Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) in Breast Cancer

INTRODUCTION Breast cancer is a heterogeneous group of tumours with distinct molecular subtypes described at the mRNA level, and specific changes in DNA, methylation and protein levels. Ductal carcinoma in situ (CIS) and related invasive tumors (IBC) show enormous similarities at mRNA/DNA levels and the key molecular steps for tumor invasion are still unknown. Alterations on lipid metabolism have been increasingly recognized as a hallmark of cancer cells and may help to understand breast cancer carcinogenesis.

OBJECTIVE We aimed to use *in situ* Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) to investigate differences in lipid profiles across invasive breast cancer (IBC) of all 4 molecular subtypes and related normal parenchyma (NBP) and ductal carcinoma in situ (DCIS).

METHODS: We selected frozen samples from 9 IBC with various ER/PR/HER2 status (ER 0 to 100%, PR 0 to 90%, HER2 0 and 3+, Ki67 10 to 70%). Unstained slides cut on cryotome at 10µm were submitted to DESI-MS analysis with no pretreatment and spatial resolution of 300µm. Molecular profiles (mass to charge [m/z] ratio versus signal intensity) were generated directly from tissues to detect changes from areas of interest. IBC (and normal breast parenchyma and DCIS whenever present) regions were identified by a breast pathologist on HE-stained sequential slides. Three areas of interest were selected in each lesion. The experiment was conducted in the full-scan mode over and *m/z* range of 100-1200. DESI mass spectra were analyzed using a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole Orbitrap Mass Spectrometer in the positive ion mode. For each sample a mass spectrum and image were generated, based in their lipid profile. The Biomap software was used to visualize the images and to export the spectrums to metaboanalyst, where the data were statiscally analyzed.

RESULTS: The images obtained by lipid profiling presented a direct correlation with HE stained slides, with some lipids clearly overlaying areas of normal parenchyma, DCIS and invasive breast cancer. The unsupervised hierarchical clustering analysis showed all technical triplicates remained grouped. All NBP but one clustered together. A triple negative IBC and its related NBP formed a distinct cluster from all other samples. The ER+/HER2 + tumor was segregated from other Luminal/HER2- tumors. DCIS and IBC from a ER+/PR+/HER2- tumor clustered together. The Ions with m/z 186.9 and m/z 303.2 was consistently related to NBP and IBC respectively.
CONCLUSION: Chemical microscopy using DESI-MS is a powerful tool and can identify distinct lipid profiles across molecular subtypes of IBC and may contribute to understand breast cancer carcinogenesis.