Targeted metabolomics of clinically characterised serum samples identifies metabolite patterns for differentiating prostate cancer progression restricted to Gleason score, Epstein criteria and TMPRSS2:ERG-gene fusion

Emeka Igwe1, Guido Dallmann1, Daniel Andreis1, Mattias Bair1, David Enot1, Therese Koal1, Georg Schäfer2, Irmgard Verdorfer3, Helmut Klocker2, Georg Bartsch2, Klaus Weinberger1, and Hans-Peter Deigner1.

1BIOCRATES Life Sciences AG, Innrain 66/2, 6020 Innsbruck, Austria; 2University Clinics for Urology, Innsbruck Medical University, Anichstraße 35, 6020 Innsbruck, Austria; 3Department of Pathology, Innsbruck Medical University, Müllnerstraße 40, 6020 Innsbruck, Austria.

Introduction
Prostate cancer is the most commonly diagnosed cancer among men and the second leading cause of cancer-related deaths. The etiology of prostate cancer remains controversial still, as environmental, hormonal and hereditary factors are implicated as key players. In addition, the current methods used to classify prostate cancer stages and/or progression clinically are often erroneous, inconsistent and non quantitative. Thus, classifying patients in gray zones (cut off) with these methods is difficult. Herein, we have employed a LC-MS/MS based targeted metabolomic approach, as opposed to metabolomic profiling, to quantitatively differentiate the severity/stages of prostate cancer with human serum samples from patients that have been clinically characterized based on Gleason scores, Epstein and TMPRSS2:ERG-fusion criteria.

Methods
Age matched serum samples were randomly chosen from the prostate cancer serum archive in Innsbruck, Austria. Blood samples from patients diagnosed with PCa and healthy controls were obtained from the Prostate Cancer Early Detection program open to the general public in Tyrol (Bartsch et al., BJU International 101 (2008), 809-816) with the informed consent of sample donors; the serum sample procurement, data management and blood collection protocols were approved by the local Ethical Review Board. Serum was obtained by centrifugation (4 min, 1800g) and frozen in 2 mL cryovials (Simport) at −80°C. A multi-parametric, (FIA)-MS/MS and LC-MS/MS high-throughput targeted metabolomic platform was used for the simultaneous quantification of 650 metabolites in 8 assays. MRM detection was performed using an 4000 Q TRAP® tandem mass spectrometry instrument (Applied Biosystems/MDS Sciex), data were quantified with Analyst 1.4.2 software (Applied Biosystems) and finally exported for statistical analysis.

Results and Discussion
Eleven metabolites differentiated prostate cancer characterized as low from high Gleason score tumours, 11 metabolites differentiated prostate cancer characterized as Epstein insignificant from Epstein significant, and 21 metabolites differentiated prostate cancer with TMPRSS2:ERG-gene fusion to those without TMPRSS2:ERG-gene fusion. Furthermore, most of the metabolites found within each classification scheme were restricted to one classification, with only octadecadienoylcarnitines (saturated and unsaturated) and hydroxykynurenine found in at least two different classifications.

In a panel of 238 metabolites analysed in this study, we establish many metabolites are affected when prostate cancer samples were compared to control samples, only few metabolic changes where observed within each classification scheme. Thus, metabolomics offers a high potential for the identification of minute alterations in pathological conditions.

Conclusion
Through the targeted metabolomics approach, we have identified serum metabolite signatures capable of differentiating prostate cancer based on Gleason score, Epstein criteria and ETS-fusion status. While several metabolites were affected when prostate cancer samples were compared to control samples, only few metabolic changes where observed within each classification scheme. Thus, metabolomics offers a high potential for the identification of minute alterations in pathological conditions.

Acknowledgments
Institut für Medizinische Genomforschung Planungsgesellschaft mbH
Kärntner Straße 21-1010 Wien
BIOCRATES Life Sciences AG
Innrain 66/2
6020 Innsbruck, Austria
Tel: +43.512.579823
Email: ignatius.igwe@biocrates.com
Website: www.biocrates.com