Supplementary Methods

Tissue microarray (TMA) of triple-negative breast cancers

Initially H&E sections were obtained from each tumor block. Areas from tumor-rich regions were marked and targeted. Using the Beecher Tissue Array Constructor, three tissue cores with a diameter of 1.0 mm were punched from different regions of the tumor and deposited into a 12 x 9 (108 cores) TMA block. Three TMAs were constructed containing 3 cores of each of 100 FFPE tumor blocks. Standard IHC markers were scored by an expert pathologist using light microscopy, a four value intensity scale (0-3+) and percentage extent. The antibody to the androgen receptor (AR, # M3562; Dako, Denmark) was used at 1:300. The antibody to HER2 (#2242; Cell Signaling) was used at 1:200. Ki67 staining and scoring was performed as described (1). An H-SCORE was calculated multiplying both parameters (range: 0-300). Only nuclear AR and membrane-localized HER2 were considered for evaluation. The antibody to Ki-67 (M7240; Dako) was used at a 1:75 dilution overnight. Briefly, FFPE tumor sections were scanned at 100x magnification, and the area containing the highest number of positive cells in each case was selected. Positive and negative tumor cells were manually counted by an expert breast pathologist (MGK or MES) at 400x; the percentage of positive cells was calculated with at least 700 viable cells. Antigen retrieval for Ki-67 was performed using HpH Buffer (pH 8.0) in a decloaking chamber (Biocare Medical, Concord, MA).

mRNA was collected from the FFPE sections, and NanoString analysis was performed as described previously (1). Molecular subtyping was carried out by the correlation of the log2 normalized transcript counts to the PAM50 microarray data centroids (2). Using this
approach, gene expression data were available in 89 tumors. Matched TMA scoring and mRNA data was available for 79 patients.

5-μm sections of the TMA were used for immunohistochemistry staining of LDHB. Slides were dried at 60°C, deparaffinized in xylene, quenched in 1% hydrogen peroxide/methanol for 10 min, and rehydrated through sequentially graded ethanols. Antigen retrieval was performed by EDTA incubation (1 mmol/L, pH 8.0) using a pressure cooker at 125°C/25 psi for 5 min. Slides were cooled for 20 min, rinsed with distilled water, and washed in Tris-buffered saline. Using a DAKO Autostainer (DAKO, Carpinteria, CA), the slides were blocked for 30 min in horse serum and incubated with LDHB antibody (Abcam, Cambridge, MA; ab85319; 1:2,000) for 1 h. The Universal ABC Elite kit (Vectastain, Burlingame, CA) with 3,3′-diaminobenzidine development was used to visualize antibody binding, and the slides were subsequently counterstained with hematoxylin. The Automated Cellular Imaging System (ACIS) III instrument (DAKO) was used to quantify the staining of LDHB. Without knowledge of clinical outcomes or subtypes, representative tumor cells from each core were marked using a free-scoring tool. The averages of the brown intensities for each case were used for the analyses. The breast cancer cell line MCF7 was used as a negative tissue control. Breast cancers without primary antibody incubation were negative controls.

**Lactate Dehydrogenase Isoenzyme Activity Gel**

To resolve the LDH tetramers and quantify activity, non-denatured electrophoresis was used as described previously (3,4) with modifications. Electrophoresis was performing using a vertical, 4% polyacrylamide gel (1.0 mm, 18 well). Cell lysates were prepared in 20 mM bicine pH 7.6, 0.6% 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonic acid (CHAPS)
and mixed with 40% glycerol in 1.5M Tris-HCl, pH 8.8. Electrophoresis in Tris-glycine, pH 8.3 (pre-chilled), was run for 120 min, 125 V, 5°C. The LDH activity was developed at room temperature in 0.1M of sodium lactate, 1.5 mM of NAD, 0.1M Tris-HCl pH 8.6, 10 mM sodium chloride, 5 mM magnesium chloride, phenazinemethosulphate (0.03 mg/mL), and nitrobluetetrazolium (0.25 mg/mL). The gels were imaged, and the LDH band intensities (area under the curve, AUC) were quantified by ImageJ software (National Institutes of Health).
REFERENCES


