effectiveness compared to decision making based solely on clinicopathologic factors.
Methods: We retrospectively analyzed data from a Romanian cohort of 1203 N0 and 230 N1, cM0/ER+/HER2- early breast cancer patients who underwent Oncotype DX testing between 2012 and 2024. All N1 patients were ≥ 55 years old and were considered to be postmenopausal. Patients were stratified based on their Recurrence Score (RS) in low (RS 0–25) and high (RS >25) genomic risk. Additionally, we conducted subgroup analysis for N0 patients based on their clinical risk classification as defined by the TAILORx and NATALEE trials.
Results: In breast cancer patients with ER+/HER2-/N0, cM0 tumors, RS 0–25 was reported in 998 (83%) while RS >25 in 205 (17%) of them. Among patients classified as high clinical risk(n = 491) based on TAILORx criteria, 357 (73%) had a RS 0–25. Conversely, in low clinical risk patients (n = 712), RS >25 was reported in 71 cases (10%). For N0 patients meeting high clinical risk criteria eligible for the NATALEE trial(n = 636), 463 (73%) had a RS 0–25. In N1 patients aged ≥ 55 years (n = 230), RS 0–25 was reported in 273 (89%) and RS>25 in 19(8%) of cases.
Conclusions: Oncotype DX consistently demonstrates its clinical utility by stratifying the majority of node-negative (N0) and postmenopausal node-positive (N1) patients as having low genomic risk (RS 0–25). This underscores its value in identifying patients with high clinical risk who might otherwise have been overtreated in the absence of genomic insights. Simultaneously, the test identifies patients with low clinical risk but high genomic risk (RS>25), who may have been undertreated if treatment decisions were solely based on clinical factors. By utilizing Oncotype DX to optimize treatment strategies, cost-effectiveness can be achieved ensuring that healthcare resources are used more efficiently while avoiding overtreatment.

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Reproducibility of equivocal HER2 in situ hybridisation (ISH) results in invasive breast cancer, and correlation with HER2 mRNA expression as determined by Mammatyper®

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Goals: Invasive breast cancers (IBC) with an equivocal HER2 immunohistochemical (IHC) score are subjected to in situ hybridization (ISH). Dual-probe ISH evaluates the mean *HER2* and CEP17 copy numbers and the *HER2*/CEP17 ratio, to exclude or confirm *HER2* amplification. IBCs are classified in five ISH groups, based on the 2018 ASCO/CAP guidelines, with ISH groups 2, 3 and 4 representing equivocal results. The goal of this study was to evaluate the reproducibility of HER2 ISH groups 3 and 4, and to correlate their HER2 mRNA expression level with the integrated IHC/ISH result.
Methods: Dual-probe ISH slides of 41 IBCs with an originally reported equivocal ISH group 3 or 4 result were re-evaluated by two independent observers, blinded for the original results. Five amplified and four non-amplified IBCs were added as controls. mRNA extraction from micro-dissected tumour cells was performed. For 32 tumours with equivocal ISH, there was sufficient mRNA to allow for RT-qPCR by using the Mammatyper® assay.
Results: HER2 ISH for amplified (ISH group 1; n = 5) and non-amplified (ISH group 5; n = 4) IBC shows no or limited inter-observer variability (80–100% concordance). Reproducibility of ISH groups 3 (n = 13) and 4 (=28) was limited: 9 (22%) and 18 (44%) out of these 41 equivocal ISH cases were classified differently after recounting by observers 1 and 2, respectively. The concordance between the integrated IHC/ISH HER2 result and the Mammatyper® HER2 status was 56% (18/32) for the original report, 59% (19/32) for observer 1, 72% (23/32) for observer 2 and 63% (20/32) for the majority opinion. Based on the majority opinion of three observers, 7 patients would have been reconsidered HER2-negative, and 1 patient would have been reconsidered HER2-positive, resulting in a treatment change for 8 out of 41 patients (20%).
Conclusions: The reproducibility of equivocal ISH groups is limited. Although HER2 ISH has long been considered as the gold standard to establish the HER2 status in IBC, the equivocal 'grey zone' between amplified and non-amplified IBCs is prone to inter-observer variability. Solutions to tackle this problem could include the use of at least three different observers for ISH evaluation, and/or assessment of the HER2 mRNA level as an alternative method.

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P053
Genomic profiling of metastatic phyllodes tumors identify potential therapeutic strategies

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Goals: Molecular targeted therapy shows promise as an additional treatment option for patients with metastatic phyllodes tumors (PTs). However, our current understanding of the molecular biology behind this therapy is not yet sufficient to fully support its use. Genetic analysis will potentially supplement classical histologic examination to improve our management of PTs. We try to find some druggable genome for future therapeutic intervention.
Methods: We obtained formalin-fixed, paraffin-embedded (FFPE) tissue samples from 10 patients diagnosed with breast phyllodes tumors. Among these patients, 6 had experienced metastasis, while the other 4 showed no signs of metastasis during follow-up. The samples were subsequently analyzed using bulk RNA sequencing.
Results: Principal component analysis (PCA) revealed distinct gene expression patterns between patients with and without metastasis. Volcano plots further highlighted significant upregulation and downregulation of specific genes when comparing the metastatic and non-metastatic groups. Gene Set Enrichment Analysis (GSEA) identified the ubiquitin–**26S proteasome** pathway as being associated with metastasis. Leading-edge analysis further demonstrated that AKT1 and mTOR were overexpressed in the metastatic group.
Conclusions: This study underscores the importance of integrating genomic analysis with traditional histological assessments to refine the diagnosis and treatment strategies for PTs. Identifying druggable genomic targets, including the ubiquitin–**26S proteasome** pathway, as well as **AKT1** and **mTOR**, presents promising opportunities for future therapeutic interventions, with the potential to improve outcomes for patients with metastatic PTs.

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