H38. Clinical Significance of Acquired Copy-Neutral Loss of Heterozygosity in Acute Myeloid Leukemia
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Introduction: Chromosomal abnormalities are important in the diagnosis and prognosis of patients with acute myeloid leukemia (AML). Identification of genomic imbalances has become more sensitive and precise with microarray techniques. In addition to copy number aberrations, copy neutral loss of heterozygosity (cnLOH) can also be detected; however, the prognostic significance is unknown. In this study, acquired cnLOH is investigated in AML patients and correlated with FLT3-ITD and NPM1 mutations, cytogenetic risk classification, and treatment response, including complete remission (CR) rate and CR duration, as well as overall survival (OS).

Methods: To identify cnLOH, we performed chromosome genomic array testing (CGAT) on DNA from bone marrow or blood specimens of AML patients using Affymetrix CytoScanHD and analyzed the data with ChAS and Nexus software. G-banding and FISH were performed on the concurrent sample whenever possible. Of 119 AML patients analyzed, 33 demonstrated cnLOH with a filter size of 10 Mb. Only recurrent cnLOH events were evaluated.

Results: The most common cnLOH is 13q seen in seven patients (21%), followed by 1p and 11p in four patients (12%) each and 21q in three patients (9%). Seventeen of the 33 patients with cnLOH (52%) achieved CR; with an average CR duration > 5.4 months. The prognosis, based on CR rate, ranked from worst to best is: 17p and 19q (0% CR) < 13q (27% CR) < 2q, 7q, 11p, and 11q (50% CR) < 1p, 2p, and 21q (100% CR). Of the latter, CR duration was longest in patients with 21q cnLOH (>7.7 months), followed by 1p (>7.2 months), and 2p (1.75 months). With an average follow-up time of 39.5 months, the average OS time for the 33 cnLOH patients is 18.7 months ranging from >3.5 months for 11q to >50 months for 2q and 21q. Of patients classified as poor or intermediate cytogenetic risk, 100% with 21q cnLOH achieved CR and 25% to 50% with 1p, 2p, 2q, and 7q cnLOH achieved CR. None of those with 19q cnLOH achieved CR despite intermediate cytogenetic risk. FLT3-ITD mutation is clearly associated with 13q cnLOH, seen in 100% of patients (6/6) tested. Presence of NPM1 mutation in 13q cnLOH cases does not appear to negate the poor prognosis in these cases.

Conclusions: Our data shed light on the prognostic impact of cnLOH in AML. Treatment outcome and OS appear poor in patients with cnLOH of 13q, 17p and 19q and better with cnLOH of 1p and 21q.

OTH02. Implementation of Value Stream Mapping (VSM) to Study RNA-Based Testing Workflow and Collect Performance Metrics in the Molecular Pathology Laboratory
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Introduction: The advent of molecular diagnostic testing has revolutionized diagnostics, prognostication and monitoring of treatment in a wide variety of clinical settings. Although the role of the molecular laboratory is expanding, the changing healthcare climate is impacting reimbursement models. Successfully adapting to these new conditions requires close inspection of laboratory work processes to distinguish value-added (activity that contributes towards the end product) vs non-value added (activity that does not contribute towards the end product, often defined as waste) steps. Value Stream Mapping (VSM) is a graphical LEAN tool that is used to improve workflow by identifying process delay(s) and studying movement of information and material in a family of related processes. We applied VSM to our laboratory's RNA-based testing menu for hematolymphoid malignancies. The goal was to study our workflow and capture baseline process performance metrics that could be utilized to validate future process improvements to accommodate increased workload.

