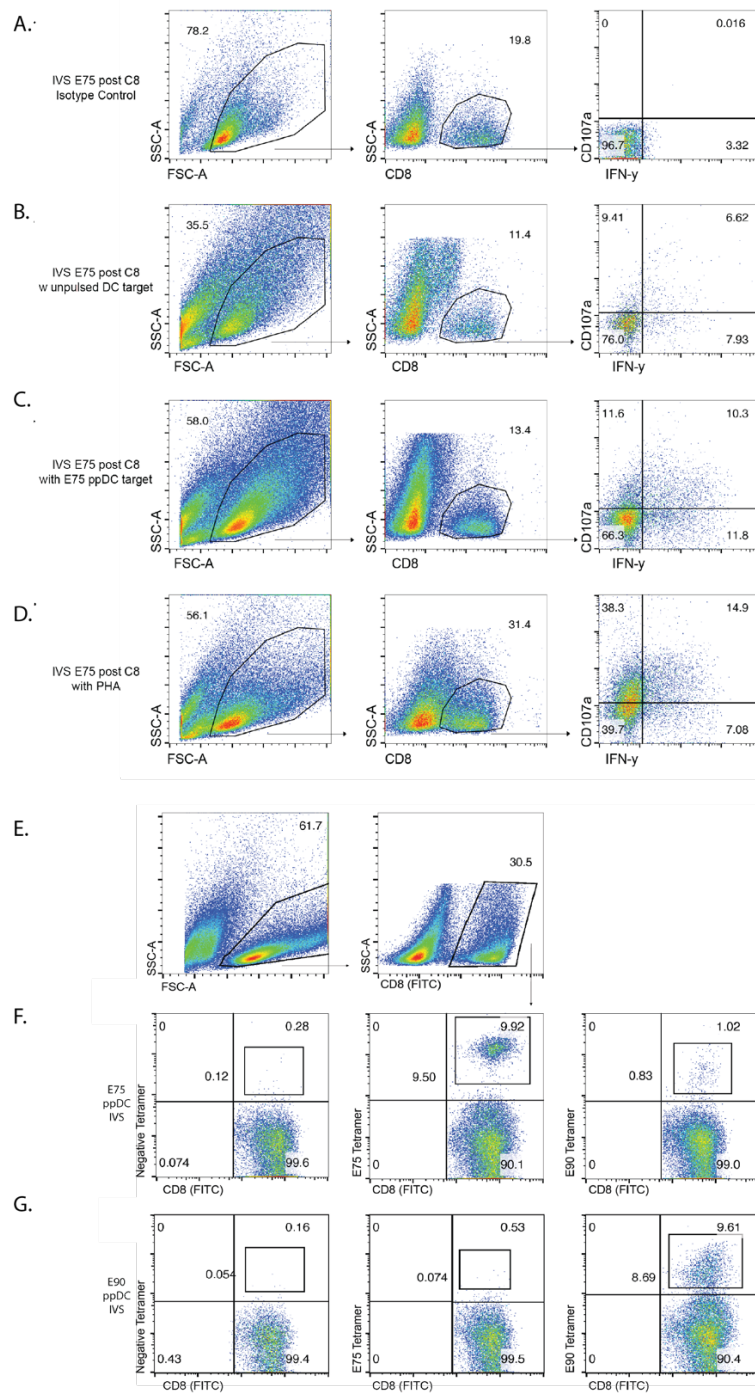
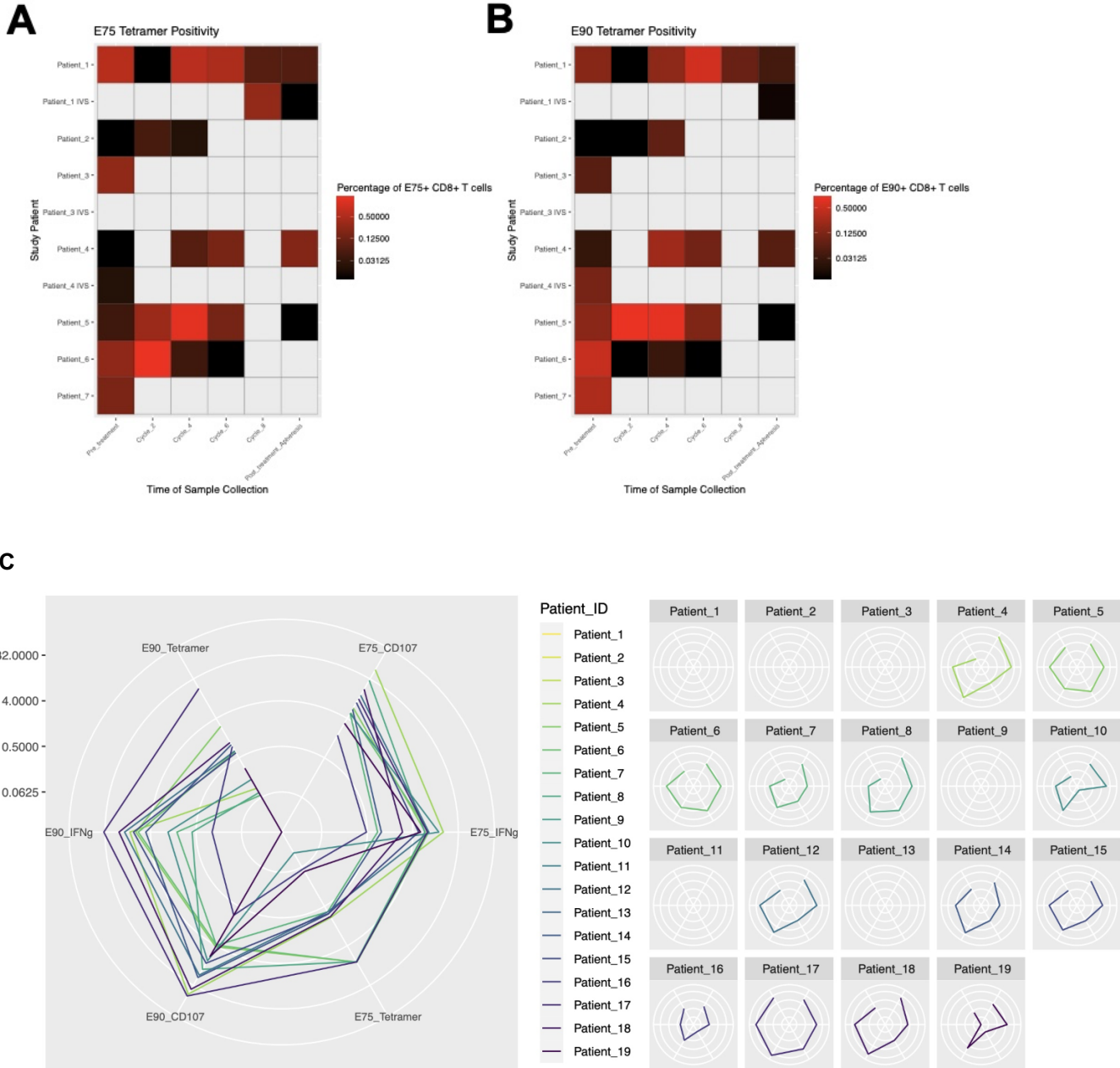


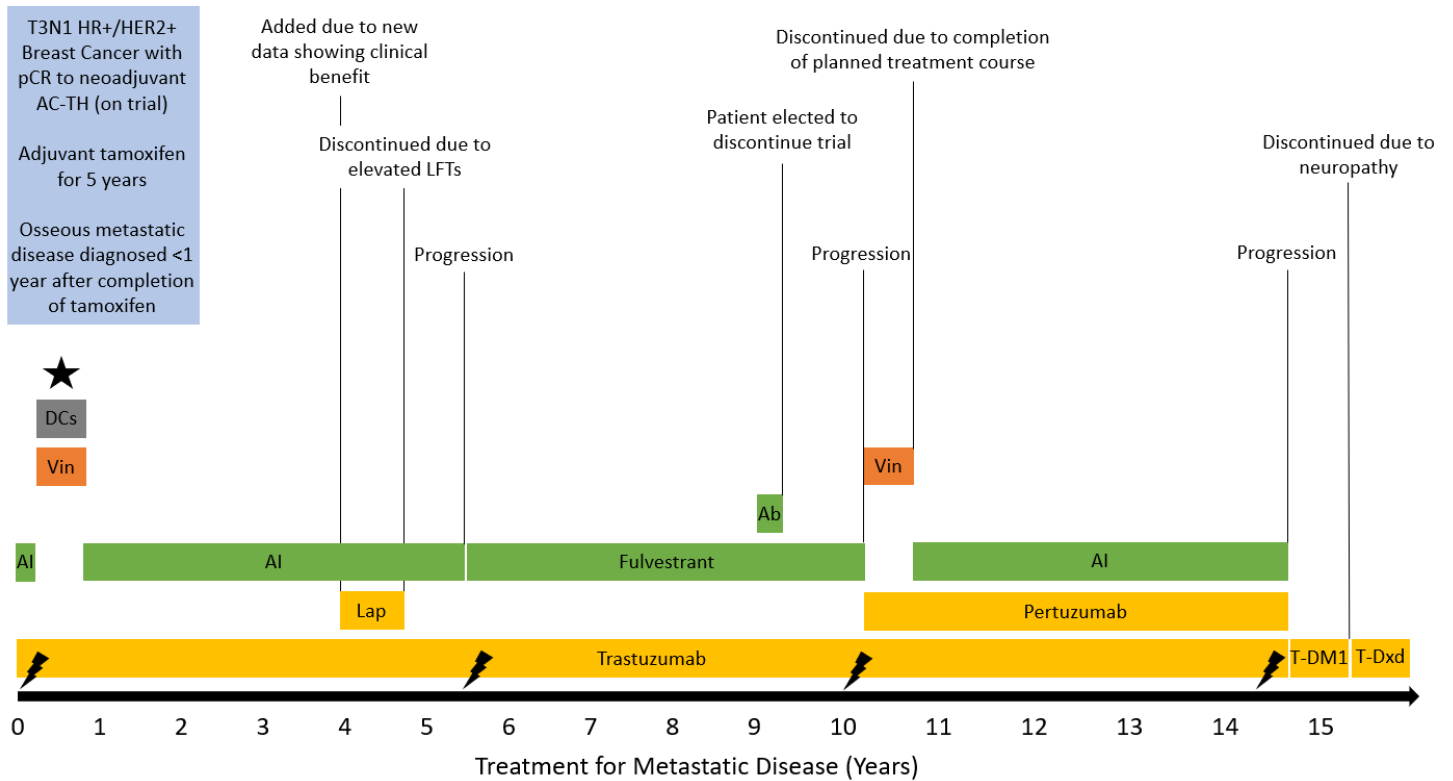
Supplementary Figure 1. Immunophenotype of CD34⁺-derived dendritic cells used for therapeutic vaccinations and in vitro stimulations. CD34⁺ selected autologous dendritic cells expanded and differentiated with GM-CSF, FLT3-ligand, Stem Cell Factor, and IL-4 and matured with the addition of Tumor Necrosis Factor (TNF), IFN- γ , and IL-6. Single stain flow cytometry gates were gated first into large, granular dendritic cells or smaller, less granular populations. (A) Large, granular dendritic cells gated on forward (FSC) versus side scatter (SSC). (B) Isotype IgG2a-PE gating control for CD11b and HLA-DR single stains. (C) Isotype IgG2b PE gating control for CD86 and CD11c single stains. (D) Isotype IgG1-PE gating control for CD40, CD80, and CD1a single stains. (E) Isotype IgG2b-FITC gating control for CD83. (F) IgG2b-FITC gating control for CD14. (G) Smaller, less granular dendritic cells gated on forward versus side scatter. (H) Isotype