# A HIGH-THROUGHPUT PLATFORM FOR PROTEOME AND PHOSPHO-PROTEOME PROFILING OF TUMOR TISSUES

**Jakob Vowinckel**[1], Karel Novy[1], Thomas Corwin[2], Jonathan Woodsmith[2], Tobias Treiber[1], Roland Bruderer[1], Lukas Reiter[1], Eike-Christin von Leitner[2], Oliver Rinner[1], Claudia Escher[1]

1) Biognosys AG, Wagistrasse 21, 8952 Schlieren, Switzerland 2) Indivumed GmbH, Falkenried 88, 20251 Hamburg, Germany

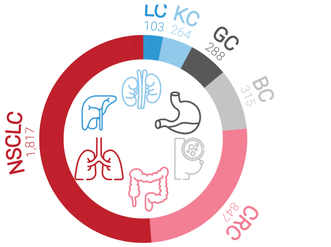
BIOGNOSYS
NEXT GENERATION PROTEOMICS

**Jakob Vowinckel, PhD**
Senior Scientific Project Manager

jakob.vowinckel@biognosys.com
www.biognosys.com

## INTRODUCTION

Precision oncology requires a detailed molecular understanding of tumor biology. Phenotype and underlying cellular functions are best characterized by the study of the proteome. However, MS-based proteome profiling is underrepresented in precision medicine compared to DNA/RNA sequencing techniques. Limitations in instrument stability, reproducibility, sample throughput and data analysis have prevented large-scale proteome characterization experiments. Recent developments in data-independent acquisition (DIA) LC-MS*/MS and robust chromatographic separation now present the opportunity to make proteomics available to routine analysis. Here we present a workflow that is capable of routine profiling 850 whole proteome (WP) or 650 phospho-proteome (PP) tumor samples per month with an average depth of 6,000 proteins (WP) or 30,000 phospho-peptides (PP), respectively. The workflow was applied across several indications as depicted on the right. NSCLC is highlighted in this poster.




* Liquid Chromatography–Mass Spectrometry

## CONCLUSIONS

- **An optimized, semi-automated workflow enables high throughput deep proteome and phospho-proteome profiling of matching tumor and normal tissue samples from Indivumed's high quality collection of fresh-frozen biospecimens.**
- **Rigorous quality control during sample collection, sample processing, data acquisition and analysis allows reproducible generation of data sets consisting of thousands of samples.**
- **On average, 5,903 protein groups and 28,819 phosphopeptides were quantified in each lung tissue sample within the NSCLC patient cohort, with up to 7,346 protein groups quantified in total.**
- **Observed protein expression and phosphorylation status of known substrates are in accordance with established knowledge and provide valuable insights to previously unknown markers relevant for tumor biology.**

## RESULTS

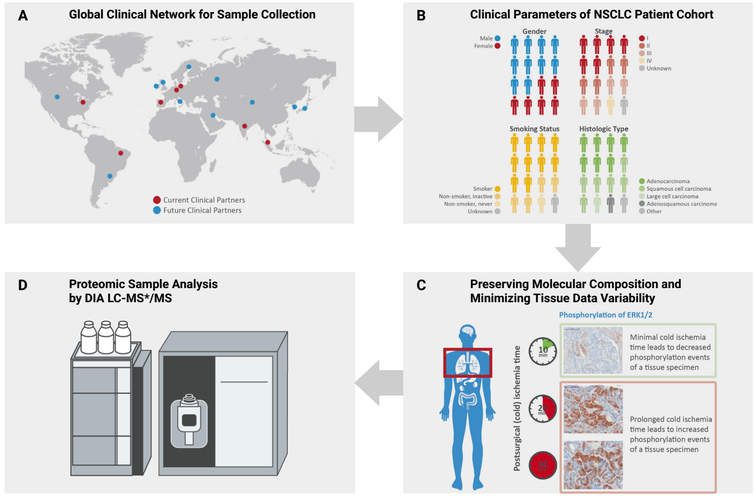



**Figure 1: IndivuType Sample Collection and Processing Workflow** (**A**) Patient bio-specimens and matching clinical data were collected through Indivumed's global clinical network in a standardized manner. (**B**) Subset of the clinical attributes for the cohort of ~800 non-small cell lung cancer (NSCLC) patients. 1 person represents 50 patients. (**C**) Indivumed follows SOP-driven standardized tissue collection approach to minimize molecular alterations resulting from post-surgery tissue collection and preservation processes that allows for an accurate representation of a patient's tumor biology. Representative immunohistochemistry for pERK 1/2 from one patient taken at three timepoints. pERK 1/2 expression levels increased from 10 minutes to 60 minutes post-surgery demonstrating early molecular changes. (**D**) Both whole-proteome and phospho-proteome profiling of tumor tissue and adjacent normal tissue from each NSCLC patient was performed using state-of-the-art liquid chromatography-tandem mass spectrometry in DIA mode.

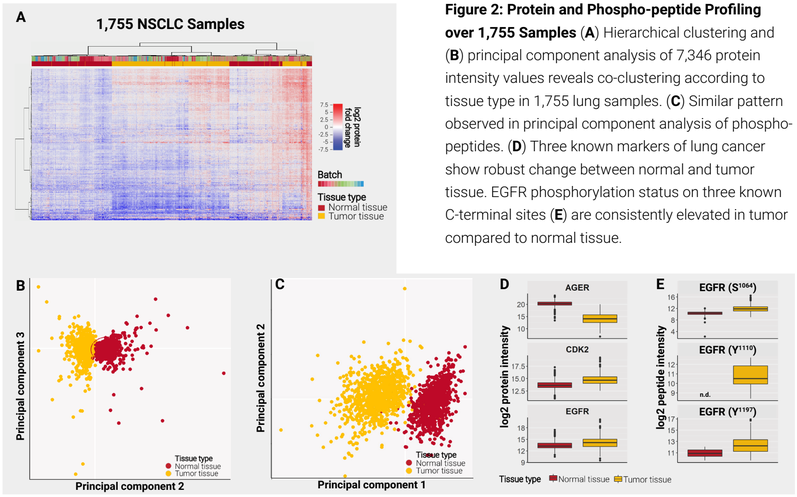



**Figure 2: Protein and Phospho-peptide Profiling over 1,755 Samples** (**A**) Hierarchical clustering and (**B**) principal component analysis of 7,346 protein intensity values reveals co-clustering according to tissue type in 1,755 lung samples. (**C**) Similar pattern observed in principal component analysis of phospho-peptides. (**D**) Three known markers of lung cancer show robust change between normal and tumor tissue. EGFR phosphorylation status on three known C-terminal sites (**E**) are consistently elevated in tumor compared to normal tissue.

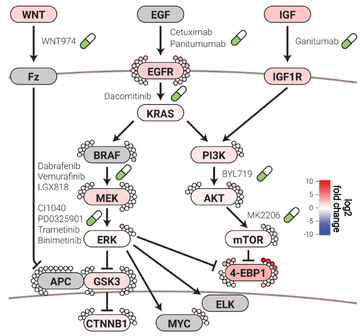



**Figure 3: NSCLC tumor biology.**
EGFR signaling pathway is well represented, with 92 out of 120 KEGG annotated proteins quantified in NSCLC tumor samples with protein and/or phospho-peptide information.