor. This entity has alternately been termed lipofibromatosis-like tumor. Following confirmation of NTRK fusion, she was treated with oral TRK inhibitor with near total response. With this NTRK-rearranged spindle cell tumor's minimal mitotic activity, absence of necrosis, and low cellularity, the behavior of this tumor was expected to be indolent rather than aggressive. However, the patient was presented for assessment and management at a recent tumor board about 8 months after her initial diagnosis as she had residual/recurrent tumor.

Results (if a Case Study enter NA): NA

Conclusion: Our case highlights the clinical utility of screening for NTRK fusions in all pediatric tumors.

Identifiable Mutations in Pancreatic Adenocarcinoma in the Veteran Population: Molecular Testing Guidelines by NCCN 2020

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Introduction/Objective: In 2019 and 2020, the National Comprehensive Cancer Network (NCCN) advanced a recommendation that all patients with metastatic, recurrent, or locally advanced pancreatic adenocarcinoma should undergo tumor gene profiling (TGP). Prior to these recommendations, TGP in targeted patients have demonstrated a high frequency of KRAS (>90%), TP53 (60-70%), CDKN2A (>50%), SMAD4, TGF- βR1, and TGF- βR2 mutations or alterations. Even less frequent mutations such as the homologous recombination repair (HRR) genes impact treatment by predicting tumor response to platinum-based therapies. However, the literature is sparse for the frequency of these mutations in patients with pancreatic adenocarcinoma undergoing generalized testing as part of the standard of care per NCCN guidance, particularly for veterans.

Methods/Case Report: For a quality assurance study, a retrospective review was performed to identify patients with pancreatic adenocarcinoma at a tertiary medical center serving veterans from January 2019 to February 2021 with TGP performed as part of their care. All of the TGP had been sent to Foundation Medicine (Cambridge MA), and the identifiable tumor mutations from the test reports were recorded to document the frequency of KRAS, TP53, CDKN2A, SMAD4, TGF- βR1, TGF- βR2 and HRR mutations or alterations.

Results (if a Case Study enter NA): There were a total of 11 patients with pancreatic adenocarcinoma who had a tumor specimen for TGP during the study period. All 11 patient tumors had KRAS mutation. 10 out of 11 had a mutation or alteration in TP53. 8 of 11 patients had a CDKN2A mutation or alteration. 7 of 11 patients had a mutation or alteration of SMAD4 though none had TGF- βR1 or TGF- βR2. 2 of 11 patients had HRR mutations (1 with FANCA and 1 with ATM).

Conclusion: Tumor mutations on generalized gene profiling per NCCN guidelines continue to identify important mutations in pancreatic adenocarcinoma for veteran patients.

Assessment of Comprehensive Mutational Profiling in T-lymphoblastic leukemia/lymphoma (T-ALL/LBL): A Single Center Experience

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Introduction/Objective: T-lymphoblastic leukemia/lymphoma (T-ALL/LBL) is a malignancy arising from immature precursor T cells with T-ALL involving bone marrow/blood and T-LBL occurring in the thymus and nodal/extranodal sites. Studies have now revealed >100 recurrently altered genes that are not necessarily disease initiating but can provide diagnostic, prognostic, and predictive information which can then be utilized in personalized therapy.

Methods/Case Report: Next-generation sequencing was performed on DNA and/or RNA extracted from blood/marrow aspirates or tissue at an external CLIA-certified, CAP-accredited laboratory. The hematology panel sequenced DNA of 406 genes, introns of 31 gene rearrangements, and RNA of 265 genes.

This retrospective single-center study highlights salient findings noted in genomic profiles of 15 T-ALL/LBL cases out of 83 total patients with ALL from 2018-2021. While the majority were B-ALL cases, T-ALL accounted for 18%, and all but 1 case were pediatric patients (ages 9-21 years).

