Prediction of therapy response in lung cancer patients: Optimization and evaluation of a robust ^{1}H-NMR metabolomics protocol

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INTRODUCTION

- Lung cancer is a very common cancer type with non-small cell lung cancer (NSCLC) representing 85% of all lung cancer patients. The main treatment for **early-staged NSCLC patients** is a surgical removal of the tumor, but in 30-55% of the cases a disease relapse occurs.

- The Prolung study is a prospective, longitudinal study at ZOL/UHasselt, which to predict **therapy response** by a metabolism-based **biomarker**.

➢ This biomarker is based on the **metabolite profile** of blood plasma, which is analyzed by **proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy**. To better understand and evaluate cancer-related aberrations in biochemical pathways, the metabolite peaks in the ^{1}H-NMR spectrum must be identified in a correct and quantified manner.

OBJECTIVES

- **Optimization of a robust ^{1}H-NMR metabolomics protocol**
  - ➢ JEOL 400MHz spectrometer

- **Multivariate statistical analysis**
  - ➢ Smokers versus ex- and non-smokers
  - ➢ Possible confounders

Figure 1: JEOL 400MHz spectrometer with autosampler

MATERIALS AND METHODS
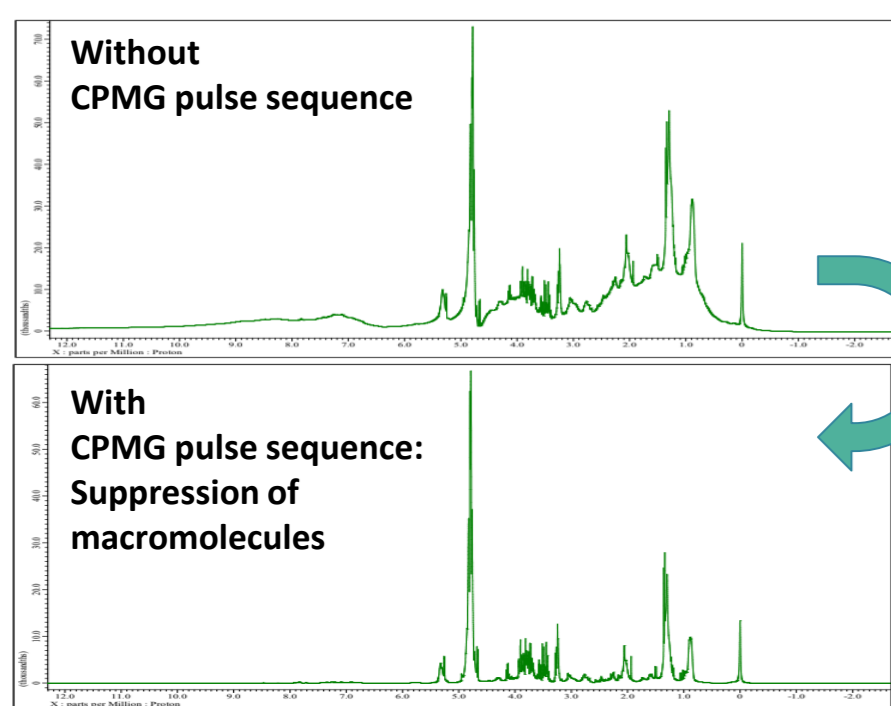
Optimization of the ^{1}H-NMR protocol

Without CPMG pulse sequence

With CPMG pulse sequence: Suppression of macromolecules

Figure 2: Effect of parameters on ^{1}H-NMR spectrum

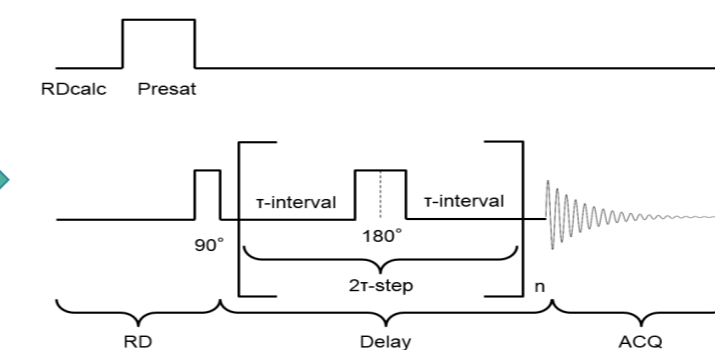|	
Buffer and Buffer capacity	D_2O/TSP, 0.15 M K_2HPO_4, 0.14 M Na_2HPO_4, 0.15 M K_2HPO_4/KH_2PO_4
Concentration and total volume	1:3, 1:2 and 1:1; 600, 700 and 800 µl
Temperature	22.5 °C → 37.5 °C
Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence	

