



Immune profiling in residual disease: refining risk stratification after neoadjuvant therapy in HER2-positive breast cancer

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The biological behavior of human epidermal growth factor receptor 2 (HER2)-positive early breast cancer (EBC) under the selective pressure of neoadjuvant HER2-directed therapy continues to reveal key insights into therapeutic sensitivity, residual disease (RD) biology, and long-term prognosis. The study by Fernandez-Martinez *et al.* (1) is among the most comprehensive investigations to date, uniquely integrating gene expression profiling from 452 patients with RD, including 169 with matched pre- and post-treatment samples, across four pivotal neoadjuvant trials. This paired-sample design, rare in HER2-positive disease, provides a powerful opportunity to examine how tumor-intrinsic and immune-related features evolve during treatment and how these dynamic changes influence event-free survival (EFS).

A central finding is the marked shift in intrinsic subtype following neoadjuvant HER2 blockade. At baseline, HER2-enriched tumors represented half of all samples, yet in RD, the most common subtype became Normal-like (49.1%), followed by Luminal A (18.9%). This transition was accompanied by significant reductions in *ERBB2* expression,

HER2 amplicon correlation, and PAM50 proliferation scores, highlighting a broad phenotypic remodeling under therapy. Interestingly, despite the shift toward luminal transcriptional profiles, ESR1 and PGR expression remained largely unchanged, suggesting heterogeneous mechanisms underlying treatment-induced reprogramming. This phenomenon aligns with prior descriptions of subtype drift following HER2-targeted therapy (2,3).

The predominance of Normal-like classification in RD likely reflects not only biological changes but also decreased tumor cellularity and increased stromal content. The authors appropriately excluded pre-treatment Normal-like tumors from analysis, recognizing that this classification often indicates low tumor purity. RNA-seq-based tumor purity estimates confirmed that Normal-like samples showed significantly lower purity than other post-treatment intrinsic subtypes, underscoring the importance of considering tumor cellularity when interpreting molecular classifications in RD specimens.

Equally important is the demonstration of adaptive

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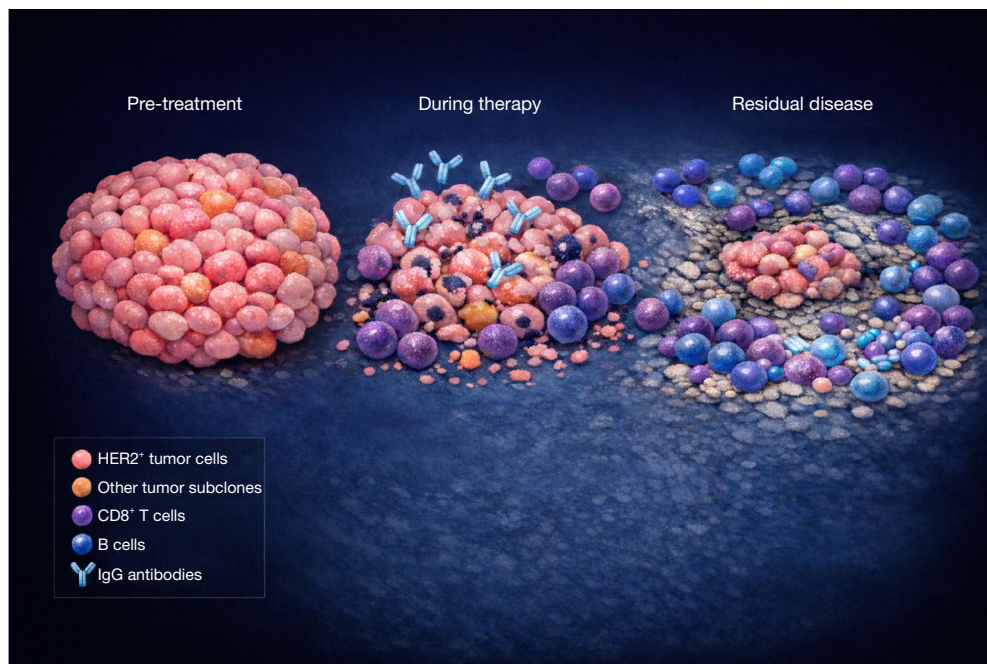


