H19. Protein-Tyrosine Phosphatase, Nonreceptor-Type 6 (*SHP1*) Expression Loss as an Alternate Drug Resistance Mechanism in Chronic Myelogenous Leukemia

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Introduction: Chronic myelogenous leukemia (CML) patients can be refractory to imatinib mesylate therapy targeting the *BCR-ABL* kinase. Resistance in the absence of detectable abl kinase mutations suggests the presence of alternative mechanisms of resistance. The protein-tyrosine phosphatase, nonreceptor-type 6 (*PTPN6, SHP1*) down-regulates (dephosphorylates) signal transduction factors required for cell proliferation. The purpose of this study is to investigate the role of *SHP1* in CML patients with and without detectable abl kinase mutations. We hypothesize that decreased expression of *SHP1* is an alternative mediator of imatinib resistance. **Methods:** Sensitivity to imatinib was defined as a three log decrease in BCRabl/abl transcript levels in12 months or less. Transcript numbers were quantified using qPCR standard curves made with synthetic oligomers. The ratio of *SHP1* transcripts/abl transcripts (*SHP1/abl*) was measured in RNA from 53 imatinib-sensitive and 47 imatinib-resistant patients with CML and ten normal controls. **Results:** Average *SHP1/abl* was similar in normal cells and CML (p=0.747). The average *SHP1/abl* ratio was lower in imatinib-resistant CML cases (18.6), than in imatinib-sensitive cases (29.2; p=0.172). *SHP1* transcript levels equal to or less than *abl* transcript levels (*SHP1/abl* <1.00) were significantly associated with resistance to imatinib (Chi-Square p=0.020). Taking account of *abl* kinase mutations, T315I, M351T, E255V and E255K, the average *SHP1/abl* ratio was highest in five sensitive cases found to have mutations, 42.7 vs 27.7 in sensitive cases (n=48) without *abl* kinase mutations. Lower levels of *SHP1* were observed in seven resistant cases with mutations (22.2). The average *SHP1*/abl ratio was lowest in imatinib-resistant cases without detectable abl kinase mutations (18.0; n=40). **Conclusions:** The results are consistent with *SHP1* loss contributing to imatinib resistance in CML. Negative regulation of signal transduction factors, including *src* kinases, by *SHP1* phosphatase would suggest a mechanism for the activity of *src* kinase inhibitors such as dasatinib or dual agents such as bafetinib. These agents may counteract loss of *SHP1* activity in imatinib-resistant cases of CML or ALL, especially those without detectable *abl* kinase mutations.

H20. Array Cytogenomics as a Diagnostic Aid for Acute Myeloid Leukemia: A Comparison of Four Different Platforms

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Introduction: Array-based cytogenomic platforms have become mainstream diagnostic tools for constitutional chromosomal abnormalities not detectable by traditional cytogenetics. However, the chromosomal complexities seen in human cancer have delayed the implementation of this technology for personalized tumor analysis. As a first step towards validating array-based cytogenomics for cancer specimens, we selected eight cases of acute myeloid leukemia (AML) with defined cytogenetics and assayed them using four competing array platforms from Illumina, Affymetrix, Nimblegen-Roche and Agilent. Here we present the results of our comparative analysis, which demonstrate the relative strengths and weaknesses of the technologies. **Methods:** Eight cases of AML representing a spectrum of cytogenetic findings were selected for molecular analysis following routine cytogenetic analysis of bone marrow aspirates. DNA was isolated pre-culture (Puregene, Gentra) and submitted for array analysis at each company's laboratory. DNA was then processed on the four array platforms, and the resulting data was returned for our review. Data analysis, including aberrant calls, was also provided by several of the participating companies to allow for comparison of our analysis pipeline with the company's analysis software. **Results:** Three of the platforms tested included both copy number and genotyping probes (Illumina, Affymetrix and Agilent), while the Nimblegen platform analyzed copy number only. Cytogenetically visible abnormalities present in a high percentage of cells were generally detectable by all platforms. Detection of abnormalities present at low levels was more complicated, with each platform having different detection limits. Inclusion of SNP probes allowed for increased sensitivity to low level events, and was crucial for detecting copy neutral loss of heterozygosity not detectable by cytogenetics. Probe density for both copy number and genotyping probes was also found to be important for accurately determining the size of affected regions. Sensitivity to detecting the percentage of cells affected by each abnormality also proved to be important in determining the clonal diversity of the tumor. **Conclusions:** Analysis of our samples on all four platforms allowed us to assess the strengths and weaknesses of each of the underlining technologies. Given the types of aberrations found in malignancies and issues related to clonality, key aspects required for successful incorporation of arrays into cancer cytogenetics include: the power to detect copy neutral LOH and aberrations present in a low percentage of cells, and the ability to accurately quantify the percentage of cells affected by each abnormality. Each platform analyzed has overlapping abilities, with no single platform performing best in all areas.

