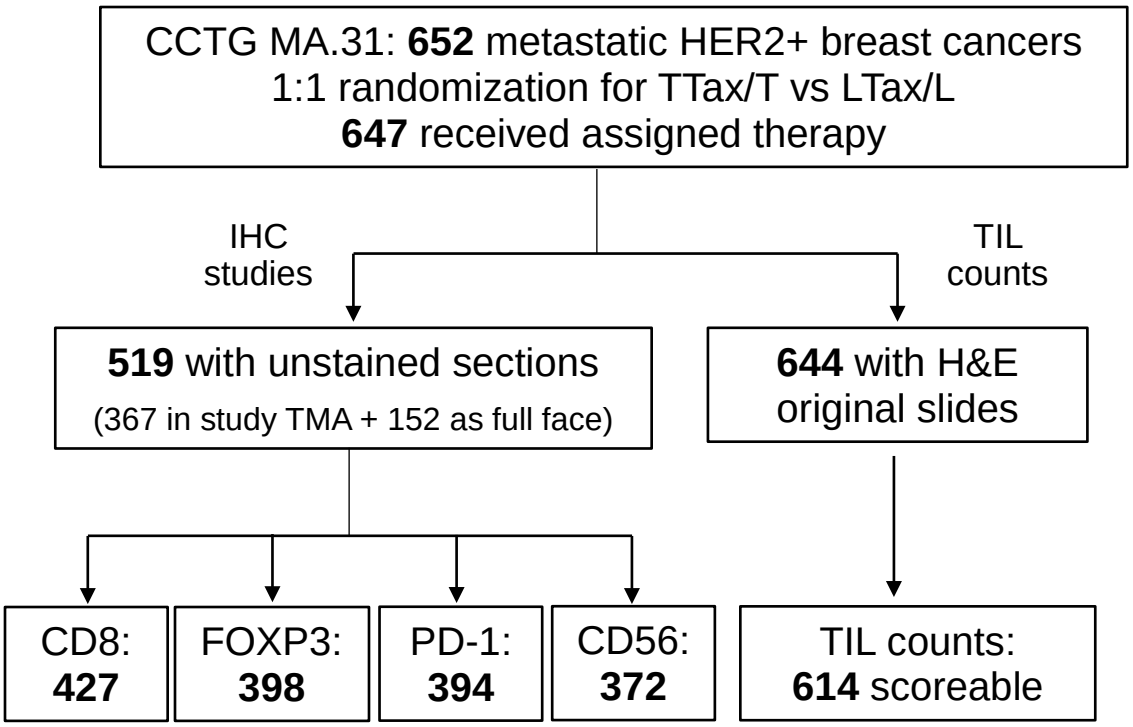
Supplementary Online Content

Liu S, Chen B, Burugu S, et al. Role of cytotoxic tumor-infiltrating lymphocytes in predicting outcomes in metastatic HER2-positive breast cancer: a secondary analysis of a randomized clinical trial. *JAMA Oncol*. Published online July 27, 2017. doi:10.1001/jamaoncol.2017.2085