Figure 1 Biological evolution during HER2-directed neoadjuvant therapy. Schematic illustration of temporal biological changes in HER2-positive breast cancer during neoadjuvant therapy. Left panel: pre-treatment tumor dominated by HER2-positive cells (pink) with minimal immune infiltration. Center panel: during therapy showing HER2-targeted antibodies (blue Y-structures) engaging tumor cells and initiating immune cell recruitment (CD8⁺ T cells in purple, B cells in blue). Right panel: residual disease demonstrating phenotypic shift from 50.3% HER2-enriched to 49.1% Normal-like subtypes, *ERBB2* downregulation, increased cellular heterogeneity (orange subclones), and robust adaptive immune activation with dense B cell, CD8⁺ T cell, and IgG infiltration. This biological transformation underlies the superior prognostic performance of immune signatures (C-index: 0.77) when assessed in post-treatment specimens. HER2, human epidermal growth factor receptor 2.

immune activation in RD samples, with significant increases in B-cell, immunoglobulin G (IgG), CD8 T-cell, NK-cell, and T-cell cooperation signatures (*Figure 1*). These observations challenge the traditional notion that RD simply represents immune-resistant tumor clones and instead suggest a more complex interplay between tumor cell elimination and immune infiltration. The upregulation of adaptive immune signatures suggests that HER2-directed therapy may prime the tumor microenvironment for immune recognition, even in cases where complete pathologic response is not achieved. This finding is consistent with accumulating evidence that HER2-targeted antibodies exert their effects not only through direct anti-tumor mechanisms but also through antibody-dependent cellular cytotoxicity and other immune-mediated processes (4,5).

Consistent with results from the KATHERINE biomarker analyses, programmed death ligand-1 (PD-L1) (CD274) expression did not differ significantly pre- and

post-treatment (6), highlighting that immune activation in RD is not necessarily paralleled by upregulation of immune checkpoints. This suggests that the immune response in RD may be qualitatively different from that typically associated with checkpoint inhibitor-responsive tumors, with potential implications for the design of immunotherapy-based escalation strategies in this setting.

From a prognostic standpoint, the study reveals a striking temporal pattern: baseline tumor-intrinsic biology is more prognostic before treatment, whereas immune-related signatures dominate prognostication in RD. Baseline HER2-enriched and basal-like tumors were associated with worse outcomes, consistent with prior analyses of CALGB 40601 (7), NeoALTTO (8), and NSABP B-41 (9). The HER2-Enriched subtype, while associated with higher pathologic complete response (pCR) rates (10), paradoxically confers worse prognosis in patients with RD (7), highlighting the importance of response as a key

