

Sustained Lymphocyte Decreases after Treatment for Early Breast Cancer: Supplementary Material

	Mean Cell Count x 10 ⁹ /L (SEM)				Test for Trend	
	Baseline	End of Treatment	3-6m post chemo	>12m post chemo	Slope	p-value
WCC	7.45 (0.17)	5.03 (0.20)	5.74 (0.18)	6.40 (0.22)	-0.3579	< 0.01
ALC	2.05 (0.06)	1.16 (0.04)	1.41 (0.06)	1.80 (0.08)	-0.0946	<0.01
ANC	4.62 (0.15)	3.29 (0.16)	3.64 (0.15)	3.92 (0.18)	-0.2720	<0.01
NLR	2.75 (0.20)	3.12 (0.15)	3.02 (0.18)	2.52 (0.17)	-0.0476	0.56
n	180	177	103	87		

Supplementary Table 1: Mean absolute white cell count (WCC), neutrophil count (ANC), lymphocyte count (ALC) and neutrophil-to-lymphocyte (NLR) in all patients, across timepoints

Mean total white cell count (WCC), absolute neutrophil count (ANC), absolute lymphocyte count (ALC) and neutrophil to lymphocyte ratio (NLR) in all patients, collected from peripheral full blood count records at baseline (before the first dose of chemotherapy), end of treatment (before the last dose of chemotherapy) and 3-6 months and at least 12 months post chemotherapy. Slope and p-value derived from mixed effects linear test for trend. Cut-off for significance $p < 0.05$. n = number of patients with FBC count available at each time point. SEM = standard error of the mean.

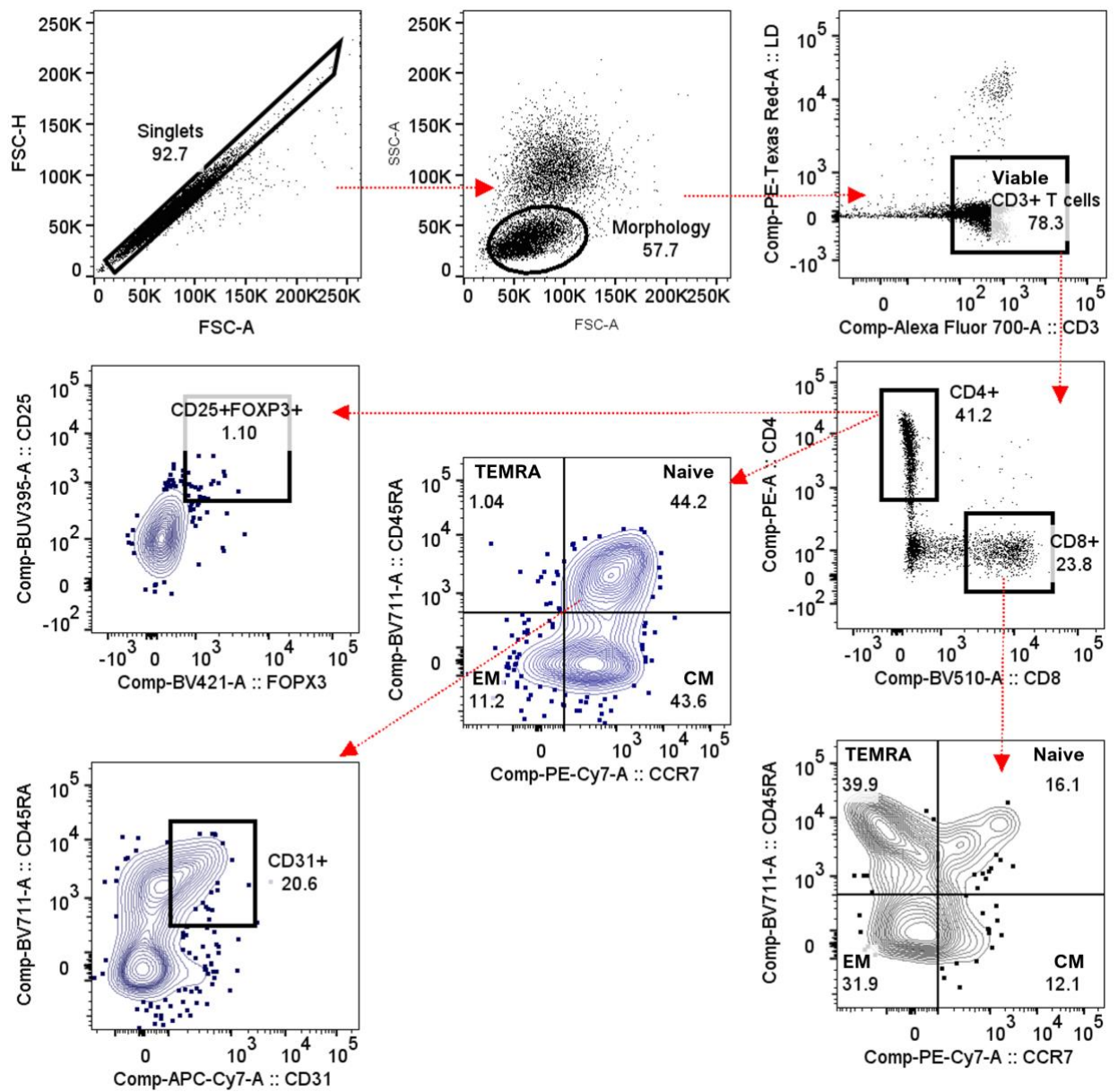
Supplementary Table 2: Clinical characteristics of patients included in the flow cytometric analysis (n = 9)

