Supplementary Methods and Results

eMethods: Analysis of high tumor mutation burden (TMB) and *PTEN* alterations in chemotherapy-treated and non-immune checkpoint inhibitor (ICI)-treated metastatic triple-negative breast cancer (mTNBC)

All clinical and genomic data were obtained from the cBioPortal site for a previous publication about advanced breast cancers from Memorial Sloan Kettering Cancer Center (http://www.cbioportal.org/study/summary?id=breast_msk_2018).[1] We included 90 patients from this study with metastatic TNBC (mTNBC), as defined by the absence of estrogen receptor and progesterone receptor expression (<1%) and lack of HER2 amplification, who underwent targeted DNA sequencing (MSK-IMPACT) on either a metastatic (62%) or primary (34%) tumor sample and were treated with single-agent chemotherapy (71%) or combination chemotherapy (29%) that was not labeled as neoadjuvant or adjuvant treatment. To further minimize neoadjuvant/adjuvant chemotherapy regimens, second-line chemotherapy treatments were included when available. We additionally analyzed overall survival (OS) in 169 patients with mTNBC treated with regimens that did not include immune checkpoint inhibitors (ICIs).

The Memorial Sloan Kettering-integrated mutation profiling of actionable cancer targets (MSK-IMPACT) is performed in a Clinical Laboratory Improvement Amendments-certified laboratory environment and is a hybridization capture-based next generation sequencing assay for targeted deep sequencing of all exons and selected introns of a predefined subset of cancer-related genes using tumor-derived DNA paired with DNA from patient-matched normal samples.[2] The majority of patients (51; 57%) had testing done using the 410-gene MSK-IMPACT version 2 (exonic coverage region = 1.016478 megabase [Mb]). Thirty-five (39%) patients were assessed

with the 341-gene MSK-IMPACT version 1 (exonic coverage region = 0.896665 Mb), and 4 (4%) patients were evaluated with the 468-gene MSK-IMPACT version 3 (exonic coverage region = 1.139322 Mb). Tumor mutation burden (TMB) was calculated by determining the number of nonsynonymous somatic mutations per megabase of exonic sequence data across all genes within the panel. All nonsynonymous mutations, including nonsense, missense, frame-shift, splice site, and nonstop changes, were considered. High TMB was defined as ≥7 mutations/Mb using the integer closest to the previously published MSK-IMPACT specific cutoff of the top 20% of TMB for this cohort,[3] and *PTEN* alterations were defined as a nonsynonymous mutation or a 1.5 or 2 copy deletion, based on prior work showing that partial *PTEN* deletions are associated with poor prognosis in breast cancer.[4] An additional analysis was completed with high TMB defined as ≥10 mutations/Mb to match the analysis presented in the main text. Only pretreatment MSK-IMPACT samples were included to ensure that all patients had MSK-IMPACT performed on samples collected before starting chemotherapy.

The associations of TMB and *PTEN* alterations with progression-free survival (PFS) and OS were analyzed. OS was defined as the date of metastatic disease diagnosis, calculated as the difference between the overall survival from invasive cancer diagnosis and the disease-free survival, until the date of death or last follow-up. Patients still alive at last follow-up were censored for OS. PFS was defined as the date of starting chemotherapy to the date of progression, death, or last follow-up. Patients alive and without progression were censored for PFS. PFS and OS were assessed using the Kaplan-Meier method and Cox proportional hazards regression. Differences in survival distributions by high TMB and *PTEN* alteration status were assessed using log-rank tests. To adjust for available clinical factors in the chemotherapy-treated mTNBC cohort, we included the following in multivariate regression models: therapy regimen

(combination chemotherapy versus monotherapy) and number of previous systemic therapies in metastatic setting (≥1 versus 0). Analyses were performed in RStudio Version 1.2.5001.

eResults: Alterations in other immunotherapy-related pathways in anti-PD-1/L1-treated mTNBC

In a directed assessment of genomic pathways related to immunotherapy response in other tumors, only 2 patients in this cohort had high amplifications in *PDL1*, and both had partial responses, one with a progression-free survival (PFS) of 6.4 months and the other with an ongoing PFS of 42.5 months, consistent with a previous report linking *PDL1* amplifications to improved immune checkpoint inhibitor (ICI) responses in solid tumors.[5] Similarly, the only patient with a nonsynonymous mutation in *POLE*, who had a tumor mutational burden (TMB) of 4.6 mutations/megabase (Mb), had an ongoing partial response with a current PFS of 19.1 months, also consistent with a previous report demonstrating longer OS in patients with *POLE* mutated-cancers treated with ICIs.[6] Only 5 patients had mutations in antigen-presentation genes, including *JAK2*, *JAK3*, and *CIITA*, and no patients had mutations in *B2M* or *JAK1*. These mutations were not consistently associated with PFS individually or in a combined univariate analysis (hazard ratio [HR] 0.52, 95% CI 0.13-2.18, p=0.38). A total of seven patients had mutations in chromatin modifier genes, including *ARID1A*, *SMARCA4*, and *PBRM1*, and these mutations were also not consistently associated with PFS individually or in a combined univariate analysis (HR 0.97, 95% CI 0.38-2.49, p=0.95).