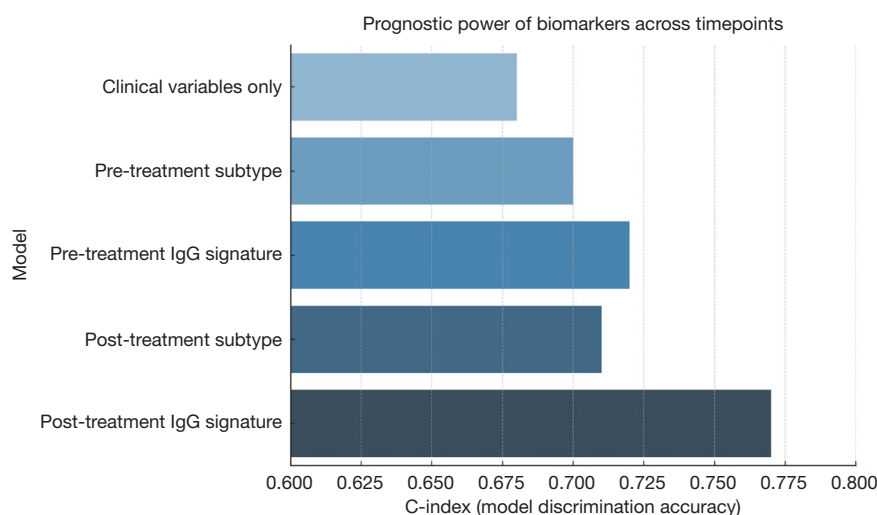


Figure 2 Prognostic performance of biomarkers assessed at different time points. Comparison of C-index values for five multivariable Cox models predicting event-free survival in 452 HER2-positive early breast cancer patients with residual disease (pooled from CALGB 40601, PAMELA, NeoALTTO, NSABP B-41). All models include baseline clinical variables (treatment arm, tumor size, nodal status). Models incorporating: (I) clinical variables only (C-index: 0.68), (II) pre-treatment intrinsic subtype (C-index: 0.70), (III) pre-treatment IgG signature (C-index: 0.72), (IV) post-treatment intrinsic subtype (C-index: 0.71), and (V) post-treatment IgG signature (C-index: 0.77, best model). Vertical dashed line marks C-index 0.70 threshold for good discrimination. The analysis demonstrates that tumor-intrinsic biomarkers perform better at baseline while immune signatures demonstrate superior prognostic value when assessed in residual disease. Patients with high post-treatment IgG expression showed 90.6% *vs.* 68% 5-year event-free survival compared to low IgG tertile. HER2, human epidermal growth factor receptor 2; IgG, immunoglobulin G.

prognostic determinant and the biological heterogeneity within this molecular subgroup.

However, in RD, virtually all evaluated immune signatures predicted EFS more strongly than tumor-intrinsic markers. The most powerful predictor was the post-treatment IgG signature, which, when incorporated into a multivariable model with clinical variables, achieved a C-index of 0.77, the highest across all models. Patients in the highest IgG tertile exhibited outstanding 5-year EFS (90.6%), whereas those in the lowest tertile had substantively poorer outcomes (68%). This striking difference suggests that B-cell infiltration and humoral immune responses play a critical protective role following HER2-directed therapy (*Figure 2*). Beyond confirming previously described biological and immune changes after neoadjuvant HER2-directed therapy, these findings provide a critical conceptual advance by demonstrating that the prognostic relevance of biomarkers in HER2-positive EBC is highly dependent on the timing of assessment. While tumor-intrinsic features retain prognostic value when measured at baseline, their discriminatory performance markedly diminishes in RD. In contrast, immune-related

gene expression signatures, particularly B-cell-related and IgG signatures, emerge as the dominant determinants of long-term outcomes when assessed post-treatment. This temporal shift reframes RD not merely as a surrogate of treatment resistance, but as a biologically distinct state in which host immune surveillance plays a central role in prognosis.

Several prior studies have reported biological and immune remodeling following neoadjuvant HER2-directed therapy, supporting the consistency of these findings. Translational analyses from trials such as NeoALTTO (8) and CALGB 40601 (7) have demonstrated dynamic changes in immune-related gene expression and tumor biology during treatment. In this context, the work by Fernandez-Martinez *et al.* extends the existing literature by showing that immune-related signatures assessed in RD, rather than baseline tumor-intrinsic features, provide superior prognostic stratification.

Potential biological mechanisms underlying immune activation in RD include treatment-induced immunogenic cell death, enhanced antigen presentation, and antibody-dependent cellular cytotoxicity mediated by HER2-targeted

agents. Even in the absence of complete pathological response, sustained adaptive immune surveillance may contribute to long-term disease control, providing a plausible biological framework to explain why immune-based biomarkers outperform tumor-intrinsic features in post-neoadjuvant prognostic models.