Methods: VSM of pre-, post-, and analytical processes was performed on specimens analyzed over 9 workdays by a team of three technologists. Specimens submitted for RNA-based testing for hematolymphoid malignancies (BCR/ABL p210, BCR/ABL p190, PML/RARA, AML1/ETO, and CBFB/MYH11 inv(16)) were tracked through each step.
VSM metrics such as lead time (time between specimen receipt and result report), cycle time (time from start of a process to its completion), and takt time (pace to meet work demand) were calculated to define the baseline performance. **Results:** Analysis of VSM metrics for 16 specimens (14 peripheral bloods, 1 bone marrow, 1 CSF) yielded a process cycle efficiency of 27%, derived from a lead time of 1224 minutes and a cycle time of 337 minutes. Takt time was 406 minutes/specimen. Seven distinct steps of value-addition were identified and the longest intermediate queue time was between RNA isolation and cDNA synthesis (330 minutes). These metrics demonstrated that the process performance was adequate for the current test volume. **Conclusions:** With VSM, baseline performance metrics were successfully captured and points of delay identified. We will now implement VSM for our other test families (example, fragment length analysis). Overall, with this technique we were able to show that our process capacity and workflow are adequate to accommodate the current customer demand and objectively measure any impact due to anticipated increase in future test volume. Furthermore, we can now systematically select, plan and implement future process improvements. We strongly recommend that molecular laboratories utilize VSM to benchmark their performance and design efficient processes.

**ST44. Screening for Lynch Syndrome: Expression of Annexin A10 Can Discriminate between Sporadic and Lynch-Associated Microsatellite-High Colorectal Carcinoma**

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**Introduction:** Differentiating sporadic microsatellite high (MSI-H) colorectal carcinoma (CRC) due to hMLH1 promoter methylation from Lynch syndrome (LS)-associated MSI-H CRC due to mutations in mismatch repair proteins is time consuming, cost intensive, and requires advanced laboratory testing. A mutation in *BRAF* has been shown to be highly specific for sporadic CRCs; however, ~30% to 50% of sporadic MSI-H CRCs are *BRAF* wild-type. Analysis of *hMLH1* promoter methylation can subsequently be used to differentiate LS and sporadic MSI-H CRC, but both tests require specialized labs and are expensive. Through previous gene expression profiling of sessile serrated polyps we identified ANXA10 as a protein highly expressed in these polyps. As sessile serrated polyps give rise to sporadic MSH-H CRC, we evaluated the ability of ANXA10 expression to discriminate between LS and sporadic MSI-H CRC. **Methods:** Sporadic MSI-H CRCs were determined by *BRAF* mutational analysis (allele specific PCR) and *hMLH1* promoter methylation in *BRAF* wild-type cases (quantitative methylation specific PCR). Expression of ANXA10 mRNA was evaluated in sporadic MSI-H and LS CRC by quantitative RT-PCR. Immunohistochemical (IHC) expression of ANXA10 (1:250, Novus biologicals) was evaluated in 72 sporadic MSI-H CRC (53 *BRAF* mutated, 19 *BRAF* wild type with *hMLH1* promoter hypermethylation) and 52 LS CRCs from 49 patients (30 definite LS and 19 presumed LS). Any nuclear or strong membranous expression in the CRC was considered positive. **Results:** A marked increase in ANXA10 mRNA was observed in sporadic MSI-H CRC compared to LS CRC (378 fold increase, p<0.001). Using IHC, ANXA10 was expressed in 27/52 (51%) *BRAF*-mutated sporadic CRC and 8/19 (42%) *BRAF* wild type/*hMLH1* methylated sporadic CRC. Only 3/52 (6%) LS CRC were positive for ANXA10 (p<0.0001). One patient had a deleterious mutation in MSH2 and another had a variant of uncertain significance in MSH6. Only 1/24 (4%) LS CRC with loss of hMLH1 expressed ANXA10. This patient did not have a deleterious hMLH1 mutation, but rather germline promoter hypermethylation of hMLH1. **Conclusions:** Expression of ANXA10 shows promise in helping screen for LS CRC. Although 3 LS patients expressed ANXA10, 2 had abnormalities in MSH2/MSH6 and can easily be distinguished from sporadic MSI-H CRC using IHC for MSH6 and MSH2. Importantly, no patient with a deleterious hMLH1 mutation expressed ANXA10. Based on these results, IHC for ANXA10 may be a useful marker to distinguish sporadic from LS MSI-H CRC.