Age (years)	Subtype	Menopausal Status	Chemotherapy Regimen	Time from last chemo to post-treatment blood draw (months)
61	TNBC	Postmenopausal	AC-Paclitaxel+Carboplatin	1
79	HER2+	Postmenopausal	Weekly paclitaxel	7
67	HR+	Postmenopausal	Docetaxel + cyclophosphamide x 4	12
74	HR+	Postmenopausal	AC-Paclitaxel	5
42	HR+	Premenopausal	AC-Paclitaxel	7
29	HR+	Premenopausal	AC-Paclitaxel	7
40	HR+	Premenopausal	Dose-dense AC-Paclitaxel	7
43	HR+	Premenopausal	Dose-dense AC-Paclitaxel	7
31	TNBC	Premenopausal	Dose-dense AC-Paclitaxel	8
Mean: 51.8				Mean: 6.8

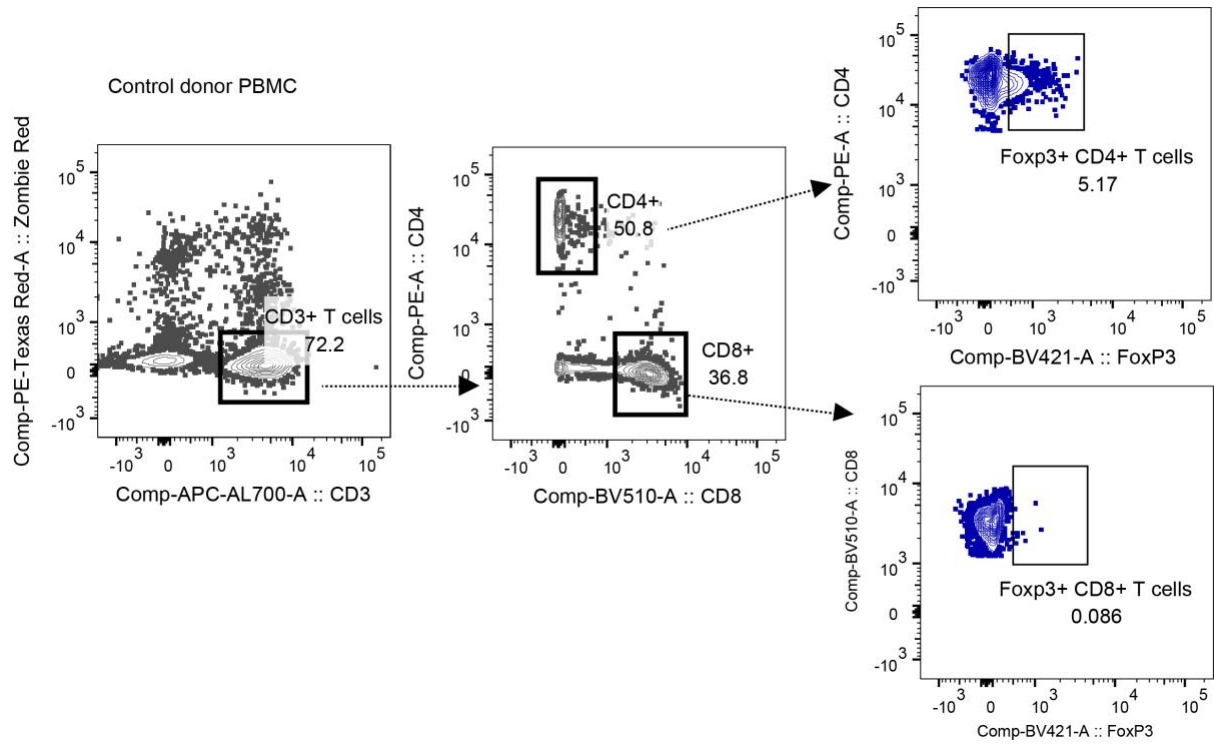
TNBC = triple negative breast cancer, HR+ = hormone receptor positive, HER2+ = Human epidermal receptor positive, AC = doxorubin + cyclophosphamide.

Supplementary Figure 1: Gating Strategy for Flow Cytometric Analyses

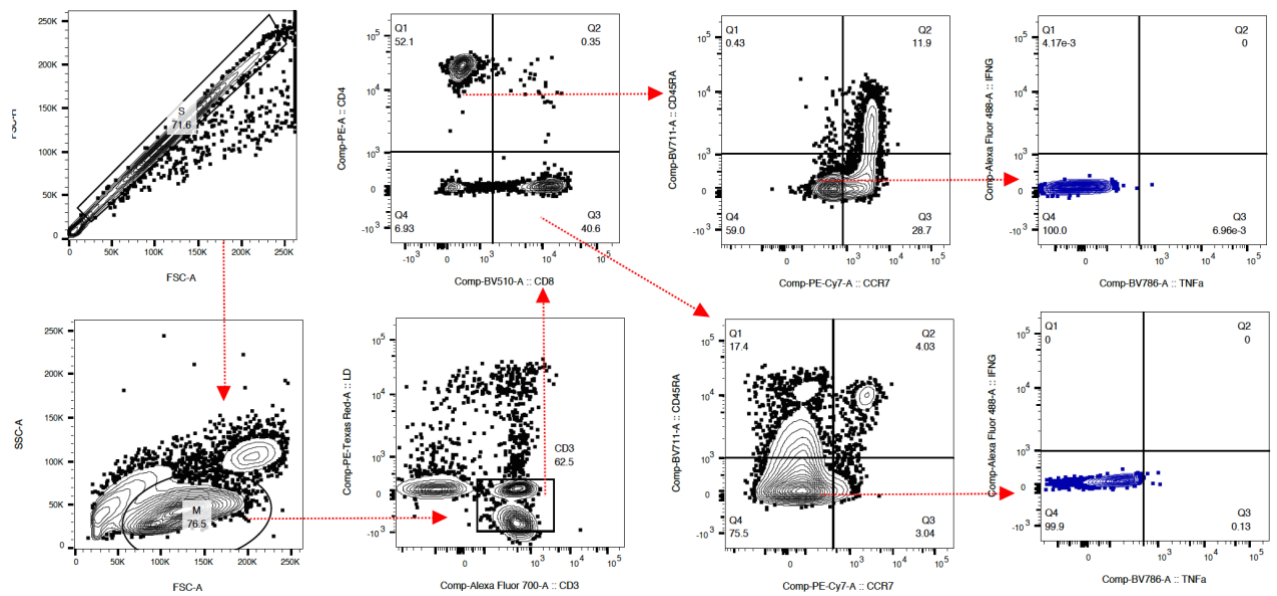
a.



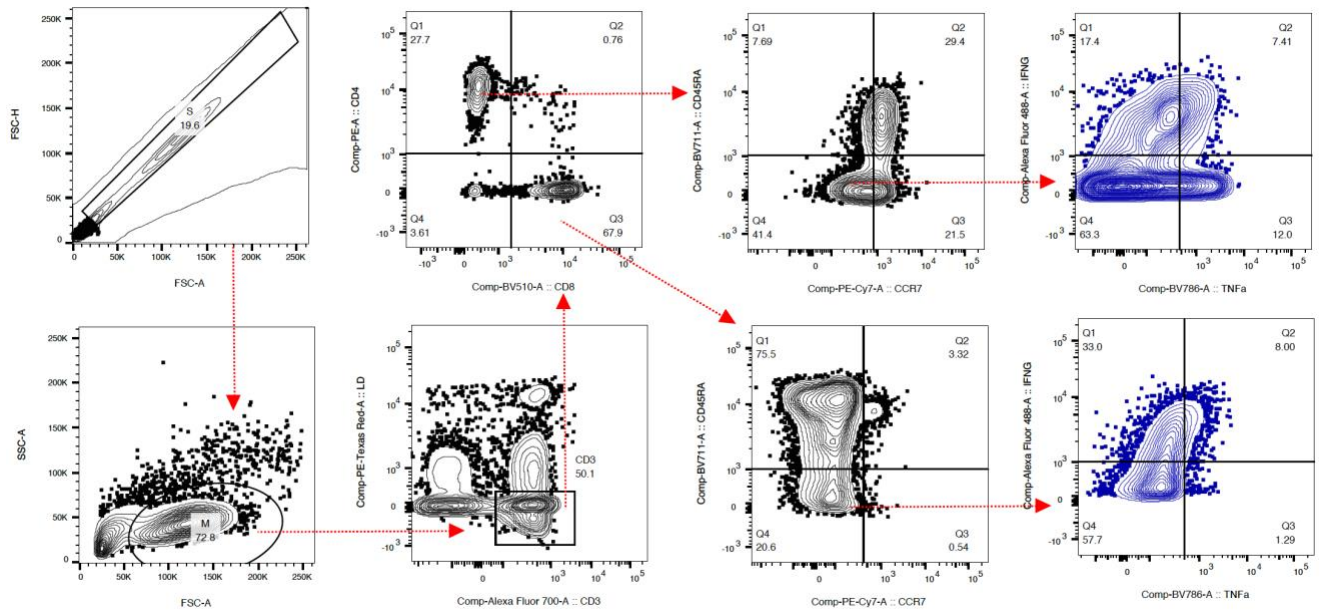
b.



c.



d.



e.

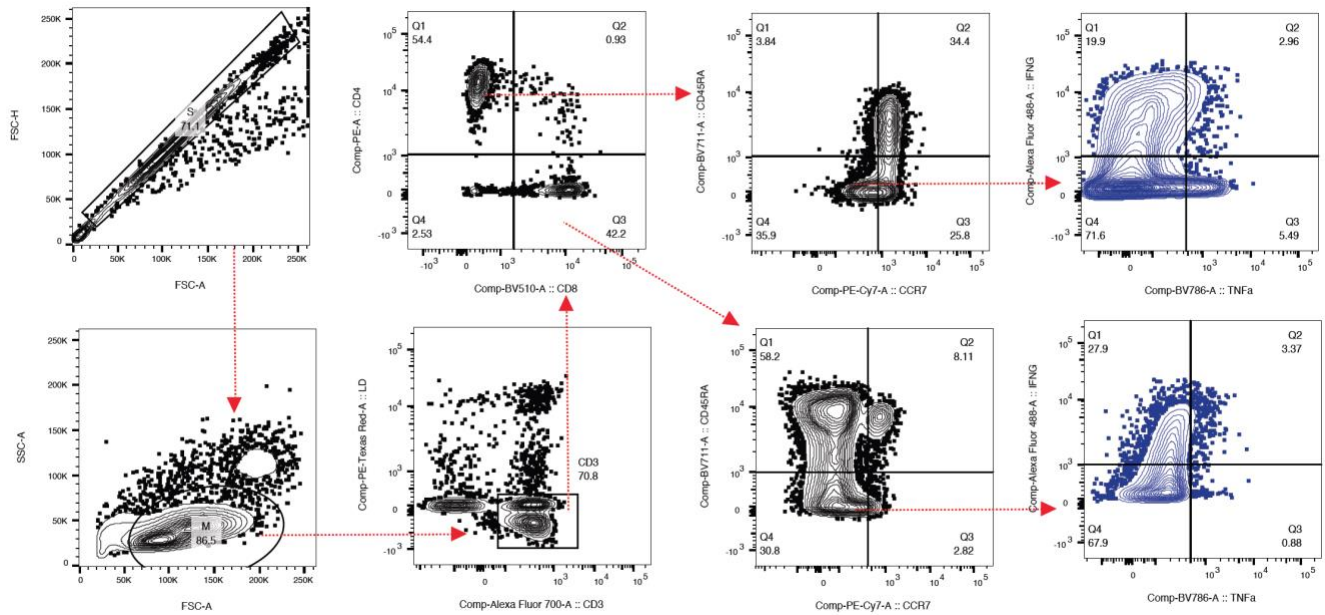


Figure Legend: Gating strategy for flow cytometry, for surface markers (**S1a**) and intracellular cytokines (**S1c-e**). First, we applied a gate to select for single cells, from which lymphocytes were selected by morphology. A viability gate (Zombie Red, [ZR]) was applied to exclude dead cells. Live (ZR negative) cells were gated for CD3 to identify CD3⁺ T cells which were split into CD4⁺ and CD8⁺ T cells. These subsets were gated according to expression of CD45RA and CCR7 to define T cell memory subsets (CD45RA⁺ CCR7⁺ naïve T cells, CD45RA⁻ CCR7⁺ central memory [CM] T cells, CD45RA⁻ CCR7⁻ effector memory [EM] and CD45RA⁺ CCR7⁻ terminally differentiated [TEMRA] T cells). These subpopulations are expressed as a percentage of CD4⁺ or CD8⁺ T cells, respectively. A gate for CD31 was applied to CD45RA⁺ CD4⁺ T cells to select recent thymic emigrants (CD45RA⁺ CD31⁺) and a gate for CD25 and FOXP3 was applied to CD4⁺ T cells to identify T regulatory cells (CD25⁺ FOXP3⁺). Recent thymic emigrants (RTEs) are expressed as a percentage of naïve CD4⁺ T cells and T regulatory cells are expressed as a percentage of CD4⁺ T cells (**S1a**). Intracellular cytokine producing cells were analysed based on standard forward (FSC) and side-scatter (SSC) profiling, live (ZR⁻) CD3⁺, CD4⁺ and CD8⁺ T cell lineages (**S1c-e**). Single-positive CD4⁺ and CD8⁺ T cell were further sub-classified into naïve (CCR7⁺, CD45RA⁺), effector memory (CCR7⁻/CD45RA⁻), central memory (CCR7⁺/CD45RA⁻) and terminally differentiated memory (CCR7⁻/CD45RA⁺) cells. The effector memory (CCR7⁻/CD45RA⁻) pool of CD4⁺ and CD8⁺ T cells were particularly examined for the individual expression of IFN- γ , TNF- α cytokines and assessed for co-expression of IFN- γ and TNF- α . Flow panels from representative samples prior to chemotherapy (**S1d**) and after chemotherapy (**S1e**) are shown. Normal healthy donor control for FOXP3 is shown in (**S1b**) and non-stimulated cells were used as control for intracellular cytokines (**S1c**).

Supplementary Figure 2: T cell subsets in post-menopausal patients

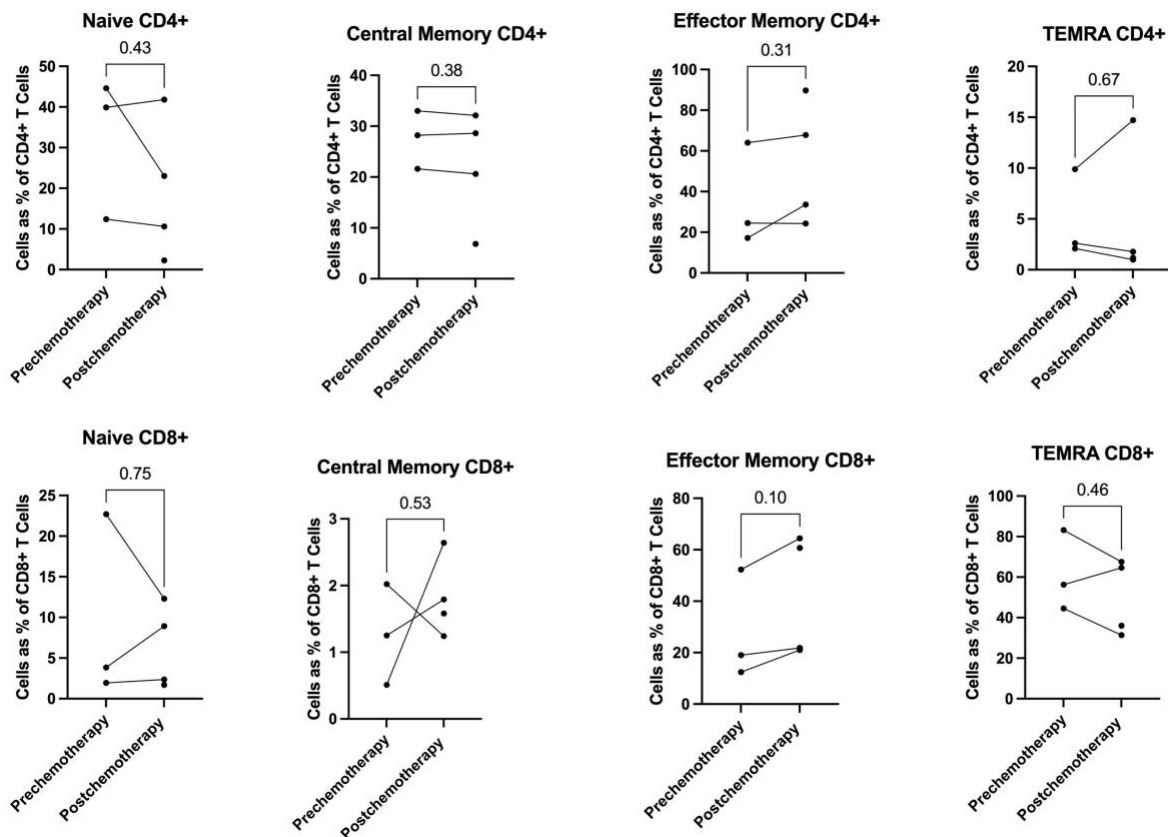


Figure Legend: T cell CD4⁺ and CD8⁺ subsets in post-menopausal patients expressed as a percentage of CD4⁺CD3⁺ and CD8⁺CD3⁺ T cells respectively. For one post-menopausal patient, there were insufficient cells in the pre-chemotherapy patient. The post-chemotherapy result for this patient is shown in the graph, but this sample was excluded from the paired statistical analysis. Individual dots represent individual patients. P-value for paired t-test is shown.

Supplementary Figure 3: CD4⁺/CD8⁺ ratio in pre- and post-menopausal patients

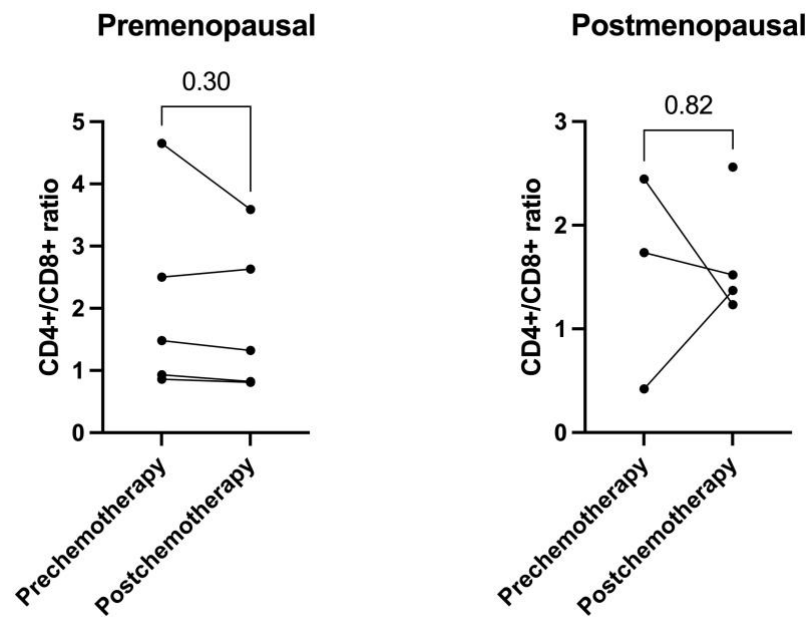


Figure Legend: Ratio of CD4⁺ to CD8⁺ T cells in pre- and post-menopausal. Coloured dots represent individual patient values. Individual dots represent individual patients. P-value for paired t-test is shown.

Supplementary Figure 4: CD4+ and CD8+ absolute cell counts in pre- and post-menopausal patients

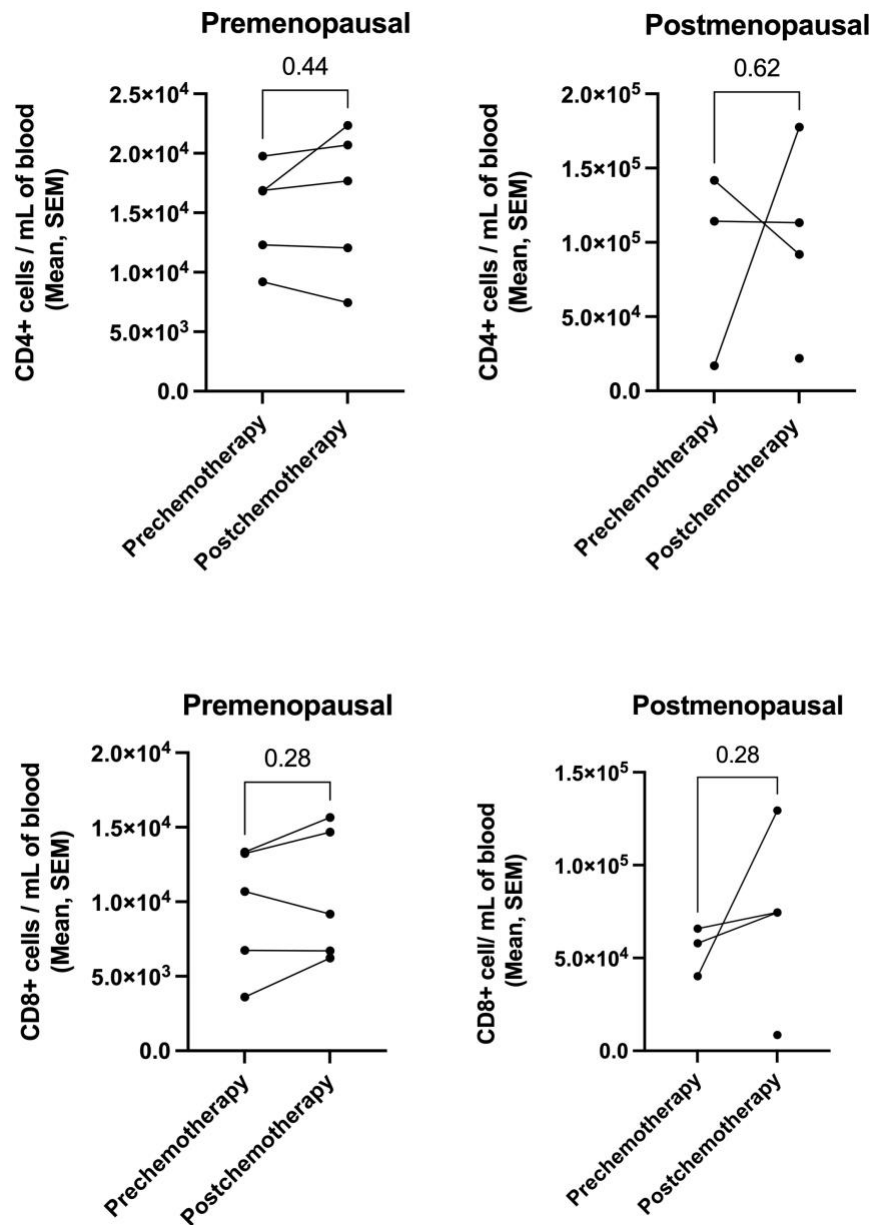


Figure Legend: Absolute numbers of CD4⁺CD3⁺ and CD8⁺CD3⁺ T cells per millilitre of blood in pre- and post-menopausal patients before and after chemotherapy. Individual dots represent individual patients. P-value for paired t-test is shown.

Supplementary Figure 5: CD4⁺ and CD8⁺ cells as a percentage of CD3⁺ cells in pre- and post-menopausal patients

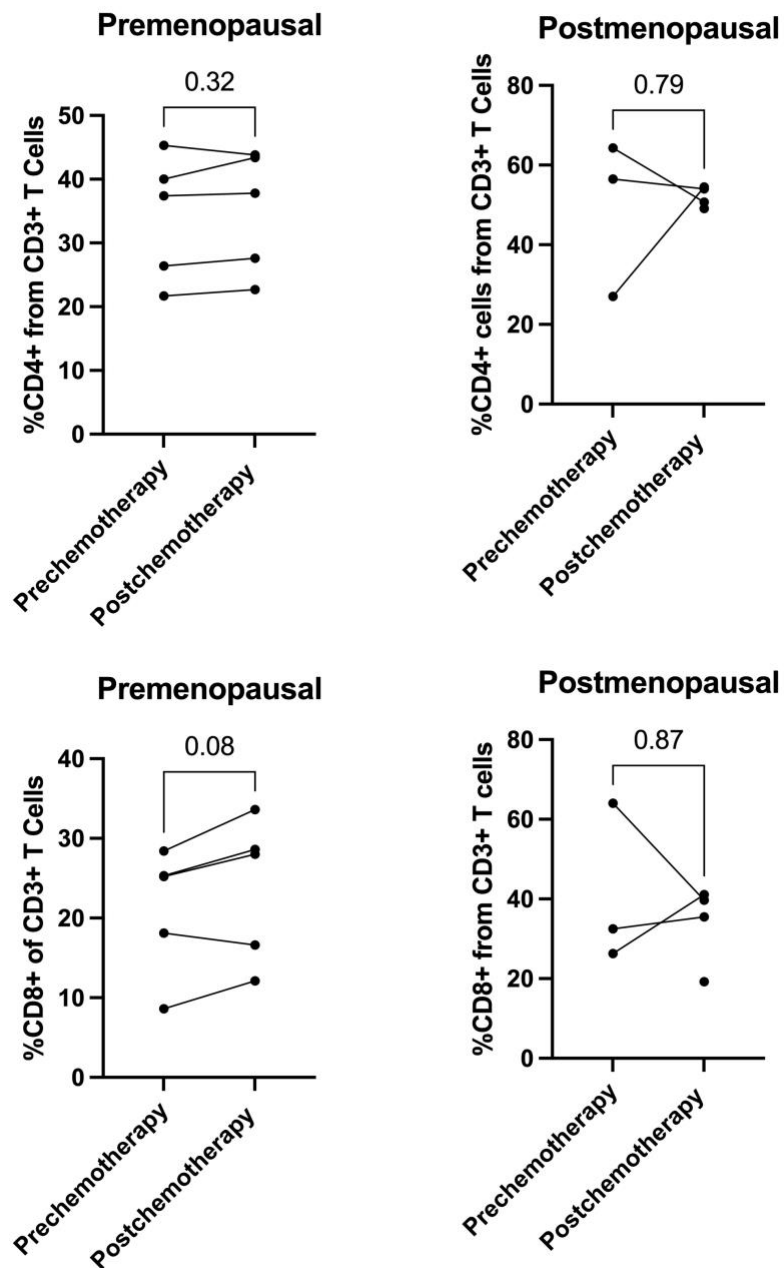


Figure Legend: CD4⁺ and CD8⁺ T cells as a percentage of CD3⁺ T cells in pre- and post-menopausal patients. Individual dots represent individual patients. P-value for paired t-test is shown.

Supplementary Figure 6: T cell subsets as absolute numbers for premenopausal patients

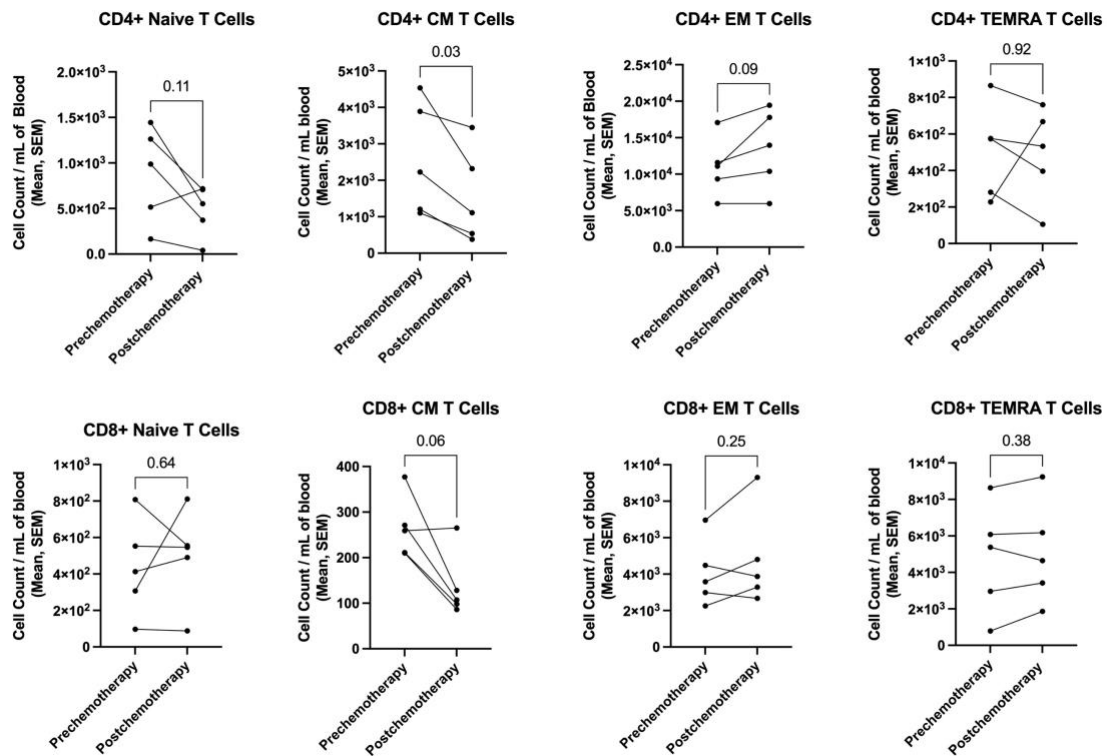


Figure Legend: Absolute numbers of CD4⁺ and CD8⁺ T cells subsets in premenopausal patients. Individual dots represent individual patients. P-value for paired t-test is shown.

Supplementary Figure 7: T cell subsets as absolute numbers for postmenopausal patients

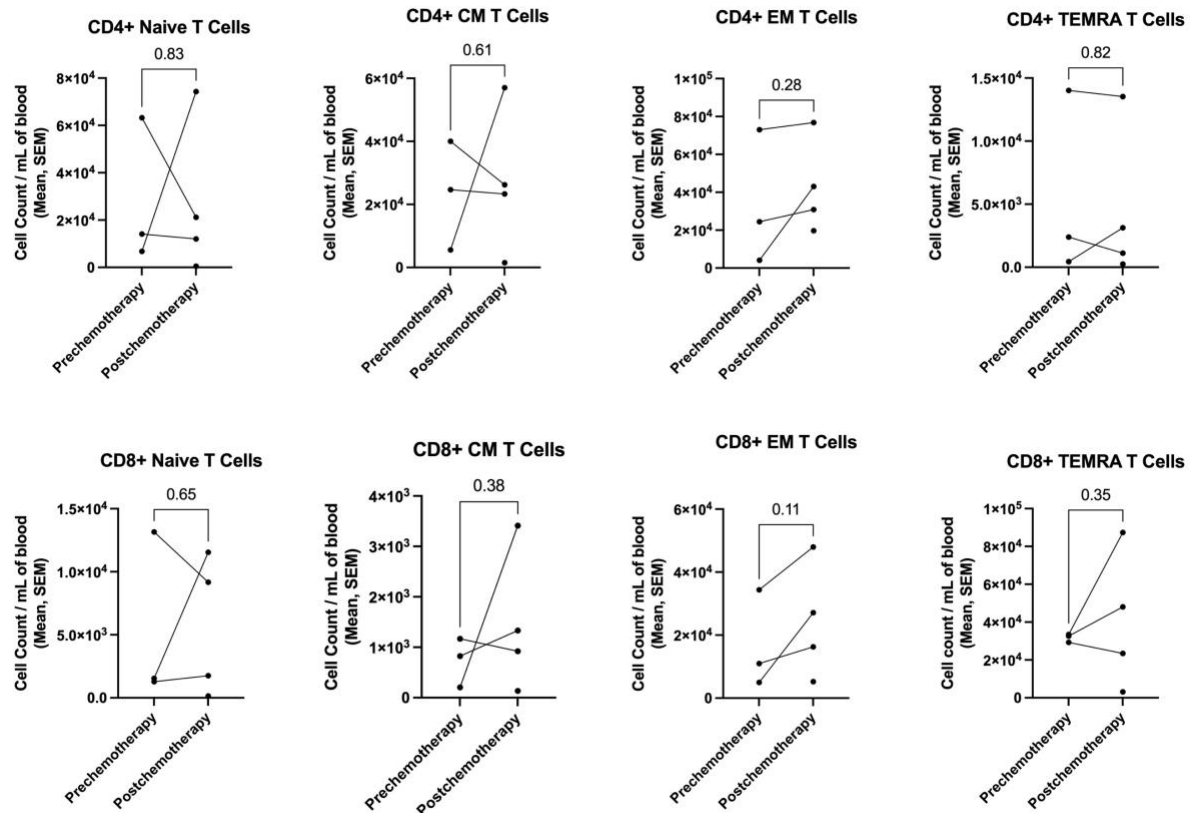


Figure Legend: Absolute numbers of CD4⁺ and CD8⁺ T cells subsets in postmenopausal patients. Coloured dots represent individual patient values. Individual dots represent individual patients. P-value for paired t-test is shown. CM: central memory, EM: effector memory, TEMRA: terminally differentiated memory cells.