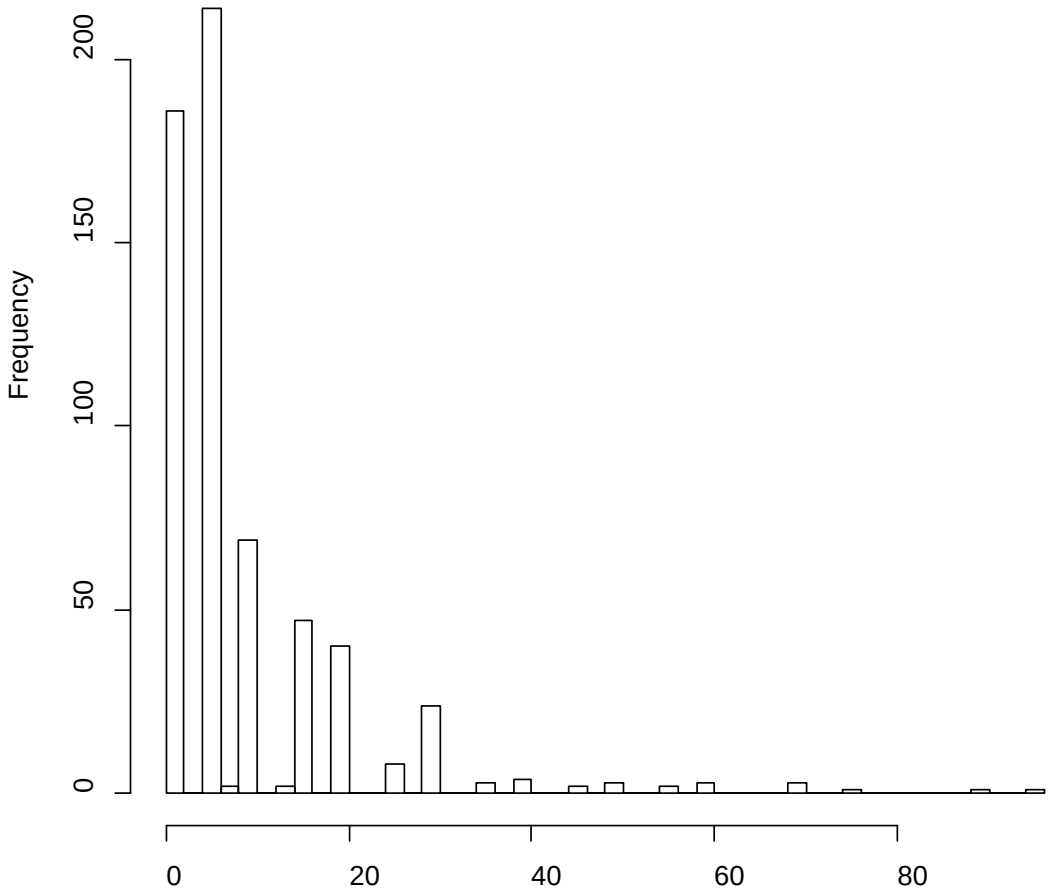
This supplementary material has been provided by the authors to give readers additional information about their work.

eFigure 1. REMARK Diagram of the Original CCTG MA.31 Trial and Biomarker Study Sets



