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## 48P An AI System for accurate Ki-67 IHC assessment in breast cancer following the IKWG whole section global scoring protocol

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**Background:** Assessment of immunohistochemical (IHC) Ki-67 expression plays a crucial role in breast cancer diagnostics for many therapy decisions. The International Ki-67 in Breast Cancer Working Group (IKWG) has recently proposed a new global scoring method on whole tissue sections to improve accuracy, involving assessment of up to four representative tumor areas with negligible, low, medium and strong proliferation levels. As precise manual assessment is time-consuming, artificial intelligence (AI) support is necessary to make this method practicable for clinical routine.

**Methods:** We developed an AI software for automated Ki-67 scoring according to the IKWG protocol. Seven pathologists assessed Ki-67 IHC status in 2 x 214 readings of whole-slide images (WSI) derived from 72 breast cancer specimens, four scanning hardware types and two clones. Pathologists selected representative areas manually, scored them with assistance of a CE-cleared AI software for region-of-interest (ROI) analysis and concluded global scores (weighted/unweighted). After a 2-week washout period, the same pathologists were presented with the same slides together with results by a fully automatic AI (AI-only): 1) selection of regions, 2) score and weight per region, and 3) two global scores (weighted/unweighted). Being able to adjust the AI-suggested results, pathologists concluded the two global scores (AI+path).

**Results:** For weighted global scoring, interrater-agreements between a) pathologist and AI+path, b) pathologist and AI-only, and c) AI-only and AI+path were 93.9%, 92.5%, and 96.7%, respectively (for unweighted scores, 91.1%/92.1%/96.3%). With AI-assistance, pathologists scored significantly faster compared to manual scoring (median time per WSI: 229 vs. 306 sec).

**Conclusions:** Used as a diagnostics assistance tool, the presented AI system showed high agreement with pathologist scores, while reducing assessment time. Likewise, also fully automatic AI use showed high agreement with human scoring. Overall, this demonstrates that the investigated AI system is suitable to enable safe implementation of the global scoring protocol in clinical routine, ultimately leading to higher accuracy and reproducibility in Ki-67 IHC scoring.

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## 49P Metformin is associated with increased intratumoural immune infiltrates in patients with primary invasive breast cancer

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**Background:** Obesity has been associated with poor clinical outcomes and chronic systemic inflammation in patients with breast cancer (BC). Metformin has demonstrated antitumorigenic effects both in pre-clinical and clinical studies. We hypothesize that chronic inflammation may lead to immune cell dysfunction in BC that can be restored via the use of metformin.

**Methods:** The effect of metformin on intratumoural (IT) immune cells was evaluated in two independent peri-surgical window studies (Dundee, n=29; Oxford, n=31) using immunohistochemistry for selected immune cell markers, pre- and post-metformin. Immune cell infiltrates were digitally quantified using Definiens Architect software and correlated to clinical parameters. Descriptive and linear regression techniques were applied using STATA software. Median densities and 95% confidence intervals were reported.

**Results:** In the Dundee cohort, metformin was associated with a 2.5 (1.8-3.1) fold increase in IT CD68+ macrophages, 2.7 (2.1-9.8) fold increase in IT CD8+ T-cells and 0.5 (0.4-0.6) fold decrease in regulatory T-cells (Tregs) in patients with primary BC. These findings were validated in the Oxford cohort where metformin was significantly correlated with higher density of IT CD8+ T-cells and reduced Treg density (adj $P<0.05$). These changes were confined to the tumour islands rather than in the stroma. Stratified analysis by BMI in both cohorts showed an increase IT CD68+ macrophages and reduction in Tregs post metformin, in patients with high BMI (adj $P<0.05$) whereas there was no difference in patients with healthy BMI (adj $P>0.05$). In the Dundee cohort, linear regression showed that metformin was significantly associated with increased IT CD8+ independently of the baseline pathological or metabolic parameters [co-efficient 7.2, 95%CI (3.2-11.2)]. In the Oxford cohort, systemic inflammation (baseline serum leptin of >17 pg/ml) was associated with lack of correlation between CD8+ and PD1+ T-cells as well as between CD16+ and CD32B+ macrophages that was restored after the administration of metformin (adj $P<0.05$).

**Conclusions:** Metformin is associated with changes in the IT immune cells. Further work to understand potential combination with immunotherapy is required.

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## 50P HER2/HER3 heterodimerization can define ER+/HER2-low breast cancer as a distinct biological entity

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**Background:** HER2-low breast cancer is emerging as a specific subtype of breast tumor against which trastuzumab-containing antibody-drug conjugates, specifically trastuzumab-deruxtecan, are effective. However, attempts to define HER2-low breast tumors as a unique biological entity have thus far concluded that low HER2 expression does not categorize these tumors as having distinct molecular features beyond low immunohistochemical (IHC) cytoplasmic membrane expression of HER2. Our aim in this study is to characterize these tumors as separate biological entities and establish whether there are other mechanisms at play in these tumors that can be exploited as therapeutical approaches beyond trastuzumab-deruxtecan.

**Methods:** We have selected a cohort of patients within the large population-based SCAN-B prospective study. We included all patients diagnosed in Region Skåne between 2010 and 2014 with a follow-up of $\geq$ 5 years. We only included tumors with histology of invasive ductal carcinoma (IDC) to ensure homogeneity. All included cases had complete RNA-sequencing profiling. Tumors were ER positive, defined as IHC staining $\geq$10% of cells, HER2 negative (0-1+ by IHC or 2+ and no amplification by ISH). The HER2-low disease included all cases with HER2 IHC staining scores of 1+ or 2+ with no gene amplification, per international guidelines.

**Results:** We have identified and included 1299 patients in this cohort. As with earlier described cohorts, HER2-low tumors do not show a worse prognosis regarding breast