Results (if a Case Study enter NA): In our pediatric cohort (14 patients; 9 males, 5 females), as in literature, NOTCH

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signaling was most frequently involved with NOTCH1 (50%) and FBXW7 (36%) mutations, followed by those in cell cycle process CDKN2A/2B (36%) and PTEN (28%) mutations. Other mutations: PHF6 (21%), BCOR and TAL1 (14%) each. The prognostic effect of mutations: NOTCH1 favorable, FBXW7 no effect but trend toward favorable when FBXW7 co-occurs with NOTCH1 while PTEN is unfavorable (3 patients had relapses). Some unusual or useful findings: a patient diagnosed initially as AML with aberrant CD3 was re-classified as early T-cell precursor ALL, supported by RELN mutation (occurs in 4% ETP-ALL). The adult with NOTCH1 and BCOR mutations in addition to BCR-ABL1 fusion was diagnosed as having T-ALL blasts with CML. We could not study detailed nuances in mutational profiles of T-ALL vs T-LBL with only 1 case of T-LBL showing FBXW7, PTEN, NF1, RB1, BCOR and NRAS mutations (latter is typically noted in pediatric T-LBL cases).

Conclusion: Clinical molecular testing in our pediatric T-ALL patients revealed gene alterations that provide refinement of diagnosis, prognosis, and risk stratification. It also contributes a useful data set for further analysis and potential use of clinically actionable therapeutic targets in some cases. Longer term follow-up incorporating therapy and outcomes information would be valuable.

The optimization of an ultra-sensitive single molecule assay for the multiplexed detection of SARS-CoV-2 antigens

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Introduction/Objective: SARS-CoV-2 antigens, including the nucleocapsid (N) protein, spike protein, and its S1 subunit have served as key biomarkers for research and diagnostic purposes. We previously developed quantitative single molecule array (Simoa) assays to measure the concentration of spike, S1 subunit and N protein in plasma samples with femtomolar limits of detection. We aimed to test antibodies that were not available early in the pandemic, reduce assay cross-reactivity, develop a multiplexed assay for spike, S1, and N protein in order to minimize the sample volume needed.

Methods/Case Report: Using the Simoa platform, a bead-based digital enzyme-linked immunosorbent assay, we cross-tested 17 S1 subunit and spike antibodies for a total of 130 antibody-pair combinations, we performed dilution linearity experiments to determine the ideal dilution factor, spike and recovery experiments, tested the assay using S1 subunit from other human coronavirus HKV1, NL63, and 229E, pre-pandemic plasma samples from patients that were sick with viral or bacterial respiratory infections. We then used the best antibody pairs to measure S1 and spike in plasma samples collected from patients with severe SARS-CoV-2. Lastly, we conjugated the best-performing capture antibodies for spike, S1 and N to beads labeled with different fluorophores to test if the assay for all three antigens could be multiplexed.

Results (if a Case Study enter NA): We observed no cross-reactivity with S1 from other coronavirus strains, no detection of S1 or spike in a cohort of 30 pre-pandemic samples and successfully developed a multiplexed assay for the detection of spike, S1, and N protein, enabling us to use 50% less sample volume.

Conclusion: Reduction of necessary sample volume is important for studies involving multisystem inflammatory syndrome in children (MIS-C), and possible adverse effects of SARS-CoV-2 vaccinations on children and young adults. An improved assay with minimal cross-reactivity will also be useful to study individuals with post-acute sequelae of SARS-CoV-2 infection (PASC).

Bioinformatics and wet laboratory analysis of rs1800562 to predict genetic aetiology of iron overload in Nigeria.

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Introduction/Objective: The most reported single nucleotide polymorphism (SNP) of the HFE gene is rs1800562, representing the substitution of Adenine for Guanine at position 847 of the HFE gene. This has been widely implicated in hereditary haemochromatosis and other conditions like altered cholesterol balance, Alzheimer's disease and cutaneous photosensitivity. Abnormal HFE protein resulting from the mutant HFE gene leads to formation of excess iron which has been postulated as likely mechanism for these diseases. Although there is evidence of iron overload in Africans, only few studies have explored possible genetic causes, and prevalence of rs1800562 is not known in West African population. Hence the need to determine the prevalence of rs1800562 in Nigeria using computational and wet laboratory approach.

Methods/Case Report: Details of rs1800562 were retrieved from Ensembl Genome Browser version 99. Severity of the consequences of this SNP on protein product was determined using bioinformatics tools including SIFT, Polyphen, Mutation Assessor, HOPE, I-mutant and MutPred2. Genotyping of rs1800562 was done In silico using restriction fragment length polymorphism (RFLP). Primer3plus was used for primer design, NCBI BLAST and SMS were used for primer validation. We used Webcutter 2.0 to determine suitable restriction enzymes. The genotyping was simulated using USCS virtual PCR and RestrictionMapper. Whole blood samples were obtained from 200 participants selected randomly

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