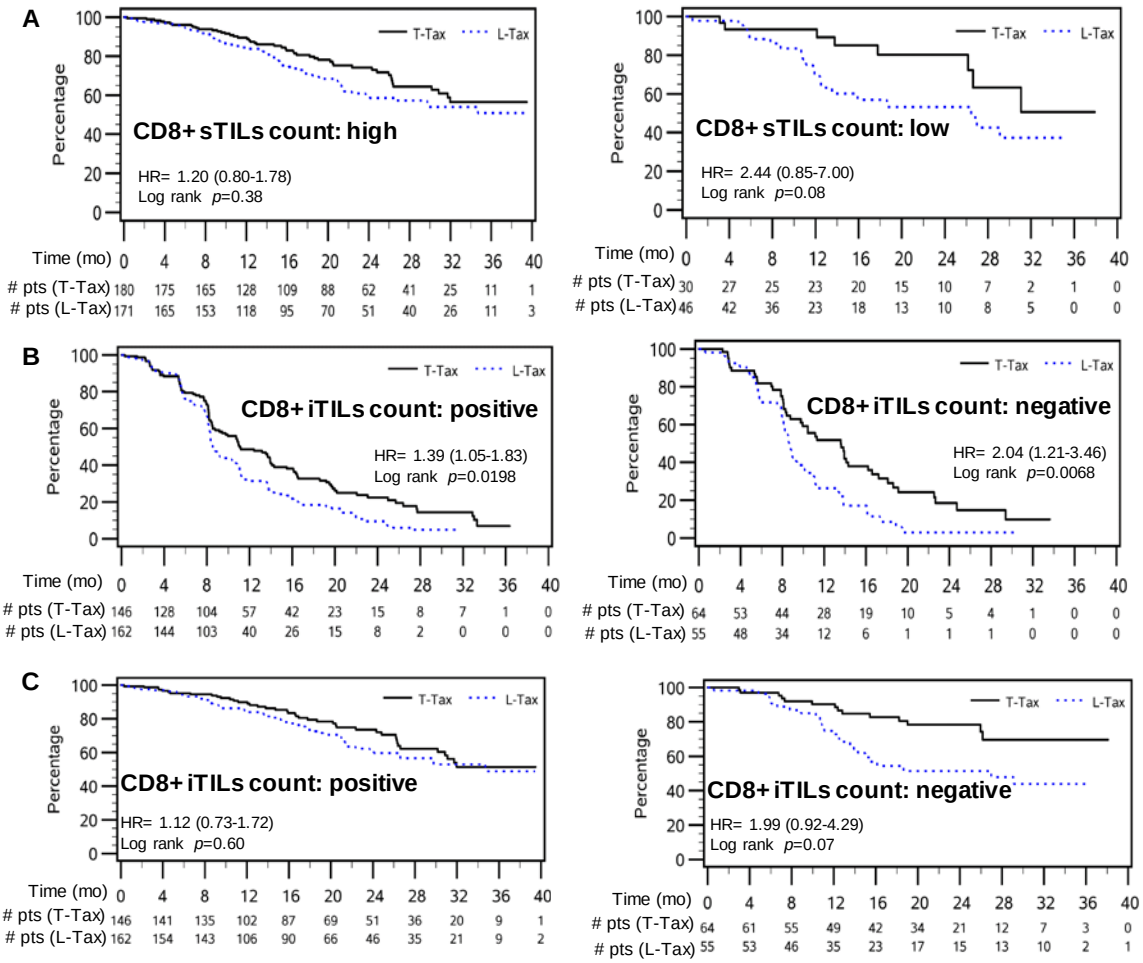
eFigure 2. Distribution of TIL Counts Across the CCTG MA.31 H&E Slide Study Set

Determined using the method of Denkert et al. (Mod Pathol 2016; 29:1155-64) based on percentage of intratumoral stromal area occupied by lymphocytes.



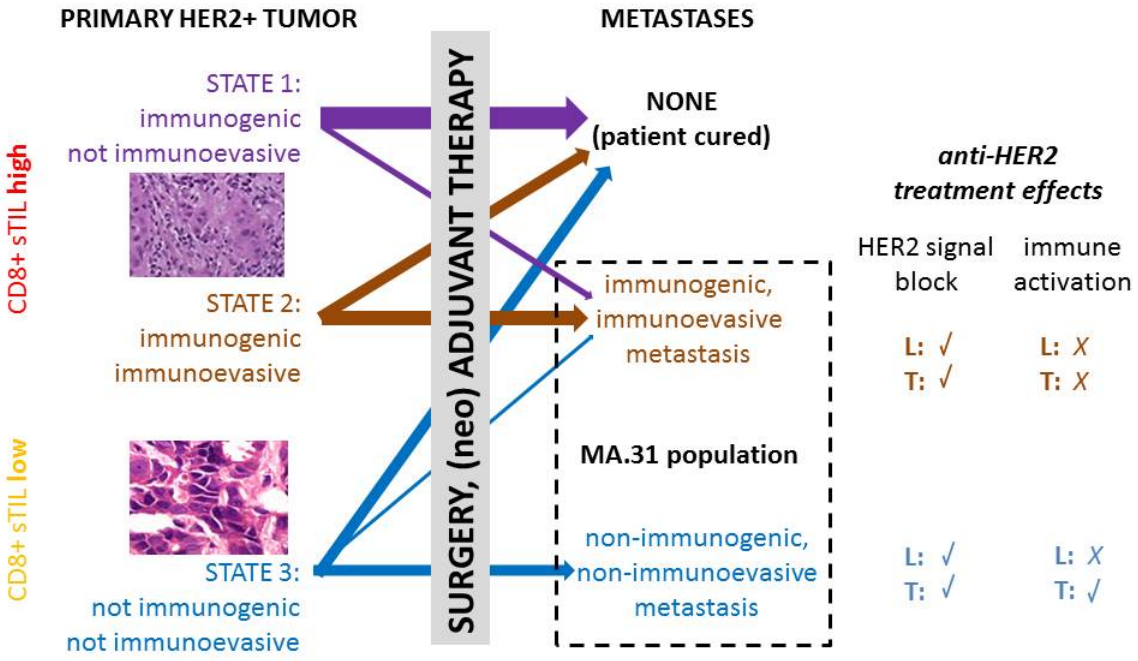
eFigure 3. Overall Survival Stratified by Treatment