Figure 3: Diagram of the CPMG pulse sequence, with the relaxation delay (RD), Spin echo delay and acquisition (ACQ)

Multivariate statistical analysis

Data previous study:
➢ **322 control subjects** (control group)
➢ 110 variables from ^{1}H-NMR measurements
➢ Clinical information (smoking status, medication, …)

Programs:
➢ Data overview: Microsoft Excel and SPSS
➢ Multivariate analysis: SIMCA
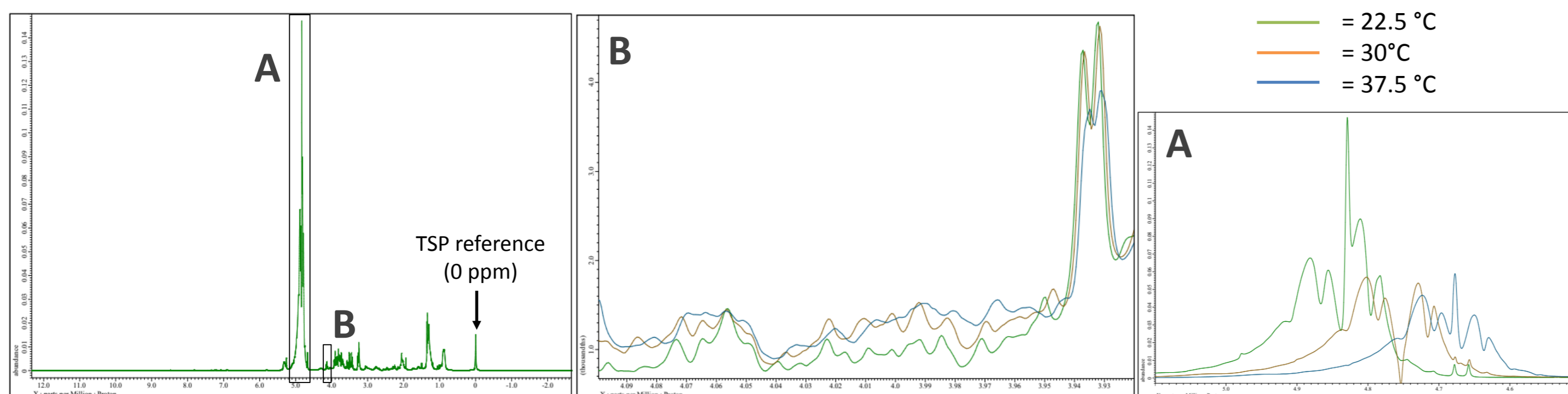
RESULTS

Optimization of the ^{1}H-NMR protocol

= 22.5 °C
= 30°C
= 37.5 °C

TSP reference (0 ppm)

Figure 4: Examples of the influence of different temperature on a ^{1}H-NMR spectrum