The prognostic superiority of immune signatures in RD compared to baseline likely reflects several biological phenomena. First, immune activation in RD may represent a successful anti-tumor immune response that, while insufficient to eliminate all malignant cells, continues to exert immune surveillance and control RD. Second, the presence of tertiary lymphoid structures and organized B-cell and T-cell cooperation may indicate an ongoing adaptive immune response capable of recognizing and eliminating micrometastatic disease. Third, immune-rich RD may be biologically distinct from immune-poor RD, representing tumors that remain immunogenic despite incomplete response to neoadjuvant therapy.

These findings have immediate translational implications. Current post-neoadjuvant management for HER2-positive RD centers on escalation with trastuzumab emtansine (T-DM1), as established by the landmark KATHERINE trial (2). In KATHERINE, T-DM1 reduced the risk of invasive disease recurrence or death by 50% compared to continued trastuzumab in patients with RD after neoadjuvant chemotherapy plus trastuzumab. However, not all RD carries equal biological risk, and universal escalation may lead to overtreatment, toxicity, and increased financial burden. Notably, even in the control arm of KATHERINE, a substantial proportion of patients with RD did not experience recurrence during the follow-up period, suggesting biological heterogeneity in RD that merits further characterization.

The immune-driven heterogeneity described here argues that RD immune profiling may refine which patients truly require therapeutic escalation. Patients with immune-hot RD, characterized by high B-cell infiltration and IgG signatures, may have sufficiently favorable prognosis to potentially de-escalate therapy, though this hypothesis requires prospective validation. Conversely, immune-cold RD may identify those who stand to benefit most from emerging strategies such as trastuzumab deruxtecan (T-DXd), tucatinib combinations, or immunotherapy-based escalation currently being evaluated in trials like DESTINY-Breast 05 (11), CompassHER2 RD, and ASTEFANIA.

The study's conclusions are also consistent with

contemporary real-world evidence. Recent investigations, including the large real-world analysis by Antonini *et al.* (12) and their systematic review and meta-analysis (13), have demonstrated that immunohistochemical (IHC) biomarker changes after neoadjuvant chemotherapy are frequent, clinically relevant, and associated with survival. These works highlight that shifts in estrogen receptor (ER), progesterone receptor (PR), and HER2 status—not only genomic signatures—carry prognostic value and should be systematically reassessed in RD. The congruence between transcriptomic changes observed in the present study and IHC conversion described in clinical practice reinforces that RD represents a biologically distinct state shaped by treatment response and selective pressure. HER2 loss or downregulation occurs in a subset of RD cases, with potential implications for the efficacy of continued HER2-directed therapy in the adjuvant setting.

Taken together, these findings strongly support integrating both molecular and IHC biomarker reassessment into routine post-neoadjuvant workflows. Current guidelines from the National Comprehensive Cancer Network (NCCN) do not mandate biomarker reassessment in RD, though this practice is increasingly adopted in academic centers. The data presented by Fernandez-Martinez *et al.*, combined with real-world evidence of phenotypic conversion, provide a compelling rationale for systematic RD characterization to inform post-neoadjuvant treatment decisions.

The strength of Fernandez-Martinez *et al.*'s work lies in its rigorous methodology: harmonized RNA-seq pipelines, large multi-trial sample size, paired tumor analyses, and robust multivariable modeling. The inclusion of samples from four independent trials with different designs—including chemotherapy-free dual HER2 blockade in PAMELA and various combinations in the other three trials—enhances the generalizability of findings. The use of standardized bioinformatics pipelines and batch correction methods ensured comparability across platforms and studies, addressing a common challenge in pooled analyses.

Limitations are acknowledged and include heterogeneity across trials, the pre-T-DM1 treatment era, and the absence of residual cancer burden (RCB) data. The trial heterogeneity, while potentially limiting, also represents a strength in demonstrating consistency of biological findings across different treatment regimens. The pre-T-DM1 era is relevant because adjuvant therapies differed from current standards; most patients received single-agent trastuzumab rather than T-DM1 in the adjuvant setting. However, this

does not diminish the biological insights regarding RD characterization and prognostication.