TTax/T vs. LTax/L in CCTG MA.31 patients with high and low levels of CD8+ sTILs in their primary tumor specimen, and progression free survival and overall survival when CD8 is assessed by counting iTIL rather than sTIL



eFigure 4. Model of Association of Immune Infiltrates in the Primary Tumor Biopsy (CD8+ sTIL) With the CCTG MA.31 Metastatic *HER2*-Positive Population

The effect of trastuzumab vs lapatinib as first-line metastatic treatment is assessed.



eTable 1. Patient Characteristics in the CCTG MA.31 Immune Biomarker Study Population

Parameter		MA.31	H&E	IHC (CD8)
No. of Patients*		**647**	**614**	**427**
Age (median)		**55.0**	**55.0**	**55.0**
Median follow-up (months)		**21.7**	**21.6**	**22.6**
HER2(+)		**100%**	**100%**	**100%**
ER negative		31%	31%	31%
Mets at presentation		42%	43%	43%
Mets only at relapse		57%	57%	56%
ECOG performance status 0		61%	61%	60%
ECOG 1		35%	35%	36%
prior anthracyclines		41%	41%	41%
prior taxanes		20%	20%	17%
prior anti-HER2		18%	18%	17%
paclitaxel q wk		43%	44%	41%
docetaxel q3wk		57%	56%	59%
lapatinib (L-Tax)		**50%**	**50%**	**51%****
trastuzumab (T-Tax)		**50%**	**50%**	**49%**
TIL score >5%			**35%**	
CD8+ stromal TILs (sTIL) ≥ 3				**82%**
CD8+ intratumoral TILs ≥ 1			-	72%
FOXP3+ sTIL ≥ 3				67% (267/398)
CD56+ sTIL > 0				3% (11/372)
PD1+ sTIL > 0				13% (51/394)

* see Figure 1 for detailed explanation of patient numbers
** no significant imbalances by treatment arm for any parameter

eTable 2. Biomarker Expression in the CCTG MA.31 Study Population, Stratified by Disease Status

Parameter	Stage IV No.	Relapsed No.	*P* Value (Fisher exact test)
TIL score >5%	94 (36%)	114 (34%)	.54
CD8+ stromal TILs (sTIL) ≥ 3	155 (83%)	196 (81%)	.61
CD8+ intratumoral TILs ≥ 1	140 (75%)	168 (70%)	.23
FOXP3+ sTIL ≥ 3	129 (74%)	139 (62%)	.01
CD56+ sTIL > 0	10 (7%)	10 (5%)	.48
PD1+ sTIL > 0	30 (18%)	20 (9%)	.009

eTable 3. Estimates of Prognostic Hazard Ratio (HR) for High vs Low Level Immune Infiltrates in the CCTG MA.31 IHC Immune Biomarker Study Population

Analysis uses univariate analysis stratified by hormone receptor status (A and B) and by disease status (C and D)

