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PTEN LOSS IN LUNG CANCER: EFFECT ON ANTI-TUMOR IMMUNE RESPONSE

PTEN is known to be a major tumour suppressor located on chromosome 10q23, working as PI3K pathway inhibitor. PTEN loss might be useful as a biomarker indicating hyperactivation of PI3K and corresponding better response to PI3K inhibitors such as Pictilisib. In order to precisely measure PTEN loss optimisation of PTEN immunohistochemistry (IHC) staining protocol and definition of threshold of protein loss is highly necessary. In previous study of NSCLC, frequencies of PTEN loss were not consistent, due to pre-analytical as well as analytical differences of IHC protocols. In order to standardize the IHC protocol and to set cut-off values, we performed an external quality assessment (EQA) using SP218, 138G6 and 6H2.1 anti-PTEN antibody clones. IHC staining were scored on webbook and tissue microarray. Followed by staining with SP218 on ETOP cohort samples (n=2245 NSCLC patients, 8980 TMA cores) for PTEN loss assessment. All cores were H-scored by pathologists and by computerized pixel-based intensity measurements calibrated by pathologists. The results of EQA indicated that all 3 antibodies differentiated 6 PTEN+ versus 6 PTEN- cases on EQA. For 138G6 and SP218, high sensitivity and specificity was found for all H-score threshold values including prospectively defined 0, calculated 8 (pathologists) and calculated 5 (computer). High concordance among pathologists in setting computer-based intensities and between pathologists and computer in H-scoring was observed. Due to over-integration of the human eye, pixel-based computer H-scores were overall 54% lower.
PTEN loss is not only an important predictive biomarker for PI3K inhibition, but it also correlates with higher PD-L1 expression in tumour epithelia. In order to understand PTEN interaction with immune system we stained TMA with 1109 cases and scored of PTEN, PDL1, CD8 markers. Results indicated invert correlation between PTEN and PDL1 -0.202 ($p<0.001$). As well as PDL1 has a positive correlation with CD8 0.141 ($p<0.001$) in chemo naive cases. Invert correlation between PTEN and PDL1 was stronger in LSCC. Following we would like to continue with understand the influence of mutational load and cytokines in combination with PTEN. In order to do it we will collect 40 cases from 2009-2012 which has fresh frozen tissue and paraffin embedded tissue. These cases will be selected according to microenvironment status. Cytokines influence on PTEN network will be access using Luminex microbeads. The same tissues would be then stained with multicolour staining for PTEN, PDL1, PanCK and CD8. The assessment will be performed in following zones - intratumoral, tumour margin and stroma.

SPECIAL TECHNIQUES AND EQUIPMENT

Imunohistochemistry (IHC), Image J, Ventana staining, Luminex microbeads