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# RE: RNA Disruption Assay as a Biomarker of Pathological Complete Response in Neoadjuvant Trastuzumab-Treated Human Epidermal Growth Factor Receptor 2–Positive Breast Cancer

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In Pritzker et al. (1), RNA disruption assay (RDA) was found to be a novel, early, on-treatment assay that has potential for clinical utility in response-guided primary systemic breast cancer (BC) therapy. This assay quantifies early chemotherapy-induced RNA disruption in breast tumors and has shown clinical utility by predicting pathological complete response (pCR) rates in the neoadjuvant treatment of BC patients (1–4). High RDA scores above 7 are associated with a higher chance of pCR than lower RDA scores. RDA has not yet been validated specifically in an independent cohort of human epidermal growth factor receptor 2 (HER2)–positive BC.

TCHL (NCT01485926) was a phase II neo-adjuvant study assessing TCH (docetaxel, carboplatin, and trastuzumab) and TCHL (TCH and lapatinib) in early-stage HER2-positive BC (5). The study's primary endpoint was to compare rates of pCR between the TCH and TCHL arms. Of the 78 patients enrolled, 23 had a core biopsy taken by an interventional radiologist, 20 days post-cycle 1 of either TCH/TCHL therapy.

These samples potentially offer a unique insight into the molecular and pathological changes that tumors undergo during the patients' initial treatment and how they relate to the final pCR status of patients. Pathological review of these samples (Figure 1, A and B) indicates that in 10 patients who later achieved a pCR at surgery, five had no tumor present in their on-treatment tumor biopsy sample and the average tumor content in those patients who achieved pCR was 10% ± 15%. In patients who either had a partial response or no response (n = 13 evaluable patient samples), the average tumor content in the on-treatment biopsy sample was 60% ± 23%, which was higher than that observed in the pCR samples ($P = .000001$). This interesting finding highlights the immediate impact that trastuzumab has on tumor content in HER2-positive BC patients.

In this cohort, RDA scores of greater than 7 in the on-treatment biopsy sample indicated a higher chance of pCR in response to TCH or TCHL chemotherapy (6 of 7 patients with an RDA >7 subsequently achieved a pCR vs 1 of 10 patients with a score <7). Figure 1C indicates that those patients who had a pCR (n = 10) had an average RDA score of 10.2 ± 5.1, which was higher than in those patients who had a partial or no response at subsequent surgery (n = 10; RDA score of 5.4 ± 2.2, P = .025).

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A

| TCHL ID | % tumor | % stroma | RDI score | Response | Arm |
|---|---|---|---|---|---|
| 2 | 69 | 31 | 4.9 | NR | H |
| 12 | 59 | 41 | 3.8 | NR | L |
| 6 | 45 | 55 | 3.0 | NR | H |
| 42 | 80 | 20 | n/a | NR | L |
| 4 | 68 | 35 | 5.8 | PR | L |
| 7 | 21 | 79 | 5.1 | PR | L |
| 11 | 58 | 43 | 10.4 | PR | L |
| 29 | 68 | 33 | 3.4 | PR | L |
| 37 | 59 | 41 | n/a | PR | L |
| 39 | 91 | 9 | 5.7 | PR | H |
| 50 | 58 | 42 | n/a | PR | H |
| 54 | 43 | 58 | 5.5 | PR | L |
| 87 | 5 | 95 | 6.8 | PR | L |
| 8 | 50 | 50 | 9.3 | pCR | L |
| 14 | 20 | 80 | 7.6 | pCR | H |
| 20 | 0 | 100 | 9.7 | pCR | L |
| 31 | 15 | 78 | 7.5 | pCR | L |
| 32 | 0 | 100 | n/a | pCR | H |
| 44 | 5 | 95 | n/a | pCR | L |
| 45 | 5 | 95 | 13.9 | pCR | H |
| 61 | 0 | 100 | n/a | pCR | L |
| 66 | 0 | 100 | 3.8 | pCR | L |
| 76 | 0 | 100 | 19.5 | pCR | H |

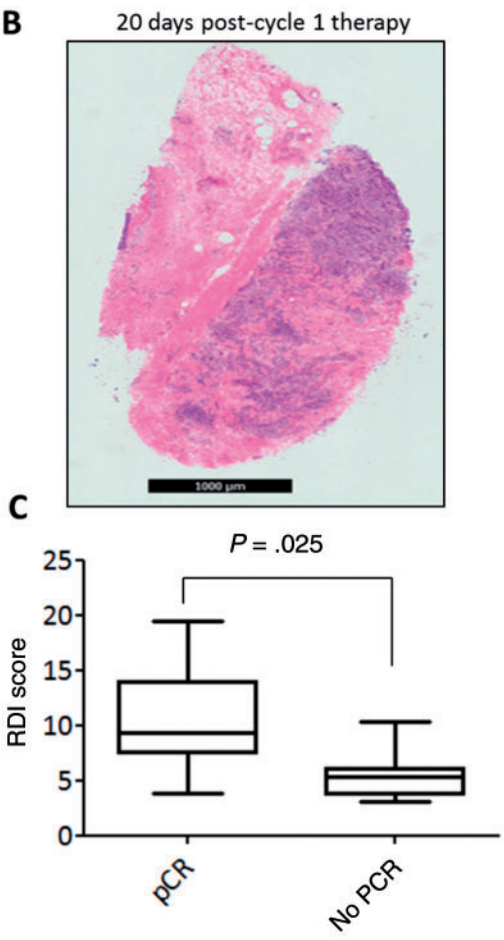


**Figure 1.** Biopsy cores taken 20 days after the initiation of neoadjuvant therapy by a radiologist were embedded in optical coherence tomography and the samples were cryo-sectioned. A single 3 μM section was taken for haematoxylin and eosin (H&E) staining and analysis and the adjacent ten 10 μm sections were cut and stored in a chilled cryovial. Following this, a second 3 μM section was then cut for H&E staining. Cut sections were stored at -80°C. We also performed RNA extractions on the cut sections using the QIAGEN RNAEasy kit, and quantified them on the Bioanalyser using the Agilent RNA 6000 Nano kit. RNA quantification files were then sent to RNA diagnostics for analysis using RNA disruption assay (RDA) as previously described. **A and B)** Pathological analysis of the H&E stained sections was conducted to assign a percentage tumor and a percentage stroma score to each sample. **C)** Correlation of RDA scores and pCR in 17 patient samples. *P* values were calculated using the Student's t test with a value of less than .05 being considered statistically significant. NR = no response; pCR = pathological complete response; PR = partial response.

Our results support the work of Pritzker et al. (1) and demonstrate the benefit of obtaining core tumor biopsies after cycle 1 of neoadjuvant treatment in HER2-positive BC patients for further study of the clinical utility of RDA. Pathological analysis of these samples will demonstrate the effect of treatment, and the use of RDA score may allow for easy and robust stratification of patients into two groups with and without a high likelihood of pCR at subsequent surgery. The RDA score may be a useful early prognostic and predictive biomarker of the likelihood of later pCR, with the potential to guide subsequent neoadjuvant treatment in an attempt to optimize pCR rates.

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## Notes

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