

Supplementary Materials

Title:

A Phase Ib trial of pembrolizumab plus paclitaxel or flat-dose capecitabine in 1st/2nd line metastatic triple negative breast cancer

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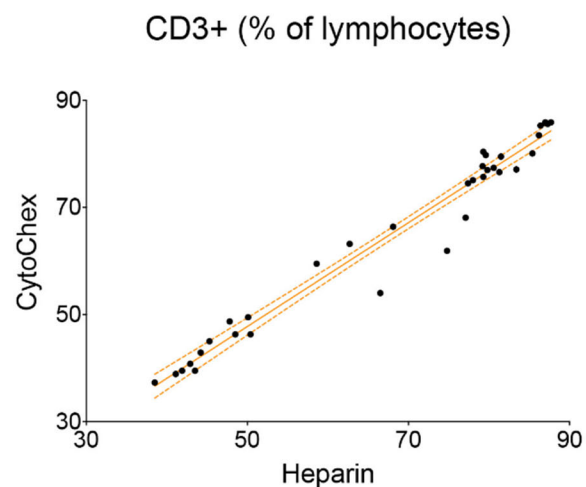
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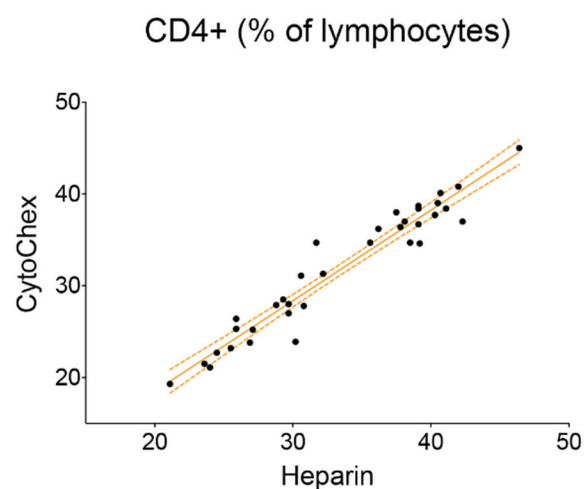
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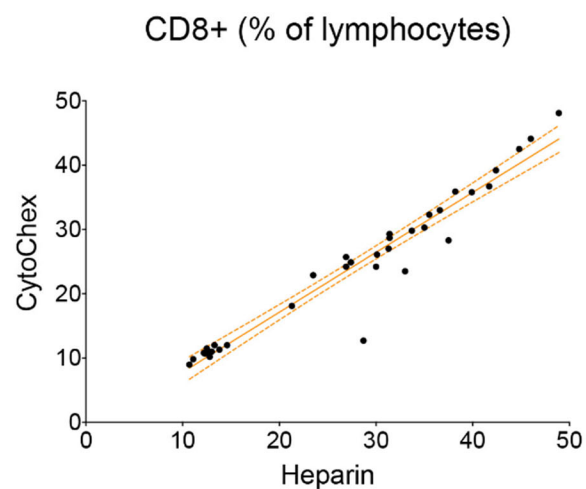
Supplementary figure 1: Correlation of real-time flow cytometry using heparin versus cytochex tubes



	Heparin vs. CytoChex
Spearman r	
r	0.9728
95% confidence interval	0.9453 to 0.9866
P value	
P (two-tailed)	<0.0001
P value summary	****
Exact or approximate P value?	Approximate
Significant? (alpha = 0.05)	Yes
Number of XY Pairs	35