In unbiased analyses to discover other pathways associated with immunotherapy response in this cohort, *PRKDC* nonsynonymous mutations and *TP53* loss, defined as tumors with both a *TP53* nonsynonymous mutation and a *TP53* one copy deletion, emerged as associated with improved PFS and overall survival (OS) in univariate and/or multivariate analyses (eTable 1).

However, only 6 patients had *PRKDC* mutations: 4 of these patients were known PD-L1-positive, 3 had high TMB, and only one had neither PD-L1 positivity or high TMB. Although these 6 patients were all treated with ICI monotherapy, *PRKDC* is a long gene measuring 187,076 base-pairs, over 6 times longer than the average gene length,[7] and has not previously been associated with immunotherapy response. Instead, germline mutations in *PRKDC*, which encodes a protein kinase the catalytic subunit crucial for DNA double strand break repair and V(D)J recombination, have been implicated in severe immunodeficiencies.[8, 9] Similarly, 6 of the 14 patients with *TP53* loss had *PTEN* alterations, and *TP53* loss is a well-known prognostic biomarker associated with worse survival in primary and metastatic breast cancer.[10, 11] Furthermore, the prevalence of *TP53* one copy deletions (29%) in our cohort was likely overestimated by OncoPanel, which lacks corresponding germline DNA, as *TP53* one copy deletions were much rarer in other breast cancer cohorts with prevalence of <1% in both 1918 metastatic breast cancers in the MSK-IMPACT cohort (http://www.cbioportal.org/study/summary?id=breast_msk_2018) and 1070 primary breast cancers in The Cancer Genome Atlas (http://www.cbioportal.org/study/summary?id=brca_tcga_pan_can_atlas_2018). Overall, the associations of *PRKDC* nonsynonymous mutations and *TP53* loss with PFS and OS did not pass multiple corrections testing by the Bonferroni or Benjamini-Hochberg methods, applied for the absence of prior evidence linking these correlates to immunotherapy response, and therefore both are likely false positives.

eReferences

1. Razavi P, Chang MT, Xu G, Bandlamudi C, Ross DS, Vasan N, et al. The Genomic Landscape of Endocrine-Resistant Advanced Breast Cancers. *Cancer Cell* **2018**;34:427-38 e6.
2. Cheng DT, Mitchell TN, Zehir A, Shah RH, Benayed R, Syed A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): A Hybridization Capture-Based Next-Generation Sequencing Clinical Assay for Solid Tumor Molecular Oncology. *J Mol Diagn* **2015**;17:251-64.
3. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat Genet* **2019**;51:202-6.
4. Lebok P, Kopperschmidt V, Kluth M, Hube-Magg C, Ozden C, B T, et al. Partial PTEN deletion is linked to poor prognosis in breast cancer. *BMC Cancer* **2015**;15:963.
5. Goodman AM, Piccioni D, Kato S, Boichard A, Wang HY, Frampton G, et al. Prevalence of PDL1 Amplification and Preliminary Response to Immune Checkpoint Blockade in Solid Tumors. *JAMA Oncol* **2018**;4:1237-44.
6. Wang F, Zhao Q, Wang YN, Jin Y, He MM, Liu ZX, et al. Evaluation of POLE and POLD1 Mutations as Biomarkers for Immunotherapy Outcomes Across Multiple Cancer Types. *JAMA Oncol* **2019** Aug 15 [Epub ahead of print]. doi: 10.1001/jamaoncol.2019.2963.
7. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, et al. The sequence of the human genome. *Science* **2001**;291:1304-51.
8. van der Burg M, van Dongen JJ, van Gent DC. DNA-PKcs deficiency in human: long predicted, finally found. *Curr Opin Allergy Clin Immunol* **2009**;9:503-9.
9. Mathieu AL, Verronese E, Rice GI, Fouyssac F, Bertrand Y, Picard C, et al. PRKDC mutations associated with immunodeficiency, granuloma, and autoimmune regulator-dependent autoimmunity. *J Allergy Clin Immunol* **2015**;135:1578-88 e5.
10. Olivier M, Langerod A, Carrieri P, Bergh J, Klaar S, Eyfjord J, et al. The clinical value of somatic TP53 gene mutations in 1,794 patients with breast cancer. *Clin Cancer Res* **2006**;12:1157-67.
11. Meric-Bernstam F, Zheng X, Shariati M, Damodaran S, Wathoo C, Brusco L, et al. Survival Outcomes by TP53 Mutation Status in Metastatic Breast Cancer. *JCO Precis Oncol* **2018**;2018.