O-48	**LC-MS-based metabolomics revealed SLC25A22 as an essential regulator in aspartate-derived amino acids and polyamines in KRAS-mutant colorectal cancer**

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Colorectal cancer (CRC) is the third most common cancer causing death worldwide. SLC25A22, which encodes the mitochondrial glutamate transporter, is overexpressed in CRC and is essential for the proliferation of CRC cells harboring KRAS mutations. However, the metabolic effect of SLC25A22 on CRC cells has not been characterized on a metabolome-wide scale. In the study, global and targeted metabolomics based on ultra-high performance liquid chromatography (UHPLC) coupled to mass spectrometry (MS) were used to evaluate effects of SLC25A22 on metabolism in KRAS-mutated CRC cells. Global metabolomic analysis of KRAS-mutant DLD1 cells with or without SLC25A22 knockdown identified 35 differentially regulated metabolites, which were primarily involved in glutaminolysis, urea cycle and polyamine metabolism. Targeted metabolomic analysis of TCA cycle intermediates, non-essential amino acids, and polyamines revealed that TCA cycle intermediates (except ?-ketoglutarate), aspartate (Asp)-derived asparagine, alanine and ornithine (Orn)-derived polyamines were all strongly down-regulated in SLC25A22 knockdown cells. Moreover, targeted kinetic analysis was performed with [U-13C5]-glutamine as the isotope tracer. The 13C-labeled urea cycle metabolites were not detected in SLC25A22 knockdown cells, suggesting the urea cycle was not triggered due to decreased levels of Orn. On the other hand, most 13C-labeled Orn-derived polyamines were significantly decreased in SLC25A22 knockdown cells and medium. Exogenous addition of polyamines could partly restore cell proliferation in SLC25A22 knockdown cells, highlighting their potential as oncogenic metabolites downstream of SLC25A22-mediated glutamine metabolism. Collectively, SLC25A22 promoted synthesis of asp-derived

O-60	**A non-targeted UHPLC-HRMS metabolomics pipeline for metabolite identification; application to cardiac remote ischemic preconditioning**

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In recent years, the amount of investigations based on non-targeted metabolomics has increased, although often without thorough assessment of analytical strategies applied to acquire data. Following published guidelines for metabolomics experiments, we report a validated non-targeted metabolomics strategy with pipeline for unequivocal metabolites identification using the MSMLS™ molecule library. We achieved an in-house database containing accurate m/z values, retention times, isotopic patterns, full MS and MS/MS spectra. A UHPLC-HRMS Q-Exactive™ method was developed and experimental variations were determined within and between 3 experimental days. The extraction efficiency as well as the accuracy, precision, repeatability, and linearity of the method were assessed, the method demonstrating good performances. The methodology was further blindly applied to plasma from Remote Ischemic Pre-Conditioning (RIPC) rats. Samples, previously analyzed by targeted metabolomics using completely different protocol, analytical strategy and platform, were submitted to our analytical pipeline. A combination of multivariate and univariate statistical analyses was employed. Selection of putative biomarkers from OPLS-DA model and S-plot was combined to jack-knife confidence intervals, metabolites VIP values and univariate statistics. Only variables with strong model contribution and highly statistical reliability were selected as discriminated metabolites. Three biomarkers identified by the previous targeted metabolomics study were found in the current work, in addition to three novel metabolites, emphasizing the efficiency of the current methodology and its ability to identify new biomarkers of clinical interest, in a single sequence. The biomarkers were identified to level 1 according to the

O-63	**Investigation of drug-induced steatosis in HepaRG cells using untargeted LC-MS metabolomics**

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Background: Accumulation of lipids in the liver (steatosis) is a frequent Drug-Induced Liver Injury. It is the first stage of Non-Alcoholic Fatty Liver Disease (NAFLD) and can progress to non-alcoholic steatohepatitis, fibrosis, cirrhosis and carcinomas, resulting in liver failure and death. Early detection and prevention of NAFLD are crucial in toxicological research and healthcare. Here, a hepatic in vitro system combined with metabolomics was used to gain more information on the mechanisms of action of sodium valproate, a reference steatogenic drug. Experimental outline: We optimised a LC-MS based metabolomics platform and exposed HepaRG cell cultures to sodium valproate at two different concentrations (IC10 and a 1/10 dilution of the IC10) and two different time points (24 h and 72 h). The intracellular metabolites were recovered in a polar and non-polar fraction using liquid-liquid extraction, both extracts were analysed with optimised untargeted LC-MS platforms. After quality control, the data were subjected to PCA and PLS-DA to select features of interest. Results: Exposure of HepaRG cultures to sub-cytotoxic levels of sodium valproate invokes a clear alteration in the metabolome. Significant differences were observed that relate to adaptive mechanisms which eventually progress to lipid accumulation and steatosis. The metabolic changes include a conversion from lower weight to higher weight lipids and alterations in the levels of polar organic acids. Conclusions: Using untargeted LC-MS metabolomics on HepaRG cultures, a sodium valproate-induced intermediary adaptation of the metabolome is observed, progressing to steatosis.