methylation in 5′ regulatory sequences. Mutation of other putative transcription factor binding sites suggests that additional factors regulate vav1 expression as well.

552 ALK Rearrangement and EGFR Copy Number Gains/mutations in Czech Non-small Cell Lung Cancer Patients

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Introduction: The predictive roles of EGFR mutations and EML4-ALK rearrangement are the most clinically relevant findings in small nevertheless significant percentage of non-small cell lung cancer (NSCLC) patients. Gefitinib and erlotinib are available for the treatment of advanced EGFR-mutated NSCLC patients. Crizotinib, dual inhibitor of ALK and C-MET, was FDA approved for advanced NSCLC patients harboring EML4-ALK fusion. Incidence of ALK rearrangement in NSCLC cases is estimated at 2%-7% and diagnostics of this aberration have become very important.

Material and Methods: Prospective consecutive cohort of stage IIIb/IV NSCLC patients, diagnosed from February 2011 to January 2012, were screened for EGFR mutations using peptide nucleic acid (PNA) clamping. EGFR and ALK copy number gains were determined using FISH (positive in average ≥3 copies of gene/nucleus) and ALK rearrangement using ALK break-apart and EML4-ALK translocation probe FISH with cut-off of ≥15% positive cells.

Results: EGFR and ALK alterations were examined in 185 NSCLC patients. All patients were Caucasians with median age 67 years (29–85) and representation of 63 women and 122 men. The most frequent subtypes were adenocarcinoma (47.8%) and squamous cell carcinoma (37.6%). Fish assay was inconclusive in 35 cases (18.9%) and EGFR mutation detection in 31 cases (23.8%). ALK and EGFR copy number. ALK rearrangement as well as mutational status of EGFR gene were determined in 130 NSCLC patients. No alteration was found in 83 cases (63.8%). EGFR copy number gain was found in 24 cases (18.5%). ALK copy number gain was observed in 21 cases (16.2%), simultaneous EGFR copy gain and ALK rearrangement was detected in 10 of them. EGFR activating mutations were found in 13 cases (10%).

ALK rearrangement was found in 5 cases (3 women, 2 man) by break-apart probe FISH. Rearrangement as well as fusion partner EML4 were confirmed by EML4-ALK translocation probe. Adenocarcinoma histology was confirmed in four cases, no histology subtype specification was described in last NSCLC case. ALK rearrangement was mutually exclusive in all cases, no concurrent EGFR mutations were found. In one case, ALK copy number gain was found simultaneously.

Conclusions: ALK rearrangement detection has become very important for NSCLC patients due to huge benefit from crizotinib therapy and should be part of routine diagnostics of NSCLC patients as well as EGFR mutation detection. Clinical relevance of ALK copy number gain needs to be clarified.

554 Cooperating Tumor Suppressor Genes on Chromosome 8p Predict Survival Outcome in Hepatocellular Carcinoma


Introduction: Melanoma is one of the most aggressive human cancers. Patients with lymph node metastasis can show highly variable clinical course, with documented presence of several and some translocations between chr9 and chr12 which their specific amplification and over-expression function as driver genes in lung compare to lymph node metastasis. Integration of these two gene lists provided us with the final list of 16 genes located on chr9 and chr12, amplified and over-expressed in lung compare to lymph node metastasis. Integration of these two gene lists provided us with the final list of 16 genes located on chr9 and chr12, amplified and over-expressed in lung compare to lymph node metastasis. The enrichment of the variety of SVs and occurrence of several translocations between chr9 and chr12 explains these amplifications which led to over-expression of genes on these chromosomes. We believe that there are gene cassettes on chr9 and chr12 which their specific amplification and over-expression function as driver cassettes for tumor recurrence. The correlation of these genes with the cancer phenotype will be addressed.

Conclusion: High-throughput sequencing technologies such as DNA-PET with increased sensitivity for rearrangement detection are used to provide comprehensive patterns of genomic aberrations underlying tumor progression and maintenance.

555 Genomics of Metastatic Progression in Cutaneous Melanoma

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Introduction: Most of the high risk, stage 4 Neuroblastoma (NB) patients experience a poor outcome despite novel multimodal therapeutic protocols, but the favorable outcome observed in a subgroup of patients at high risk indicates that additional prognostic markers must be identified and validated to assign the patients to the most appropriate risk category. Methylation of the Protocadherin B (PDCDHB) cluster analyzed in a cohort of patients spanning from Stage 1 to Stage 4 seemed to be a really promising biomarker in a Neuroblastoma. We analyzed this biomarker specifically in stage 4 patients at high risk.

Material and Method: We developed a pyrosequencing assay to measure the methylation level of 17 genes of the PDCDHB cluster and we analyzed 106 tumors of high risk stage 4 NB patients comparing the results with stage 4 at lower risk and stage 1 at very low risk NB patients.