Final Results Optimization	
Buffer (pH)	0.15 M K_2HPO_4/KH_2PO_4 (7.4)
Total sample volume	700 µl
Plasma concentration	1:1
Temperature	25°C
Power of water suppression	67 dB
Relaxation delay	3 s
Presaturation time	3 s
Spin-echo delay	64 ms
Acquisition time	2.18 s
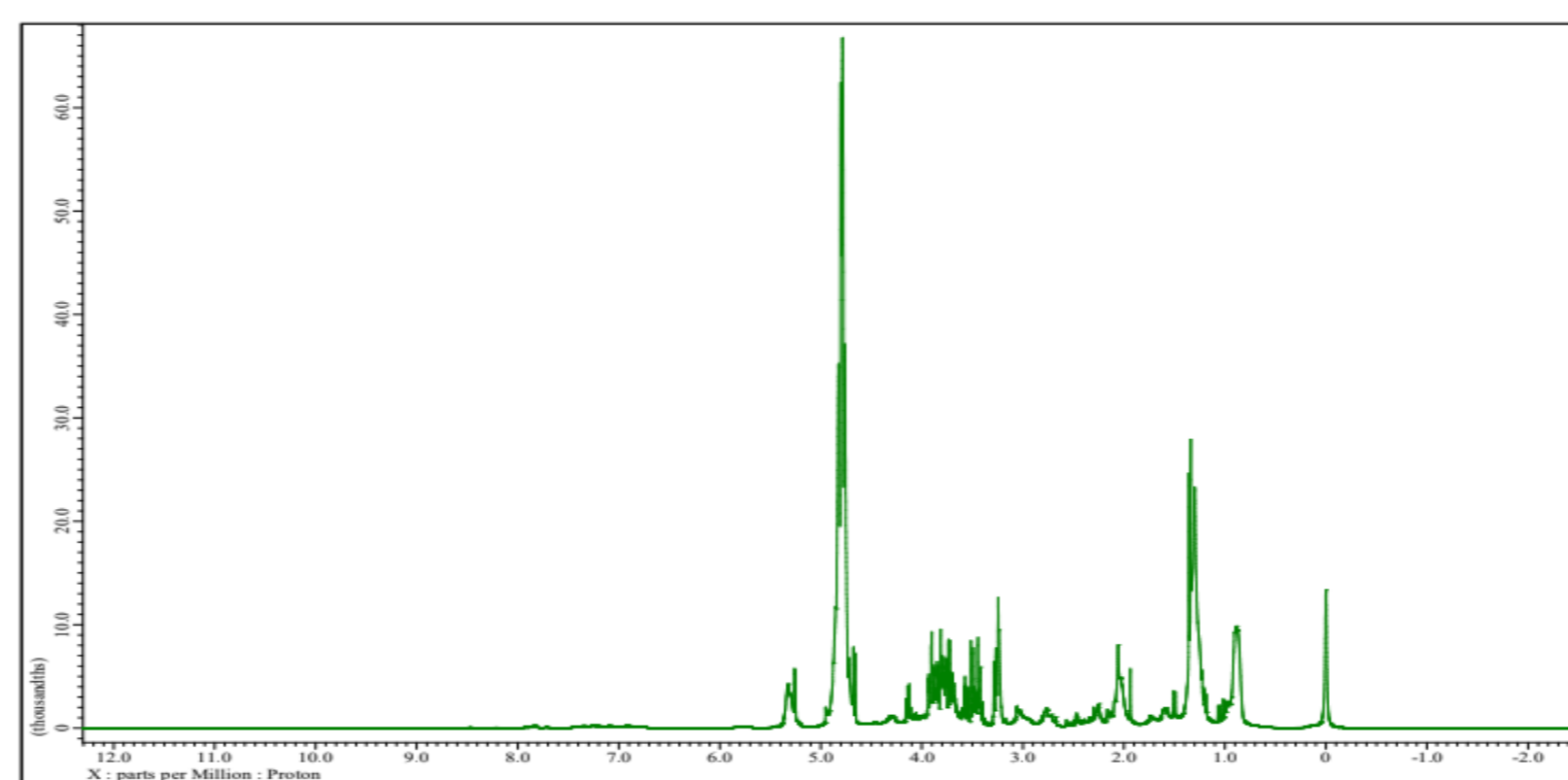
Figure 5: Final ^{1}H-NMR spectrum of human blood plasma with a CPMG pulse sequence