A. Hormone Receptor Positive (ER or PR)

Immune biomarker	Stratified HR		*P* Value
	HR	95% CI	
CD8+ sTIL: ≥ 3 vs. < 3	0.84	0.57-1.22	.35
CD8+ iTIL: ≥ 1 vs. < 1	0.79	0.56-1.10	.16
FOXP3+ sTIL: ≥ 3 vs. < 3	0.78	0.56-1.09	.14
FOXP3+ iTIL: ≥ 1 vs. < 1	0.76	0.55-1.03	.08
PD-1+ sTIL: > 0 vs. = 0	0.88	0.53-1.45	.61
PD-1+ iTIL: > 0 vs. = 0	0.81	0.50-1.31	.39
Overall H&E sTIL: ≥ 5% vs. < 5	1.05	0.81-1.37	.70

B. Hormone Receptor Negative (ER or PR)

Immune biomarker	Stratified HR		*P* Value
	HR	95% CI	
CD8+ sTIL: ≥ 3 vs. < 3	1.29	0.70-2.38	.42
CD8+ iTIL: ≥ 1 vs. < 1	1.16	0.39-1.96	.57
FOXP3+ sTIL: ≥ 3 vs. < 3	1.20	0.71-2.04	.50
FOXP3+ iTIL: ≥ 1 vs. < 1	1.08	0.64-1.82	.77
PD-1+ sTIL: > 0 vs. = 0	0.77	0.36-1.66	.51
PD-1+ iTIL: > 0 vs. = 0	1.17	0.60-2.32	.64
Overall H&E sTIL: ≥ 5% vs. < 5	0.94	0.63-1.40	.78

C. Relapsed

Immune biomarker	Stratified HR		*P* Value
	HR	95% CI	
CD8+ sTIL: ≥ 3 vs. < 3	0.83	0.54-1.29	.41
CD8+ iTIL: ≥ 1 vs. < 1	0.95	0.65-1.39	.80
FOXP3+ sTIL: ≥ 3 vs. < 3	0.92	0.64-1.31	.64
FOXP3+ iTIL: ≥ 1 vs. < 1	0.98	0.69-1.38	.90
PD-1+ sTIL: > 0 vs. = 0	0.96	0.53-1.72	.89
PD-1+ iTIL: > 0 vs. = 0	1.14	0.55-2.36	.72
Overall H&E sTIL: ≥ 5% vs. < 5	1.20	0.89-1.62	.23

D. Stage IV at Diagnosis

Immune biomarker	Stratified HR		*P* Value
	HR	95% CI	
CD8+ sTIL: ≥ 3 vs. < 3	1.01	0.64-1.61	.96
CD8+ iTIL: ≥ 1 vs. < 1	0.76	0.51-1.14	.18
FOXP3+ sTIL: ≥ 3 vs. < 3	0.85	0.57-1.28	.44
FOXP3+ iTIL: ≥ 1 vs. < 1	0.66	0.46-0.96	.03
PD-1+ sTIL: > 0 vs. = 0	0.83	0.50-1.38	.47
PD-1+ iTIL: > 0 vs. = 0	0.70	0.43-1.12	.13
Overall H&E sTIL: ≥ 5% vs. < 5	0.96	0.71-1.31	.82

eTable 4. Exploratory Additional Stratified Full Multivariate Analysis for PFS

Parameter	Hazard Ratio	95%CI	*P* Value (χ^2 test)
Treatment: L-Tax vs T-Tax	2.54	1.42-4.53	.002
CD8+sTILs *Interaction*	1.28 0.53	0.78-2.11 0.28-0.98	.33 .044
Age (Continuous)	0.86	0.67-1.11	.25
Performance Status	0.72	0.57-0.92	.008
Stage IV vs relapsed	1.07	0.81-1.42	.64
Hormone receptor positive vs negative	0.81	0.63-1.05	.11
Weight (Continuous)	1.00	0.99-1.01	.96

Includes the primary biomarker CD8, randomized treatment arm, age, and factors considered to be potential confounders: ECOG performance status, disease status (metastatic at presentation vs. relapse of disease that was localized at diagnosis), hormone receptor status (ER or PR positive vs. both negative) and weight