IgG2a-PE gating control for CD11b and HLA-DR single stains. (I) Isotype IgG2b PE gating control for CD86 and CD11c single stains. (J) Isotype IgG1-PE gating control for CD40, CD80, and CD1a single stains. (K) Isotype IgG2b-FITC gating control for CD83. (L) IgG2b-FITC gating control for CD14



Supplementary Figure 2. Quantification of in vitro (re)stimulation of CD8⁺ T cells responses post cycle 8 vaccination. A-G show quantification following IVS re-stimulation with E75 peptide-pulsed autologous dendritic cells and 6 hr incubation with unpulsed, E75, or E90 peptide-pulsed autologous dendritic cell (ppDC) targets or PHA control. (A) IgG1 Isotope control gating: forward scatter versus side scatter IVS cultured cells were gated on CD8 lymphocytes and then CD107/a/b versus IFN- γ . (B) E75 IVS lymphocytes co-cultured for 6 hrs. with autologous, un-pulsed DCs target cells: forward scatter versus side scatter IVS cultured cells were gated on CD8⁺ lymphocytes and then CD107/a/b versus IFN- γ . (C) E75 IVS lymphocytes co-cultured for 6 hrs. with autologous, E75-ppDC target cells: forward scatter versus side scatter IVS cultured cells were gated on CD8⁺ lymphocytes and then CD107/a/b versus IFN- γ . (D) E75 IVS lymphocytes stimulated 6 hrs with PHA as a positive control for the ability of the cultured cells to upregulate CD107 and generate intracellular IFN- γ . E-F show quantification of following re-stimulation with E75 or E90 peptide-pulsed autologous dendritic cells. (E) Forward scatter (FSC) versus side scatter (SSC) in vitro stimulated (IVS) cultured cells gated on CD8 lymphocytes. (F) IVS cultures re-stimulated with E75 peptide-pulsed dendritic cells (ppDCs) were tested with negative tetramer, E75 tetramer, or E90 tetramer. (G) IVS cultures restimulated with E90 ppDCs were tested with Negative Tetramer, E75 Tetramer, or E90 tetramer.



Supplementary Figure 3: Immune monitoring results in the LCCC0310 and LCCC0418 study cohorts. Leukapheresis samples were analyzed prior to first vaccination and at the end of the study. Peripheral blood samples were analyzed on the days of cycles #2, #4, #6, and #8 in vaccinated patients. Heatmaps show (A) frequency of E75-specific CD8⁺ T cells, (B) frequency of E90-specific CD8⁺ T cells in LCCC0310. Gray boxes represent time points when samples were not collected. (C) Radial plots showing best response immune monitoring measurements in LCCC0418 patients.



Supplementary Figure 4. Clinical course for exceptional responder. Bone-directed therapy was also given throughout treatment (denosumab followed by romosozumab-aqqg after pathologic fracture femur fracture). Star indicates dendritic cell vaccine clinical trial. Lightning bolts indicate treatments with palliative radiation (to T10-L1, T6-T8, L4, right iliac). pCR = pathological complete response; AC-TH = doxorubicin, cyclophosphamide, paclitaxel, trastuzumab; AI = aromatase inhibitor (given with ovarian function suppression prior to bilateral salpingo-oophorectomy); DCs = dendritic cells (vaccine); Vin = vinorelbine chemotherapy; Lap = lapatinib; LFTs = liver function tests; Ab = abemaciclib; T-DM1 = trastuzumab emtansine; T-Dxd = trastuzumab deruxtecan