

## **Near Real-time Stratification of PIK3CA Mutant Breast Cancers Using the iKnife**

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Cancer pathogenesis is associated with profound changes in cellular metabolism, but whether monitoring these changes can be used for real time diagnosis and therapy decision-making is largely unknown. The intelligent surgical knife (iKnife) allows for sampling of the aerosol ("smoke"), to detect the metabolomic composition of cauterized tissue via Rapid Evaporative Ionisation Mass Spectrometry (REIMS). This method has been used successfully for instantaneous classification of normal versus cancerous tissue, but the evolving metabolic landscape of tumour cells carries significantly more information than just the benign to malignant classification. Our data demonstrate that the iKnife allows for real time stratification of many clinically relevant features of breast cancer cells, including their stage, migration capacity, or exposure to metabolic conditions mimicking the tumour microenvironment. Of note, it also stratifies breast cancer into two major subtypes (lipogenic and non-lipogenic) with distinctive lipid signatures and overall lipid content. We demonstrate that this lipogenic subtype is highly enriched in PIK3CA mutations and depends predominantly on mTORC2 activation, opening up opportunities to investigate the mechanistic basis underlying potential metabolic vulnerabilities. Overall, our findings show that iKnife couples metabolic rewiring with a novel mode of real time diagnosis of tumour characteristics, and highlight an unidentified role for mTORC2 activation in lipid biosynthesis that promotes aberrant growth in PIK3CA mutant tumours.