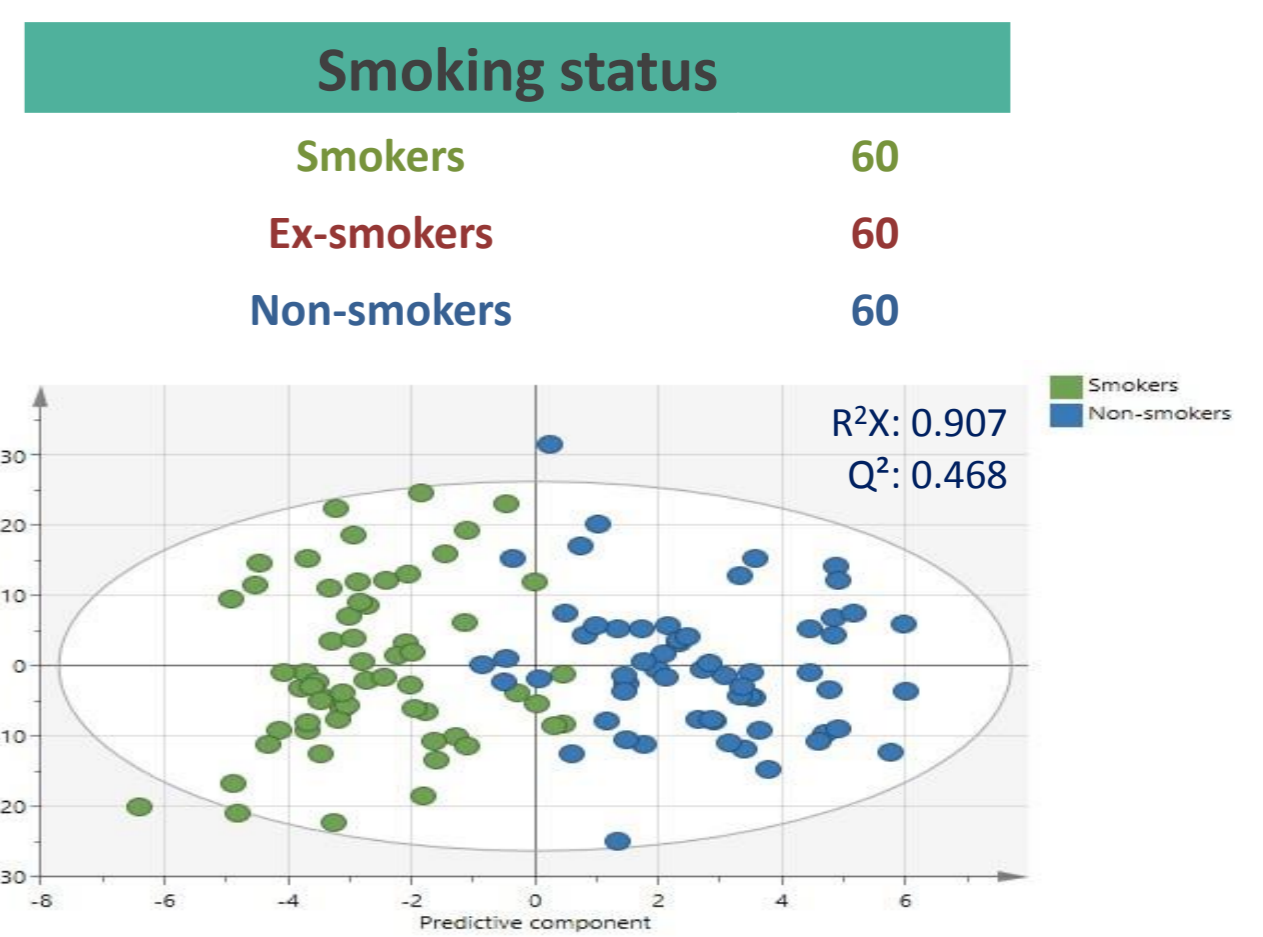### *Multivariate statistical analysis*

Smoking status	
Smokers	60
Ex-smokers	60
Non-smokers	60

R^2X: 0.907
Q^2: 0.468

Figure 6: OPLS-DA score-plot of smokers and non-smokers
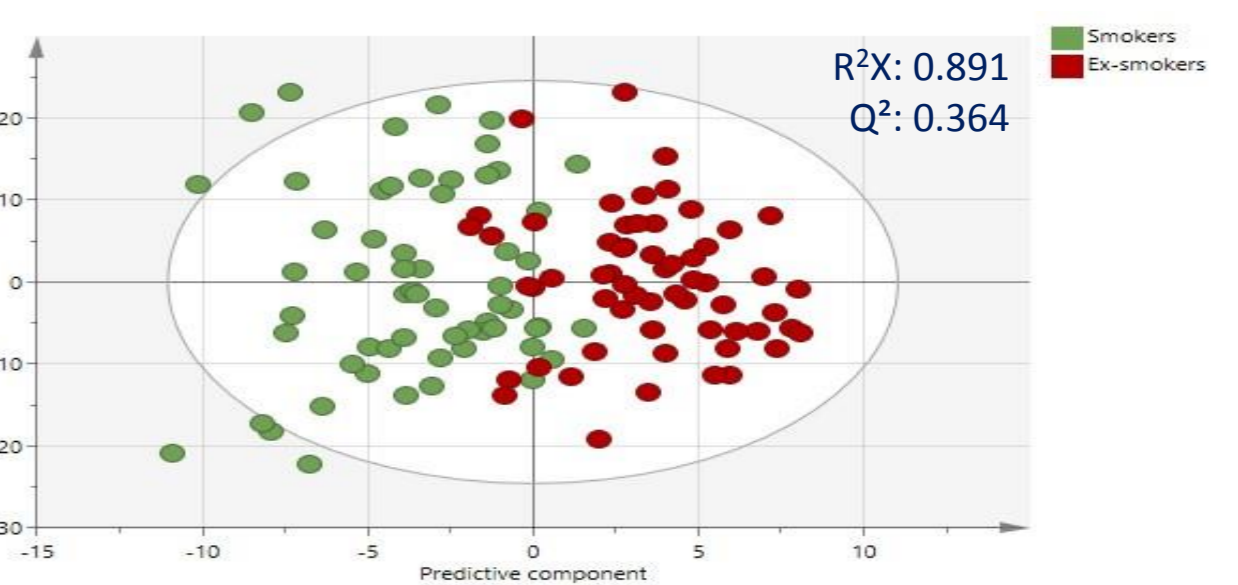

R^2X: 0.891
Q^2: 0.364

Figure 7: OPLS-DA score-plot of smokers and ex-smokers

CONCLUSION

The optimization resulted in a robust ^{1}H-NMR metabolomics protocol for blood plasma. The multivariate statistical analysis has shown that a discrimination can be made between smokers and ex- or non-smokers based on the metabolite profile. Also, no possible confounders in the smoking status model are found.

FUTURE GOALS

The next part for the optimization is the identification of the plasma metabolite peaks by performing **spiking experiments**. Afterwards, the **final measurements** of the plasma samples, collected from lung cancer patients during the Prolung study, need to be analysed.

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