NMR-metabolomics to predict therapy response of resectable lung cancer

The Prolung Project

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INTRODUCTION

- Lung cancer is the most deadliest type of cancer worldwide, with **non-small cell lung cancer (NSCLC)** representing about 85% of all cases. When diagnosed in an early stage (I-IIIA), surgical resection of the tumor is currently the treatment of choice. Unfortunately, even when early diagnosed, **disease recurrence rates are high** and overall 5-year survival is low, ranging from 73% (stage I) to 24% (stage IIIA).
- **Metabolomics**, or the study of low-molecular weight metabolites, is based on the fact that cancer cells distinguish themselves from normal cells by **reprogramming their cell metabolism**. Consequently, the cancer cell metabolite concentrations are altered. These modifications also influence the plasma metabolite concentrations, which in turn can be measured with **^{1}H-NMR spectroscopy**.
- By application of **spiking methodology**, the ^{1}H-NMR spectrum of **human blood plasma** can be divided into multiple well-defined integration regions. With this technique, a relevant amount of a known metabolite of interest is added to a reference plasma sample, originating from a healthy subject.
- Combination of this method with **multivariate statistics**, where each integration region represents a variable, led to the construction of a **classification model**.
- A previous study with a large patient cohort showed that this model enables **discrimination between lung cancer patients and healthy controls**. With external validation in an independent study cohort, high predictive accuracy of the model was confirmed.

HYPOTHESES

1) The metabolite profile serves as a predictive biomarker for early disease progression after complete surgical resection.

Analysis of plasma metabolite profiles

2) The level of dissimilarity between the metabolite profile before and after surgery predicts disease recurrence.

→ Comparison of the plasma metabolite profiles before surgery:

NSCLC patients with **disease relapse within one year**
versus
NSCLC patients with a **recurrence-free survival**

→ Follow-up evaluation of the metabolite profiles in function of time.

RESEARCH METHODS

BLOOD SAMPLING

Blood samples will be donated two times before and five times after surgery during a follow-up period of one year. All samples will be handled and stored in a **Biobank**.

^{1}H-NMR SPECTROSCOPY

Spiking methodology with specific metabolites of interest will be performed using a **600 MHz NMR spectrometer**. This way, the ^{1}H-NMR spectrum of human blood plasma will be divided into well-defined integration regions.

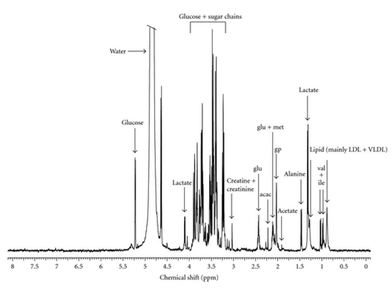
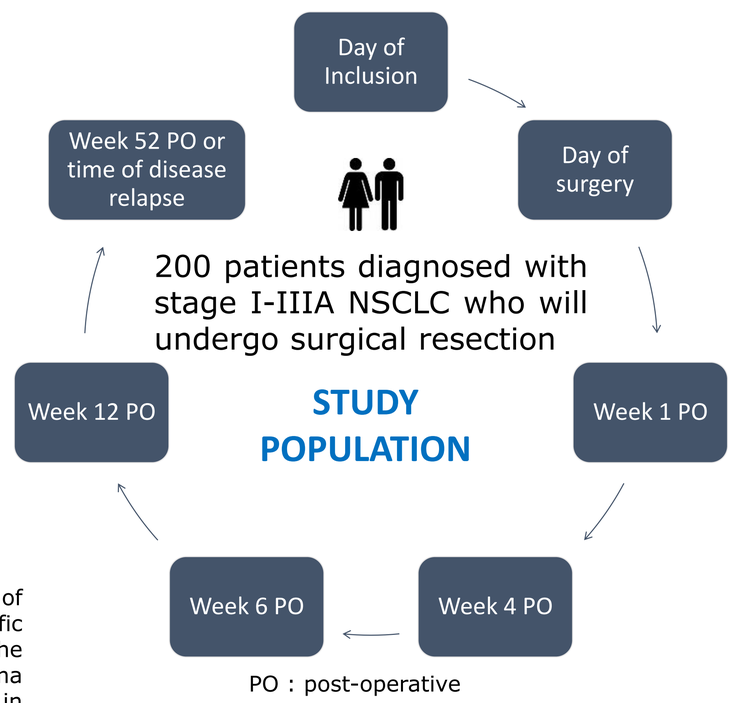


Figure 1: The ^{1}H-NMR spectrum of human blood plasma. Specific metabolites can be assigned to the different peaks. Spiking the plasma can segment the ^{1}H-spectrum in integration regions that represent the relative concentration of a single or multiple metabolites.

Compared to the 400 MHz spectrum, a 600 MHz NMR spectrometer will give rise to an improved spectral resolution and accurate definition of integration regions with less signal overlap. This might result in an increased number of **integration regions representative for a single metabolite**, which might provide new insights for cancer-related pathway analysis.



MULTIVARIATE STATISTICS

A **multivariate OPLS-DA classification model** will be constructed, where each variable is represented by a defined integration region. The classifier in figure 2 shows the discrimination between lung cancer patients and healthy control subjects. Specificity and sensitivity levels of 92% and 78%, respectively, were obtained.

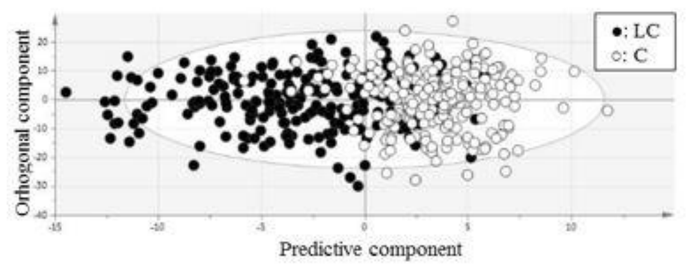


Figure 2: OPLS-DA score plot shows discrimination between lung cancer patients and healthy controls. This classifier was constructed with data from a training cohort consisting out of 233 lung cancer patients and 226 controls. **C:** controls; **LC**: Lung Cancer patients; **OPLS-DA:** orthogonal partial least squares – discrimination analysis.

Louis E. et al. Detection of lung cancer through metabolic changes measured in blood plasma. Journal of Thoracic Oncology. 2016;**11**(4):516-523

BIOCHEMICAL PATHWAY ANALYSIS

Previous research shows that plasma of lung cancer patients is characterized by an **altered level of specific metabolites**, such as an increased level of plasma glucose and a decrease in plasma lactate concentration. These results confirm an increased glycolytic flux and suggest an enhanced gluconeogenesis involved in the reprogrammed cell metabolism of lung cancer.

SCIENTIFIC GOALS OF THE PROLUNG PROJECT

- ^{1}H-NMR based metabolic fingerprinting of plasma allows detection of lung cancer.
- Plasma metabolite analysis with a 600 MHz spectrometer will contribute to a better understanding of the disturbed biochemical pathways in lung cancer.
- NMR-metabolomics can be implemented in the field of cancer research.
- The future results might lead to the development of a **metabolism-based prognostic biomarker**.

(i) Carry the potential to serve as additional **screening tool** for lung cancer by more correctly defining the selection of persons at risk.
(ii) Deliver an innovative contribution to personalized medicine by **improved characterization** of lung cancer patients & **prediction of treatment response**.

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