H21. *TP53* DNA-Binding Motif Mutation Is Found in High-Risk, Untreated Chronic Lymphocytic Leukemia Patients with Chromosome 17p Deletion

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Introduction: Interstitial deletion of chromosome 17p (17p-) detected by fluorescence in situ hybridization (FISH) and p53 mutations are unfavorable prognostic markers in chronic lymphocytic leukemia (CLL) and are associated with significantly poorer response to chemoimmunotherapy and decreased overall survival. We applied SNP-based whole genome copy number variation (CNV) analysis to characterize CLL patients with 17p-. DNA sequencing was used to identify mutations in the *TP53* gene. **Methods:** We performed CNV analysis and *TP53* sequencing on 6 patients with early-intermediate stage, untreated CLL who had high risk for disease progression based on molecular and immunophenotypic markers. All six patients had 17p- and 13q- detected by FISH. CLL cells and normal cells were separated from patient peripheral blood by immunomagnetic beads. CNV analysis was performed on purified genomic DNA from CLL and normal cells for each patient to distinguish acquired copy number changes in malignant cells from polymorphic CNVs in the human genome. The Illumina human 660w-quad beadchip, a SNP-based microarray, was used for CNV analysis. Data was analyzed by CNV partition and PennCNV software. *TP53* mutation analysis was performed by dideoxy sequencing of PCR products amplified from *TP53* exons 4-9. **Results:** CNV analysis detected 17p- in 6 out of 6 patients with 17p- by FISH. One sample had 17p- in 20% of nuclei by FISH, which is near the current detection level of SNP-based copy number analysis (estimated at 15% to 20%). Four patients had hemizygous deletions covering the entire p arm of chromosome 17. Two patients had hemizygous interstitial deletions of 17p that spanned 17 Mb and 580 Kb respectively. All 17p deletions included the region of *TP53*. Additionally, patients with 17p- had numerous acquired copy number aberrations (CNAs) in the CLL genome located on chromosomes 1, 2, 3, 4, 9, 10, 13, 15, 18, and 20. These CNAs included hemizygous deletions, homozygous deletions, duplications, and uniparental disomy. Four of the six 17p- patients had *TP53* mutations located in the p53 DNA-binding motifs (DBMs), specifically between amino acids 109 and 286. **Conclusions:** SNP-based CNV analysis enables detailed characterization of complex copy number aberrations in the CLL genome, including large and small interstitial deletions of chromosome 17p. The majority of our CLL patients with 17p- also have *TP53* mutations involving the DBMs, which have been associated with especially poor prognosis. Analysis of *TP53* mutation status complements SNP-based CNV evaluation. *TP53* screening is essential in CLL patients requiring treatment for progressive disease.

H22. Detection of Minor Clones with Internal Tandem Duplication Mutations of *FLT3* Gene in Acute Myelogenous Leukemia Using Delta-PCR

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Introduction: Internal tandem duplication (ITD) mutations of the *FLT3* gene have been associated with poor prognosis in acute myelogenous leukemia (AML). Detection of minor clones or minimal residual leukemia clones with ITD mutations may be essential. In clinical diagnostic laboratories, ITD mutations are usually detected using PCR followed by capillary electrophoresis with an analytic sensitivity of 5% to 10% blasts. Since the potential amplicon size varies greatly and multiple mutations may be present, PCR detection of ITD mutations using capillary electrophoresis may be challenging, particularly when the peak height is low. **Methods:** In our previous study, we developed a triple-primer strategy, delta-PCR, to ensure PCR specificity and to improve sensitivity. The primer design is analogous to semi-nested PCR, but all three primers are used simultaneously in a single-step reaction. The internal primer functions as a confirmatory "probe", since it was designed at a defined distance (the delta) from the external primer. A pair of amplicons differing in size by the delta indicates an ITD mutation. In the current study, delta-PCR was used to detect ITD mutations with a sensitivity of 0.1% leukemic cells. 109 cases with newly diagnosed or relapsed AML were analyzed, including serial samples from 3 cases. **Results:** Delta-PCR was able to detect single or multiple ITD mutations with an allele burden (peak height ratio of mutant allele to wild-type allele) of the dominant clone ranging from 0.2% to more than 100% among all 31 cases with previously documented ITD mutations, a 0.01% allele burden among 1/75 cases with previously reported ITD-negative results, and a 0.1% allele burden in 1 of 3 cases with previously reported ITD-negative results in the initial diagnostic specimens and ITD-positive results at the same size in follow-up specimens. These data indicate