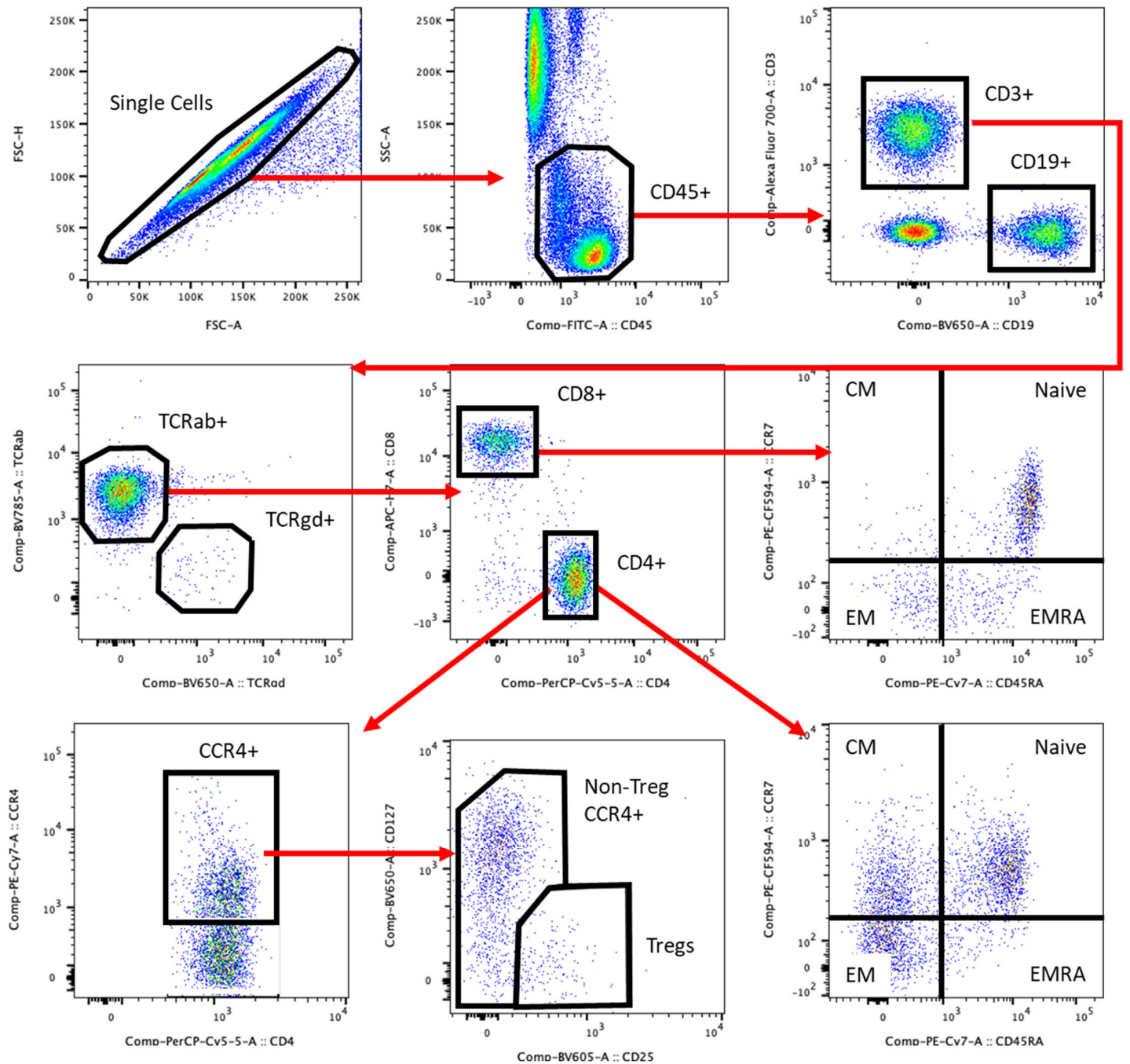
	Heparin vs. CytoChex
Spearman r	
r	0.9382
95% confidence interval	0.8778 to 0.9692
P value	
P (two-tailed)	<0.0001
P value summary	****
Exact or approximate P value?	Approximate
Significant? (alpha = 0.05)	Yes
Number of XY Pairs	35



	Heparin vs. CytoChex
Spearman r	
r	0.9697
95% confidence interval	0.9392 to 0.9851
P value	
P (two-tailed)	<0.0001
P value summary	****
Exact or approximate P value?	Approximate
Significant? (alpha = 0.05)	Yes
Number of XY Pairs	35

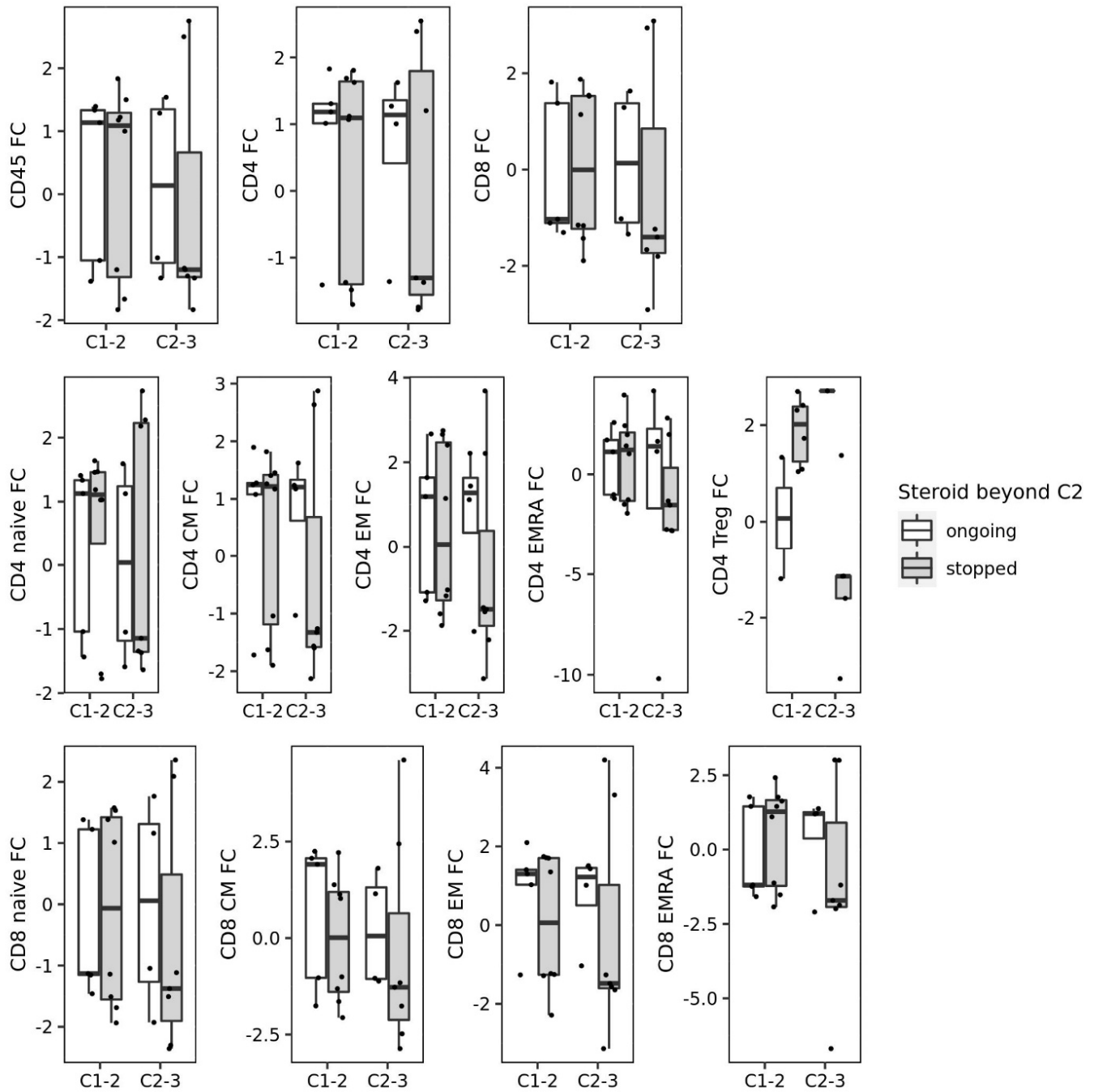
Caption: The above correlation plots illustrate the association of cell counts using Cytochex versus heparin tubes, both in real time without cryopreservation. The solid line represents the linear trend estimate, with dotted lines being the associated 95% confidence interval. P values correspond to a 2-sided t-test of means

Supplementary Figure 2: Flow cytometry gating strategy of peripheral blood mononuclear cells



Caption: Live cells were gated on FSC/SSC (singlets) and CD45⁺. CD3⁺ T cells and CD19⁺ B cells were then gated, followed by TCRab⁺ and the indicated CD4⁺ and CD8⁺ T cell subsets, including naïve (CD45RA⁺/CCR7⁺), central memory (CM) (CD45RA⁻/CCR7⁺), effector memory (EM) (CD45RA⁻/CCR7⁻), effector memory cell re-expressing CD45RA (EMRA) (CD45RA⁺/CCR7⁻) T cells, and CD4⁺ T regulatory (CCR4⁺, CD127^{low}, CD25^{high}) cells (Tregs).

Supplementary figure 3: Influence of continuation v. cessation of dexamethasone on peripheral immune cell counts



Caption: All subjects received prophylactic steroids during cycle 1 of paclitaxel, whereas some subjects stopped prophylactic steroids starting in cycle 2. Fold changes in cell counts varied significant across subjects, preventing definitive assessment of steroid effect, however the analysis does not show a consistent lymphodepleting effect of ongoing steroids.

Legend: C1-2: fold change in cell count comparing cycle 2 to cycle 1; C2-3: fold change in cell count comparing cycle 3 to cycle 2. Treg: T regulatory cell, CM: central memory; EM: effector memory; EMRA: effector memory re-expressing CD45RA⁺.

Supplementary table 1: Physician-reported adverse events attributed to pembrolizumab

Adverse event	Patients, No. (%)	
	Capecitabine (n=14)	Paclitaxel (n=15)
Any grade 1-2	6 (43%)	3 (20%)
Any grade 3-4	1 (7%)	5 (33%)
Adrenal insufficiency		
3-4	0	1 (7%)
Anemia		
3-4	0	1 (7%)
Arthralgia		
1-2	1 (7%)	0
Cough		
1-2	2 (14%)	0
Dry mouth		
1-2	1 (7%)	0
Dyspnea		
1-2	1 (7%)	0
Fatigue		
1-2	4 (29%)	2 (13%)
3-4	0	2 (13%)
Hot flashes		
1-2	0	1 (7%)
Hyperbilirubinemia		
3-4	0	1 (7%)
Hyperglycemia		
3-4	1 (7%)	0
Hypothyroidism		
1-2	3 (21%)	0
Motor neuropathy		
3-4	1 (7%)	0
Mucositis		
1-2	0	1 (7%)
Neutropenia		
1-2	0	1 (7%)
Sepsis		
3-4	0	1 (7%)

Caption: Toxicities are scored using the CTCAEv4.0. Adverse events were documented at all scheduled assessments weekly for the first 12 weeks, every 3 weeks thereafter and more frequently as clinically indicated.

Legend: CTCAEv4.0– Common Terminology Criteria for Adverse Events version 4.0

Supplementary table 2: Dose reductions for capecitabine and paclitaxel

Dose level	0	-1	-2	-3	-4
Capecitabine	2000 mg BID	1500 mg BID	1300 mg BID	1150 mg BID	1000 mg BID
% at week 4	93% (13/14)	7% (1/14)			
% at week 8	87% (11/13)		8% (1/13)	8% (1/13)	
% at week 12	40% (4/10)	40% (4/10)	10% (1/10)	10% (1/10)	
% at week 16	13% (1/8)	50% (4/8)	13% (1/8)	13% (1/8)	13% (1/8)
% at week 20		38% (3/8)	25% (2/8)	13% (1/8)	25% (2/8)
% at week 24		43% (3/7)	14% (1/7)	14% (1/7)	29% (2/7)
% at week 28		50% (3/6)	17% (1/6)	17% (1/6)	17% (1/6)
% at week 32		40% (2/5)	20% (1/5)	20% (1/5)	20% (1/5)
% at week 36		40% (2/5)	20% (1/5)	20% (1/5)	20% (1/5)
% at week 40		40% (2/4)		25% (1/4)	25% (1/4)
% at week 44		40% (2/4)		25% (1/4)	25% (1/4)
% at week 48		67% (2/3)			33% (1/3)
Paclitaxel	80 mg/m² d1,8,15	80 mg/m² d1,8	70 mg/m² d1,8	60 mg/m² d1,8	50 mg/m² d1,8
% at week 4	93% (14/15)				
% at week 8	90% (9/10)		10% (1/10)		
% at week 12	67% (6/9)		22% (2/9)	11% (1/9)	
% at week 16	63% (5/8)		25% (2/8)		
% at week 20	50% (2/4)	25% (1/4)	25% (1/4)		
% at week 24	33% (1/3)		33% (1/3)	33% (1/3)	

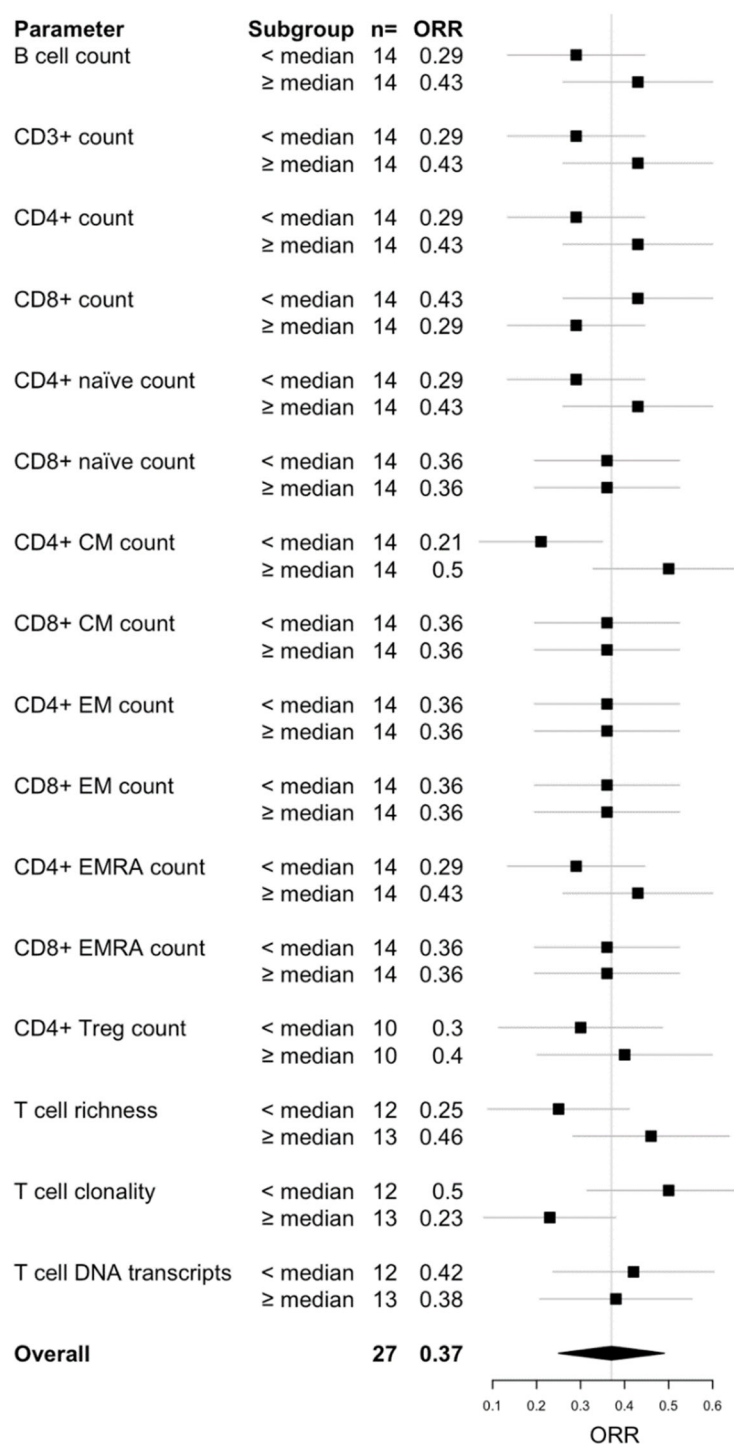
Caption: Percentages of patients receiving doses of chemotherapy, at prespecified dosing levels. Only weeks with >3 patients are listed. Dosing of capecitabine involved 7 days of twice daily administration, with 7 days off of each 14-day cycle. Dosing of paclitaxel involved infusions on days specified of each 3-week cycle.

Legend: BID – twice daily

Supplementary table 3: Guidance on management of diarrhea/colitis for combination capecitabine plus pembrolizumab, adopted from clinical protocol and study outcomes

#	Management recommendations
1	Dose interruptions may be required and are permitted according to standard practice, with clinical determination guiding whether dose modification is necessary. Dose reductions should be permanent and not re-escalated.
2	Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).
3	All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
4	For Grade 2 diarrhea/colitis, dosing may be held per standard practice. For diarrhea that persists greater than 3 days, administer oral corticosteroids.
5	For Grade 3 or 4 diarrhea/colitis that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids.
6	For persistent grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.

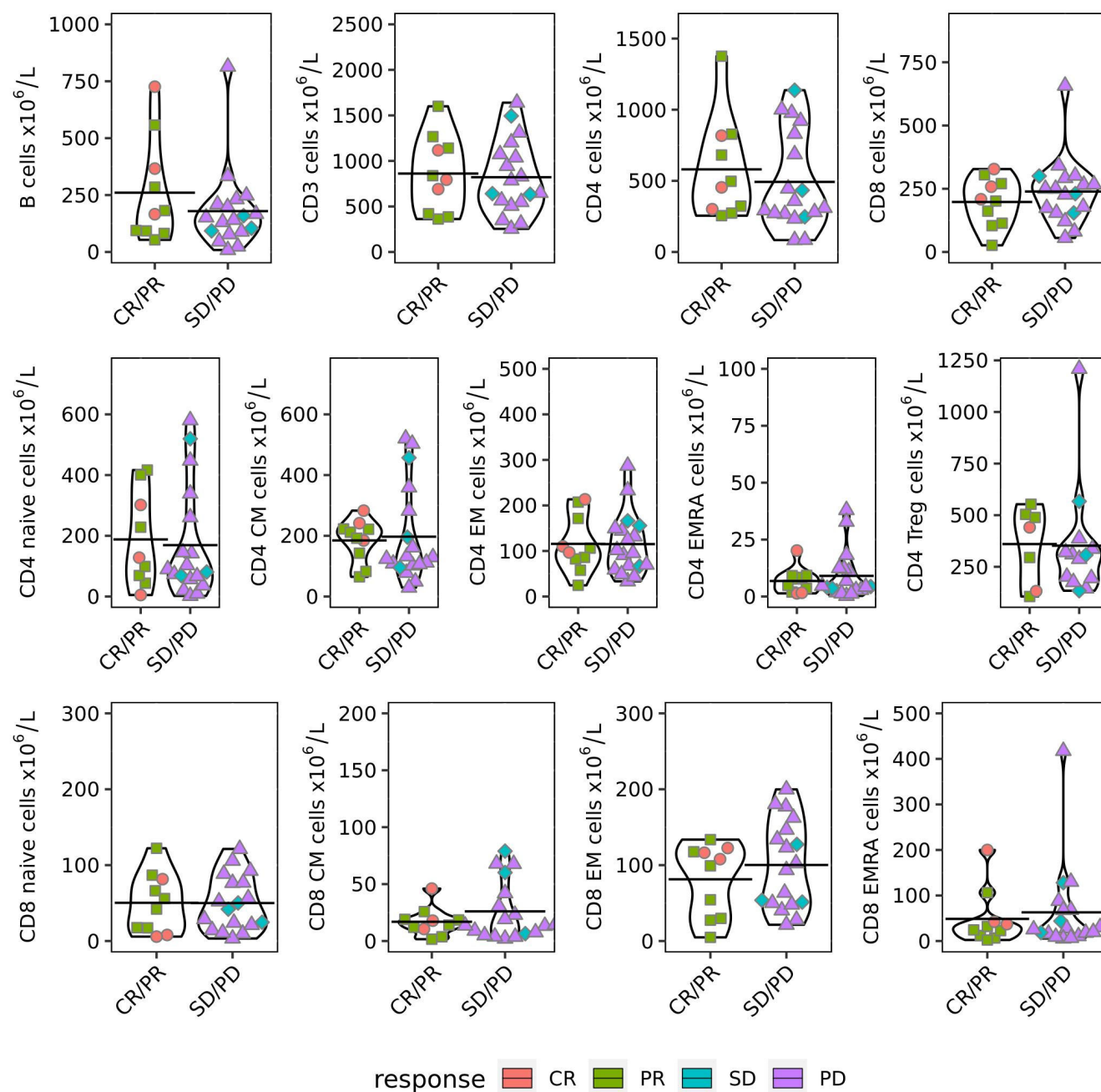
Supplementary figure 4: Associations of response with peripheral blood immune biomarkers



Caption: Intervals represent the 80% confidence interval of the point estimate of response rate. Statistical testing is not conducted given the exploratory nature of the assessment.

