SUMMARY (Identification of optimal algorithm for effective diagnostics of non-small cell lung carcinoma with *ALK* gene rearrangement – implementation of the method and practical experience with routine diagnostics)

INTRODUCTION: Smaller subgroup of non-small cell lung carcinoma (NSCLC) is represented by tumours with carcinogenesis iniciated by fusion of *ALK* gene with another partner (usually *EML4*). Patients with *ALK* gene rearrangement treated with ALK inhibitors have significantly prolonged survival. Since *ALK* gene rearrangement is described according to the current WHO classification in 4-5% of NSCLC, it is necessary to find the optimal way of identifying patients eligible for this targeted therapy in routine diagnostic practice.

AIM: In the retrospective part of the study a) to find an immunohistochemical (IHC) detection procedure of ALK protein with specificity and sensitivity high enough to use this antibody as screening method for selecting NSCLC cases for fluorescence in situ hybridization (FISH) testing of *ALK* gene rearrangement and b) to determine diagnostic yield of "small" biopsies, i.e. endobronchial, transbronchial and transthoracic biopsies and cytoblocks, for *ALK* gene rearrangement testing. In the prospective part of the study a) verification of the selected IHC method of ALK protein detection in routine testing of patients with NSCLC and b) correlation of ALK status with tumor morphology and selected clinical data.

METHODS: In the retrospective part of the study, 170 EGFR-non-mutated cases of NSCLC were IHC (three variants) and FISH tested. Tissue microarray (TMA) technique for samples processing was used. In the prospective part of the study, 557 cases of NSCLC were tested by selected IHC variant and 76 cases by FISH.

RESULTS: There were 8/154 (5,2 %) found by FISH in the retrospective part of the study. The optimal IHC method of ALK protein detection (clone 5A4 (Novocastra, Newcastle, UK)) was selected after correlation of FISH and IHC examination results. With this method there were 24/557(4.3 %) cases with *ALK* gene rearrangement detected in the prospective part of the study. Sensitivity and specificity of the best IHC method were 100 % and 99 % in the retrospective part and 100 % and 80 % in the prospective part, respectively. Depending on IHC variant, diagnostic yield of "small" biopsies was between 74 – 80 % retrospectively, and 88 % prospectively. No case with *ALK* gene rearrangement detected prospectively had *EGFR* mutation.

CONCLUSIONS: High diagnostic yield of "small" biopsies confirms that ALK status testing can be used in this type of specimen. Prevalence of 5.2 % in the retrospective part (*EGFR*-nonmutated cases) and 4.3 % in the prospective part (without known *EGFR* mutation status), tumor morphology (solid and acinar type, mucinous type or at least partial mucin production (extra- and/or intracellular) as well as lower average age and male/female ratio of patients with ALK positive tumors in the prospective part (57.5 yrs vs. 65.2 yrs; 8 men and 16 women vs. 336 men and 197 women) are consistent with global data.