Introduction:
Adenocarcinoma and squamous cell carcinoma are the most frequent non small cell lung cancer (NSCLC) subtypes and the leading cause of lung cancer-related deaths. Overall survival rate of patients with lung cancer is still very low despite several improvements of existing therapeutic efforts. Although predicted and real outcomes can vary significantly, the assessment of tumor size, lymph node status and the presence of metastasis are currently determined prognostic and treatment modality. Biomarkers with reliable prognostic significance are therefore of utmost importance. Nonetheless, and due to a possible lack of correlation between level of proteins and their corresponding mRNAs, the development of a biomarker screen based directly on enzymatic activities is a promising option enabling the selection of therapeutically treatable enzymes (1, 2).

Protein phosphorylation is an important and ubiquitous post-translational modification in all eukaryotic biological systems with over 500 protein kinase coding motifs identified in human. In general, protein kinases are involved in signal transduction cascades and related kinases may have many common substrates. Besides having common substrates, subtle differences in kinase activities may also determine the relevant in vivo signaling pathways. Emerging drugs showing great promise on specific sets of NSCLC patients are targeting protein tyrosine kinases (PTK) activities. Nonetheless, PTK inhibitors proved to be only of temporary relief since the development of secondary resistance leads to high rates of treatment failure. It is therefore becoming important to identify biomarkers with reliable prognostic significance and also, ideally, therapeutically treatable, that may improve both the prognosis and TNM staging and increase the limited pool of patients that may benefit of the application of the PTK inhibitors for treating lung adenocarcinoma. Here, we used the PTK inhibitor gefitinib and analysed ex vivo the PTK enzymatic activities of human lung adenocarcinoma biopsies as potentially important biomarkers in lung cancer biology.

Study and Methods:
Established in January 2005, the tumor bank of the Division of Thoracic Surgery at the University Hospital Zurich (UHZ) consists of fresh frozen matched pairs from malignant adenocarcinoma and non-neoplastic lung biopsies. All clinical interventions, follow up treatments, disease specific survival and outcomes were archived and written consent was required from every participating patient. The clinical characteristics of the 53 patients with early stage 1 and 2 lung adenocarcinoma are summarized in Table 1.

Statistical analysis
We performed quantitation and quality control investigation with the dedicated BiomarkerR software (version 5.2; PamGene, ’s-Hertogenbosch, The Netherlands). The tool PLS-DA (partial least square discriminant analysis) will create class prediction models. Alternatively, the original “training set” samples were also tested by leave-one-out cross validation. Both analytical approaches resulted in a prediction score called the PAMindex. Finally, for each peptide we applied a correction for the differences of observation between groups of specimens embedded with or without optimal cutting temperature media (OCT).

Conclusions:
Protein phosphorylation is an important and ubiquitous post-translational modification in eukaryotic biological systems. In this project we screened for tyrosine kinase activities on stage I and stage II adenocarcinoma biopsies in the presence of the PTK inhibitor and we present the feasibility of biopsies of stage I adenocarcinoma to discriminate between long-term and short-term survivors: We created a 76-point ‘response-signature’ for each patient’s kinase. For the malignant versus the malignant treated with the PTK inhibitor biopsies, the response signatures presented in the form of a heatmap showed that the long-term survivors had a response signature indicating a strong inhibitory effect of the PTK inhibitor whereas the short-term survivors cohort was mainly classified with a response signature indicating a weak inhibitory effect at equivalent concentration of the PTK inhibitor. When we characterized the 76-point ‘response-signature’ we detected 26 peptides substrates significantly inhibited with the kinomes of the long-term survivors than with the kinomes of the short-term survivors. Each of the activity profiles analysed may be more appropriate for a specific type of tumor or patients characteristics. With this idea to obtain a better discrimination in the samples analysed we used here the PTK inhibitor in the assay as a tool to create the inhibition profiles. The reasons for that are mainly technical and biological. The technical advantage to use an inhibition profile over a non-neoplastic profile is that the malignant profile can be used as a baseline over the inhibited malignant profile. The biological advantage is that some inhibitors may be more efficient on a signaling pathway which is important for the discrimination between samples phenotypes (i.e. long-term versus short-term survivors). In the case of our study the PTK inhibitor is already described in the clinic as a treatment for adenocarcinoma. We also observed that the long-term survivors and short-term survivors were properly discriminated in the two distinct types of samples, the OCT and non-OCT groups. We could correct for those inhibitory effect of OCT after application of a correction showing that our model would probably be tested implemented after standardization of frozen tissue biopsy storage protocols. Nonetheless, we obtained about 73% of the samples correctly predicted after OCT correction and a 10 fold cross validated PAMIndex based on the 76-point “response-signature” of the pooled TNM stage I lung adenocarcinoma biopsies. The 26 peptides substrates significantly more inhibited in the kinomes of the long-term survivors, a biological and mechanistic interpretation of the observed differences is still of interest.

References: