JAMA Oncology | Brief Report

# Association of T-Cell Receptor Repertoire Use With Response to Combined Trastuzumab-Lapatinib Treatment of HER2-Positive Breast Cancer

## Secondary Analysis of the NeoALTTO Randomized Clinical Trial

Ryan L. Powles, MS; David Redmond, PhD; Christos Sotiriou, MD, PhD; Sherene Loi, MD, PhD; Debora Fumagalli, MD, PhD; Paolo Nuciforo, MD; Nadia Harbeck, MD, PhD; Evandro de Azambuja, MD, PhD; Severine Sarp, MD, PhD, MSc; Serena Di Cosimo, MD; Jens Huober, MD; Jose Baselga, MD, PhD; Martine Piccart-Gebhart, MD, PhD; Olivier Elemento, PhD; Lajos Pusztai, MD, PhD; Christos Hatzis, PhD

**IMPORTANCE** Dual anti-HER2 blockade increased the rate of pathologic complete response (pCR) in the Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimisation (NeoALTTO) trial, and high immune gene expression was associated with pCR in all treatment arms. So far, no marker has been identified that is specifically associated with the benefit from dual HER2 blockade.

**OBJECTIVE** To examine if use of the T-cell β chain variable genes adds to the potential association of immune gene signatures with response to dual HER2 blockade.

**DESIGN, SETTING, AND PARTICIPANTS** In the NeoALTTO trial, HER2-positive patients recruited between January 5, 2008, and May 27, 2010, were treated with paclitaxel plus either lapatinib or trastuzumab or both as neoadjuvant therapy. In this study, RNA sequencing data from baseline tumor specimens of 245 patients in the NeoALTTO trial were analyzed and reads were aligned to *TRBV* gene reference sequences using a previously published Basic Local Alignment Search Tool T-cell receptor mapping pipeline. Total *TRBV* gene use, Shannon entropy, and gene richness were calculated for each tumor, and nonnegative matrix factorization was used to define *TRBV* co-use metagenes (TMGs). The association between *TRBV* metrics, tumor genomic metrics, and response was assessed with multivariable logistic regression. Statistical analysis was performed from January 23 to December 2, 2017.

**MAIN OUTCOMES AND MEASURES** The association between *TRBV* use metrics and pCR.

**RESULTS** Among the 245 women with available data (mean [SD] age, 49 [11] years), total *TRBV* use correlated positively with a gene expression signature for immune activity (Spearman ρ = 0.93; $P < .001$). High use of *TRBV*11-3 and TMG2, characterized by high use of *TRBV*4.3, *TRBV*6.3, and *TRBV*7.2, was associated with a higher rate of pCR to dual HER2-targeted therapy (*TRBV*11-3 interaction: odds ratio, 2.63 [95% CI, 1.22-6.47]; $P = .02$; TMG2 interaction: odds ratio, 3.39 [95% CI, 1.57-8.27]; $P = .004$). Immune-rich cancers with high TMG2 levels (n = 92) had significantly better response to dual HER2-targeted treatment compared with the single therapy arms (rate of pCR, 68% [95% CI, 52%-83%] vs 21% [95% CI, 10%-31%]; $P < .001$), whereas those with low TMG2 levels did not benefit from dual therapy. High TMG2 levels were also associated with a higher rate of pCR to the combined therapy in immune-poor tumors (n = 30; pCR, 50% [95% CI, 22%-78%] vs 6% [95% CI, 0%-16%]; $P = .009$).

**CONCLUSIONS AND RELEVANCE** Use patterns of *TRBV* genes potentially provide information about the association with response to dual HER2 blockade beyond immune gene signatures. High use of *TRBV*11.3 or *TRBV*4.3, *TRBV*6.3, and *TRBV*7.2 identifies patients who have a better response to dual HER2 targeted therapy.

**TRIAL REGISTRATION** ClinicalTrials.gov Identifier: NCT00553358

*JAMA Oncol*. 2018;4(11):e181564. doi:10.1001/jamaoncol.2018.1564
Published online June 14, 2018.

Supplemental content

**Author Affiliations:** Author affiliations are listed at the end of this article.

**Corresponding Author:** Christos Hatzis, PhD, Breast Medical Oncology, Yale Cancer Center, Yale School of Medicine, 333 Cedar St, PO Box 208032, New Haven, CT 06520 (christos.hatzis@yale.edu).

The Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimisation (NeoALTTO) trial demonstrated that trastuzumab, a monoclonal antibody against HER2, and lapatinib, a small-molecule tyrosine kinase inhibitor of the EGFR and HER2 family, administered together with paclitaxel chemotherapy in the neoadjuvant setting increased rates of pathologic complete response (pCR) by approximately 20% in patients with early-stage HER2-positive breast cancer compared with either treatment alone.[1] A higher tumor-associated lymphocyte count[2] and a higher expression of immune signatures[3] were associated with higher rates of pCR but not with specific benefit from dual treatments. In this study, we examine whether specific T-cell receptor (TCR) species could indicate an association with response to a specific treatment arm.

The TCR recognizes peptide antigens bound to major histocompatibility complex molecules and is composed of 2 different protein chains, α and β (**Figure 1**A). Each TCR possesses unique antigen specificity determined by the structure of the antigen-binding site formed by the α and β chains. T-cell receptor diversity arises from the random combinatorial joining of variable, joining, and diversity genes to generate each complete chain that defines antigen specificity. We estimate the diversity of T-cell populations in the tumor microenvironment by assessing the messenger RNA expression of the T-cell receptor β chain variable (*TRBV*) gene from previously published RNA sequencing data[3] of pretreatment biopsies of patients in the NeoALTTO trial.

### Key Points

**Question** Does the use of specific T-cell receptor β variable region (*TRBV*) genes add to the association of immune markers with anti-HER2 therapy in HER2-positive breast cancer?

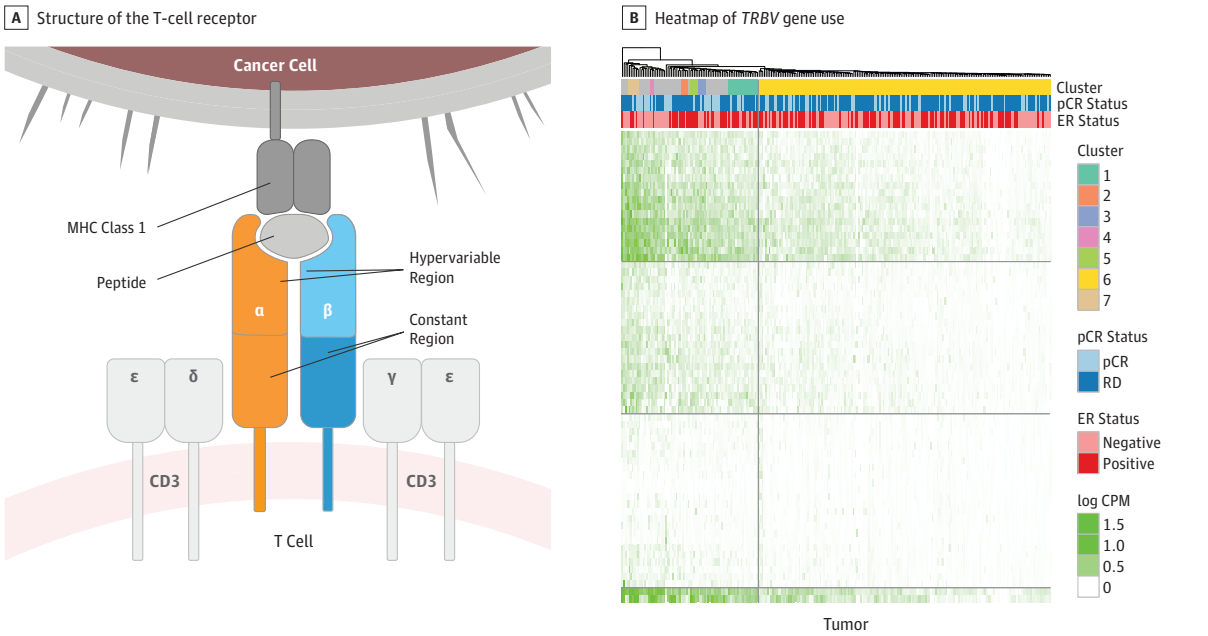
**Findings** In this biomarker study of data from the NeoALTTO trial, high levels of a *TRBV* metagene consisting of 3 individual *TRBV* genes identified a subgroup of patients who respond poorly to single HER2 blockade treatments, irrespective of overall immune signature expression, but who experience a significant benefit from dual anti-HER2 blockade.

**Meaning** Direct measurements of *TRBV* use is an independent biomarker for response to dual HER2-targeted therapy.

## Methods

A total of 455 patients with HER2-positive early-stage breast cancer were randomized to 3 treatment arms in the NeoALTTO trial.[1] Baseline biopsies were performed on 423 of these patients, and RNA was successfully extracted from 254 patients, of which 245 had matching clinical data (eFigure 1 in the Supplement).

**Figure 1. T-Cell Receptor (TCR) Structure and T-Cell β Chain Variable (*TRBV*) Gene Use**




A, Structure of the TCR. The TCR recognizes peptide antigens presented by the major histocompatibility complex (MHC) class 1. The TCR is a heterodimer of 2 subunits, TCRα and TCRβ, with each subunit consisting of a constant region that functions to anchor the receptor to the cell membrane and a hypervariable region that functions in antigen recognition. In this study, the hypervariable regions of the TCRβ subunit were characterized. B, Heatmap of *TRBV* gene use. Heatmap of log counts per million (CPM) of 245 patients across 65 *TRBV* genes. Significantly stable clusters (top row) are identified through bootstrap resampling of hierarchical clustering. Pathologic complete response (pCR; middle row) indicates patients who had a pCR in light blue and those with residual disease (RD) in dark blue. Estrogen receptor (ER) status (bottom row) indicates patients who are ER positive in dark red and patients who are ER negative in light red.

Table. Multivariable Logistic Regression of *TRBV* Features, Clinical Covariates, and Gene Expression Signatures Associated With pCR

| | One-Marker TMG2 Model | | One-Marker *TRBV*11-3 Model | | Two-Marker Model | |
|---|---|---|---|---|---|---|
| Characteristic | OR (95% CI) | *P* Value[a] | OR (95% CI) | *P* Value[a] | OR (95% CI) | *P* Value[a] |
| Covariate term | | | | | | |
| Age | 0.98 (0.96-1.02) | .47 | 0.99 (0.96-1.02) | .60 | 0.99 (0.96-1.02) | .65 |
| ER status | 0.30 (0.14-0.60) | .001 | 0.37 (0.18-0.73) | .005 | 0.32 (0.15-0.66) | .002 |
| Tumor size (≥T3 vs T2) | 0.72 (0.35-1.44) | .36 | 0.70 (0.34-1.40) | .32 | 0.72 (0.35-1.44) | .36 |
| Arm (combination vs single) | 4.51 (2.21-9.59) | <.001 | 3.69 (1.86-7.47) | <.001 | 4.51 (2.61-9.87) | <.001 |
| Grade (1-2 vs 3) | 0.73 (0.37-1.44) | .36 | 0.69 (0.34-1.36) | .28 | 0.68 (0.33-1.35) | .27 |
| Nodal status (N0 or N1 vs ≥N2) | 1.31 (0.55-2.98) | .53 | 1.13 (0.47-2.61) | .77 | 1.27 (0.53-2.95) | .58 |
| TIL | 0.65 (0.41-0.99) | .05 | 0.72 (0.46-1.08) | .13 | 0.68 (0.43-1.04) | .09 |
| Immune[3] | 1.25 (0.76-2.06) | .38 | 1.28 (0.83-2.02) | .27 | 1.05 (0.62-1.78) | .86 |
| GGI | 1.98 (1.24-3.31) | .006 | 1.97 (1.24-3.25) | .005 | 1.95 (1.22-3.25) | .007 |
| Stroma1 | 1.44 (0.95-2.24) | .09 | 1.41 (0.93-2.20) | .12 | 1.46 (0.96-2.28) | .09 |
| TCR terms | | | | | | |
| TMG2 | 0.93 (0.50-1.61) | .80 | NA | NA | 0.96 (0.48-1.78) | .90 |
| TMG2 × arm[b] | 5.38 (2.05-17.38) | .002 | NA | NA | 5.03 (1.62-18.46) | .009 |
| *TRBV*11-3 | NA | NA | 0.99 (0.56-1.69) | .98 | 1.17 (0.61-2.18) | .62 |
| *TRBV*11-3 × arm[b] | NA | NA | 2.72 (1.23-7.04) | .02 | 1.76 (0.70-4.89) | .25 |
| Likelihood ratio test[c] | NA | <.001 | NA | .004 | | <.001 |

Abbreviations: ER, estrogen receptor; GGI, Genomic Grade Index; NA, not applicable; OR, odds ratio; pCR, pathologic complete response; TCR, T-cell receptor; TIL, tumor-associated lymphocyte; TMG, *TRBV* metagene; *TRBV*, TCR β chain variable region.

[a] Calculated using the Wald test.

[b] Indicates interaction term in the model.

[c] Calculated using a $\chi^2$ test for nested models with and without TCR terms used in each model.

Data for multivariable analysis were available for 225 patients (eTable 1 in the Supplement). We adapted a previously published method[4] to map aligned reads from bulk RNA sequencing data against a database of human *TCR* genes to identify use of variable region *TCR* sequences (the software is available at https://github.com/ElementoLab/TCRVseq). The total *TRBV* load, *TRBV* richness, and *TRBV* entropy were used to characterize *TRBV* use in tumors and to identify co-use of *TRBV* metagenes using nonnegative matrix factorization (eAppendix 1 in the Supplement). Features of *TRBV* use were evaluated for association with pCR using multivariable logistic regression adjusted for clinical covariates (eAppendix 2 in the Supplement). This biomarker study was approved by the Human Investigations Committee of Yale Cancer Center. Written informed consent was obtained from all patients at entry into the NeoALTTO trial, which also covered future biomarker research.

## Results

### Characterization of *TRBV* Variant Use

The read length of standard RNA sequencing does not allow reconstructing the entire T-cell β chain; for this reason, we refer to *use* of a specific *TRBV* gene in TCRs in a tumor instead of TCR gene expression. Hierarchical clustering with bootstrap resampling identified a stable cluster of tumors that showed minimal use of most *TRBV* genes (Figure 1B). Patients in this immune cold cluster tended to have a lower rate of pCR compared with the remaining patients (28% [95% CI, 21%-35%] vs 38% [95% CI, 28%-50%]; *P* = .10 determined by use of the Fisher exact test). Overall, a median of 3.85 (range, 0.137-29.8) counts per million of *TRBV* genes were detected across samples, with a median of 53 different variants (range, 5-65 different variants) observed across tumors (eFigure 2 in the Supplement). The overall *TRBV* load was highly correlated with tumor-associated lymphocyte counts (eFigure 3 in the Supplement) and was significantly higher in patients who achieved a pCR (*P* = .009 determined by use of the Wilcoxon rank-sum test).

We found the use of different *TRBV* genes to be highly correlated (median pairwise Pearson correlation, 0.43 [range, -0.09 to 0.82]) (eFigure 4 in the Supplement) and used nonnegative matrix factorization to define 4 metagenes (TMG1-TMG4) that captured the broader *TRBV* use patterns across tumors (eFigures 5 and 6 in the Supplement). Each metagene contained at most 1 gene from each *TRBV* subgroup (eFigure 7 and eTable 2 in the Supplement), implying less than 75% sequence identity between genes of a given metagene. All 4 metagenes representing median use of the *TRBV* genes were significantly higher in patients with pCR (eFigure 8 in the Supplement).

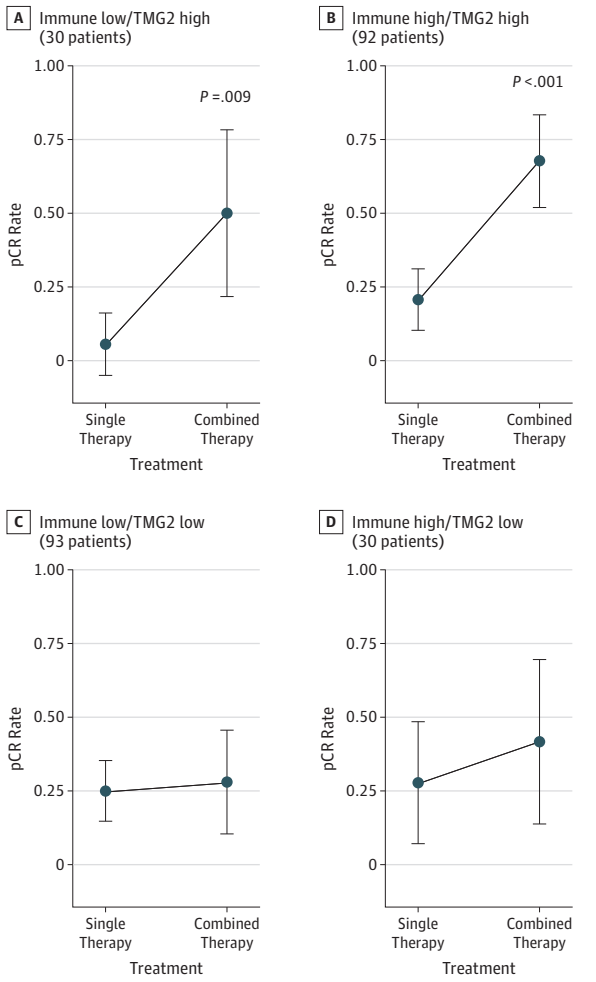
Metrics for *TRBV* were generally correlated with other measures of immune activity but not with the rate of somatic mutation or proliferation metagene expression (eFigures 3 and 9 in the Supplement). Tumor-associated lymphocyte counts were available for 225 tumor samples in the cohort[2] and found to be positively correlated with a previously reported immune signature (ρ = 0.43; *P* < .001), *TRBV* load (ρ = 0.37; *P* < .001), and the levels of all 4 TMGs (TMG1, ρ = 0.22; TMG2, ρ = 0.28; TMG3, ρ = 0.39; and TMG4, ρ = 0.34).

We assessed whether the global *TRBV* metrics, individual *TRBV* gene use, and TMGs, are associated with pCR in estrogen receptor–adjusted logistic regression. Only *TRBV*11-3 use (odds ratio, 1.67 [95% CI, 1.26-2.27]; adjusted *P* = .04) and meta-

gene TMG2 (odds ratio, 1.49 [95% CI, 1.14-1.96]; adjusted $P = .04$) were significantly associated with pCR in the overall cohort (eTable 3 in the Supplement). In multivariable logistic regression that included clinical variables and known prognostic signatures (eAppendix 2 in the Supplement), TMG2 and *TRBV*11-3 had statistically significant treatment-arm interaction terms, indicating that higher use is associated with greater probability of pCR in patients treated with dual HER2 blockade compared with patients in the single treatment arms (**Table**). When both TMG2 and *TRBV*11-3 were considered together, only the TMG2 interaction term for combined therapy vs single treatments remained significant (interaction odds ratio, 5.03 [95% CI, 1.62-18.46]; $P = .009$) (Table).

To examine the added information on association with pCR provided by TMG2, patients were dichotomized (median split) by TMG2 level and by immune signature expression into high and low groups. Patients with high TMG2 levels and immune-rich tumors (92 of 245 [37.6%]) had a significantly higher pCR rate in the dual HER2-targeted arm compared with the other arms (68% [95% CI, 52%-83%] vs 21% [95% CI, 10%-31%]; $P < .001$ determined by use of the Fisher exact test) (**Figure 2**). A high TMG2 level was also associated with a higher rate of pCR to combined therapy in immune-poor tumors (30 of 245 [12.2%]), where the rate of pCR was 6% (95% CI, 0%-16%) with monotherapy vs 50% (95% CI, 22%-78%) with dual HER2 blockade ($P = .009$ determined by use of the Fisher exact test). The interaction of TMG2 by treatment was also significant for the lapatinib vs combined therapy but not for trastuzumab vs combined therapy (eTables 4 and 5 in the Supplement) in 2-marker models, suggesting that the T-cell metagene may be associated with a specific trastuzumab benefit in the context of comparison with combined therapy. High use of the single variable-gene *TRBV*11-3, which is not included in any of the 4 TMGs, showed the same characteristics as TMG2 in immune-rich and immune-poor cancers (eFigure 10 in the Supplement).

**Figure 2. Rates of Pathologic Complete Response (pCR) Compared Between Treatments Dichotomized (Median Split) by Immune Enrichment Signature and *TRBV* Metagene 2 (TMG2) Level**




Significant differences between groups determined by use of the Fisher exact test. Error bars indicate 95% CI.

## Discussion

Our analysis indicates that use of a very small number of *TRBV* genes (*TRBV*4.3, *TRBV*6.3, *TRBV*7.2, and *TRBV*11.3) appears to be associated with selective response to dual treatment with trastuzumab and lapatinib, both in immune-rich and immune-poor cancers. The small number of *TRBV* genes involved suggests the possibility that only a small number of genes, and possibly a small number of TCRs, are needed to estimate the immunogenic activity associated with trastuzumab response and subsequent sensitivity to dual HER2 blockade, even when overall immune infiltration is modest. These *TRBV* genes have very high use in normal CD4-positive follicular helper T cells[5] that have been associated with better outcomes after therapy,[6] which may suggest as a possible mechanism that previous infections in some patients could prime the $T_{FH}$ effector cells for responses that augment the outcome of HER2-targeted therapy regimens.[7]

### Limitations

One limitation of our study is that, owing to the insert size of the RNA sequencing complementary DNA library and the limits on read length in current sequencing technology, we were unable to fully reconstruct the T-cell β chain to identify specific rearranged TCRs from bulk RNA sequencing data. Moreover, these findings are exploratory and will need to be confirmed in additional cohorts, and potentially with dual regimens combining trastuzumab with pertuzumab. Finally, this study was not designed to assess whether the pCR benefit translates to better survival outcomes in these patients.

## Conclusions

Use of a small number of *TRBV* genes potentially provides information about the association with response to dual HER2 blockade beyond immune gene signatures. If confirmed in additional cohorts, the *TRBV* signature could help identify patients who do poorly with single anti-HER2 treatments, but benefit from dual blockade.

## ARTICLE INFORMATION

**Accepted for Publication:** March 20, 2018.

**Published Online:** June 14, 2018. doi:10.1001/jamaoncol.2018.1564

**Author Affiliations:** Breast Medical Oncology, Yale Cancer Center, Yale School of Medicine, New Haven, Connecticut (Powles, Pusztai, Hatzis); Computational Biology and Bioinformatics Program, Yale University, New Haven, Connecticut (Powles); Institute for Computational Biomedicine, Department of Physiology and Biophysics, Weill Cornell Medical College, New York, New York (Redmond, Elemento); Breast Cancer Translational Research Laboratory, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium (Sotiriou, Fumagalli, de Azambuja, Piccart-Gebhart); Division of Cancer Medicine and Research, Peter MacCallum Cancer Center, East Melbourne, Victoria, Australia (Loi); Molecular Oncology Laboratory, Vall d'Hebron Institute of Oncology, Barcelona, Spain (Nuciforo); Department of Obstetrics and Gynecology, University of Munich, Munich, Germany (Harbeck); Novartis AG, Basel, Switzerland (Sarp); Department of Oncology, Istituto Nazionale Tumori, Milan, Italy (Di Cosimo); Department of Obstetrics and Gynecology, University of Ulm, Ulm, Germany (Huober); Breast Medical Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York (Baselga); Caryl and Israel Englander Institute for Precision Medicine, Weill Cornell Medical College, New York, New York (Elemento).

**Author Contributions:** Dr Hatzis had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Mr Powles and Dr Redmond contributed equally to the study.
*Study concept and design:* Powles, Redmond, Di Cosimo, Elemento, Pusztai, Hatzis.
*Acquisition, analysis, or interpretation of data:* Powles, Redmond, Sotiriou, Loi, Fumagalli, Nuciforo, Harbeck, de Azambuja, Sarp, Di Cosimo, Huober, Baselga, Piccart-Gebhart, Elemento, Hatzis.
*Drafting of the manuscript:* Powles, Redmond, Nuciforo, Hatzis.
*Critical revision of the manuscript for important intellectual content:* Powles, Redmond, Sotiriou, Loi, Fumagalli, Harbeck, de Azambuja, Sarp, Di Cosimo, Huober, Baselga, Piccart-Gebhart, Elemento, Pusztai, Hatzis.
*Statistical analysis:* Powles, Redmond, Elemento, Hatzis.
*Obtained funding:* Sotiriou, Pusztai, Hatzis.
*Administrative, technical, or material support:* Fumagalli, Harbeck, de Azambuja, Huober, Pusztai.
*Study supervision:* Loi, Di Cosimo, Baselga, Piccart-Gebhart, Elemento, Hatzis.

**Conflict of Interest Disclosures:** Dr de Azambuja reported receiving travel grants from GlaxoSmithKline and Roche and honoraria from Roche, outside this work. Dr Sarp reported being an employee of Novartis. Dr Baselga reported receiving personal fees from Roche. Dr Piccart-Gebhart reported receiving personal fees from Roche. Drs Sotiriou and Piccart-Gebhart reported being co-inventors of the Genomic Grade Index. No other disclosures were reported.

**Funding/Support:** This project was supported by Investigator Awards from the Breast Cancer Research Foundation and by a Yale Cancer Center Core Grant (NCI P30 CA16359-34) to Drs Hatzis and Pusztai and by a Susan G. Komen Foundation grant to Dr Pusztai.

**Role of the Funder/Sponsor:** The funders/sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

## REFERENCES

1. Baselga J, Bradbury I, Eidtmann H, et al; NeoALTTO Study Team. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial [published correction appears in *Lancet*. 2012;379(9816):616]. *Lancet*. 2012;379(9816):633-640.

2. Salgado R, Denkert C, Campbell C, et al. Tumor-infiltrating lymphocytes and associations with pathological complete response and event-free survival in HER2-positive early-stage breast cancer treated with lapatinib and trastuzumab: a secondary analysis of the NeoALTTO Trial [published correction appears in *JAMA Oncol*. 2015;1(8):1172]. *JAMA Oncol*. 2015;1(4):448-454.

3. Fumagalli D, Venet D, Ignatiadis M, et al. RNA sequencing to predict response to neoadjuvant anti-HER2 therapy: a secondary analysis of the NeoALTTO randomized clinical trial. *JAMA Oncol*. 2017;3(2):227-234. doi:10.1001/jamaoncol.2016.3824

4. Redmond D, Poran A, Elemento O. Single-cell TCRseq: paired recovery of entire T-cell alpha and beta chain transcripts in T-cell receptors from single-cell RNAseq. *Genome Med*. 2016;8(1):80.

5. Gong Q, Wang C, Zhang W, et al. Assessment of T-cell receptor repertoire and clonal expansion in peripheral T-cell lymphoma using RNA-seq data. *Sci Rep*. 2017;7(1):11301.

6. Gu-Trantien C, Loi S, Garaud S, et al. CD4$^+$ follicular helper T cell infiltration predicts breast cancer survival. *J Clin Invest*. 2013;123(7):2873-2892.

7. Hale JS, Youngblood B, Latner DR, et al. Distinct memory CD4$^+$ T cells with commitment to T follicular helper- and T helper 1-cell lineages are generated after acute viral infection. *Immunity*. 2013;38(4):805-817.