The absence of RCB is particularly notable. RCB has emerged as an important prognostic tool, categorizing patients based on the extent of RD in breast and lymph nodes (14). However, RCB assessment requires standardized pathologic evaluation and is not universally performed. Moreover, emerging data suggest that biomarker-driven models—especially immune-based ones—may ultimately complement or surpass RCB in prognostic accuracy. The current study demonstrates that molecular characterization of RD provides prognostic information with a C-index of 0.77 for the best model, which compares favorably to previously reported performance metrics for RCB alone. Future studies integrating RCB with molecular biomarkers may achieve even greater prognostic precision, enabling truly personalized post-neoadjuvant management.

An important consideration is the potential clinical implementation of RD immune profiling. While gene expression assays require specialized processing and are more costly than routine IHC, the prognostic value demonstrated here may justify their use in selected cases. Alternatively, surrogate IHC markers of immune infiltration, such as stromal tumor-infiltrating lymphocytes (TILs), CD8, or CD20, might provide accessible approximations of the immune signatures identified in this study. Previous work has shown correlation between TILs and immune gene expression signatures (15), though gene expression signatures generally provide superior prognostic discrimination (16). Development of streamlined, clinically feasible assays for immune characterization in RD represents an important area for future investigation. From a pragmatic standpoint, surrogate and more accessible biomarkers—such as stromal TILs, CD8-positive cytotoxic T cells, and CD20-positive B cells assessed by immunohistochemistry—may represent feasible alternatives or complementary approaches. These tools could enable broader risk stratification across diverse healthcare settings while preserving the central biological insights revealed by high-dimensional immune profiling.

Collectively, the study advances a compelling paradigm: while baseline tumor-intrinsic subtype remains important for predicting pCR and initial risk stratification, post-treatment immune activation within RD is the dominant determinant of long-term outcomes in HER2-positive EBC. This temporal shift in prognostic determinants has profound implications for biomarker assessment strategies. It suggests that optimal risk prediction requires dynamic,

longitudinal assessment rather than reliance solely on baseline tumor characterization. This paradigm shift reinforces the concept that cancer is not a static entity but an evolving ecosystem shaped by therapeutic pressure and host immune responses.

The findings also raise intriguing questions about the mechanisms underlying immune activation in RD. Does HER2-directed therapy directly stimulate immune responses through immunogenic cell death, antigen release, or other mechanisms? Are immune-rich RD samples enriched for specific tumor neoantigens or germline immune-related genetic variants? Does the composition of the baseline immune microenvironment predict which tumors will develop immune-activated RD? Addressing these questions will require integrative analyses incorporating genomics, proteomics, and spatial profiling of the tumor microenvironment.

Looking forward, this work provides a strong rationale for integrating RD molecular profiling into future HER2-positive treatment algorithms. As post-neoadjuvant strategies evolve, immune-informed personalization will likely become essential to balancing escalation and de-escalation in HER2-positive disease. Ongoing and future trials should incorporate RD biomarker assessment to enable identification of predictive markers for novel therapies. For example, immune-cold RD might preferentially benefit from ADC escalation or addition of other targeted agents, while immune-hot RD might be candidates for de-escalation or immunotherapy-based approaches.

Fernandez-Martinez *et al.* deliver a landmark contribution to the field, offering biologically grounded, clinically actionable insights that will shape future research and potentially upcoming clinical practice guidelines. Their work underscores that RD is not merely the remainder of disease after therapy—it is a new biological state, distinct from the pre-treatment tumor, shaped by therapeutic selection pressure and characterized by unique prognostic determinants. The immune contexture of RD may provide the clearest lens through which to predict patient outcomes and tailor treatment, moving the field toward truly precision medicine in HER2-positive breast cancer.

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