Legend: ORR: overall response rate; Treg: T regulatory cell, CM: central memory; EM: effector memory; EMRA: effector memory re-expressing CD45RA+.

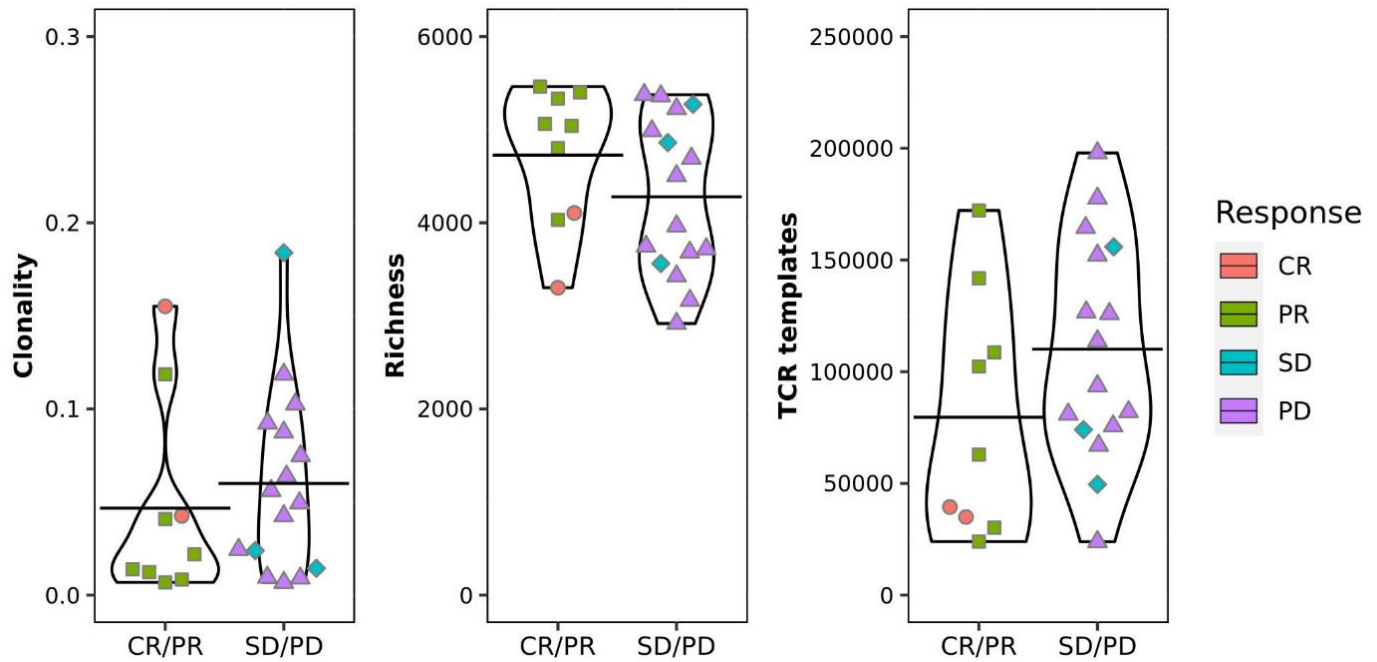
Supplementary figure 5: Baseline peripheral blood biomarkers according to response



Caption: No apparent differences in peripheral baseline cell counts were observed according to response. Means are illustrated by the horizontal line.

Legend: CR: complete response; PR: partial response; SD: stable disease; PD: progression of disease; Treg: T regulatory cell, CM: central memory; EM: effector memory; EMRA: effector memory re-expressing CD45RA+.

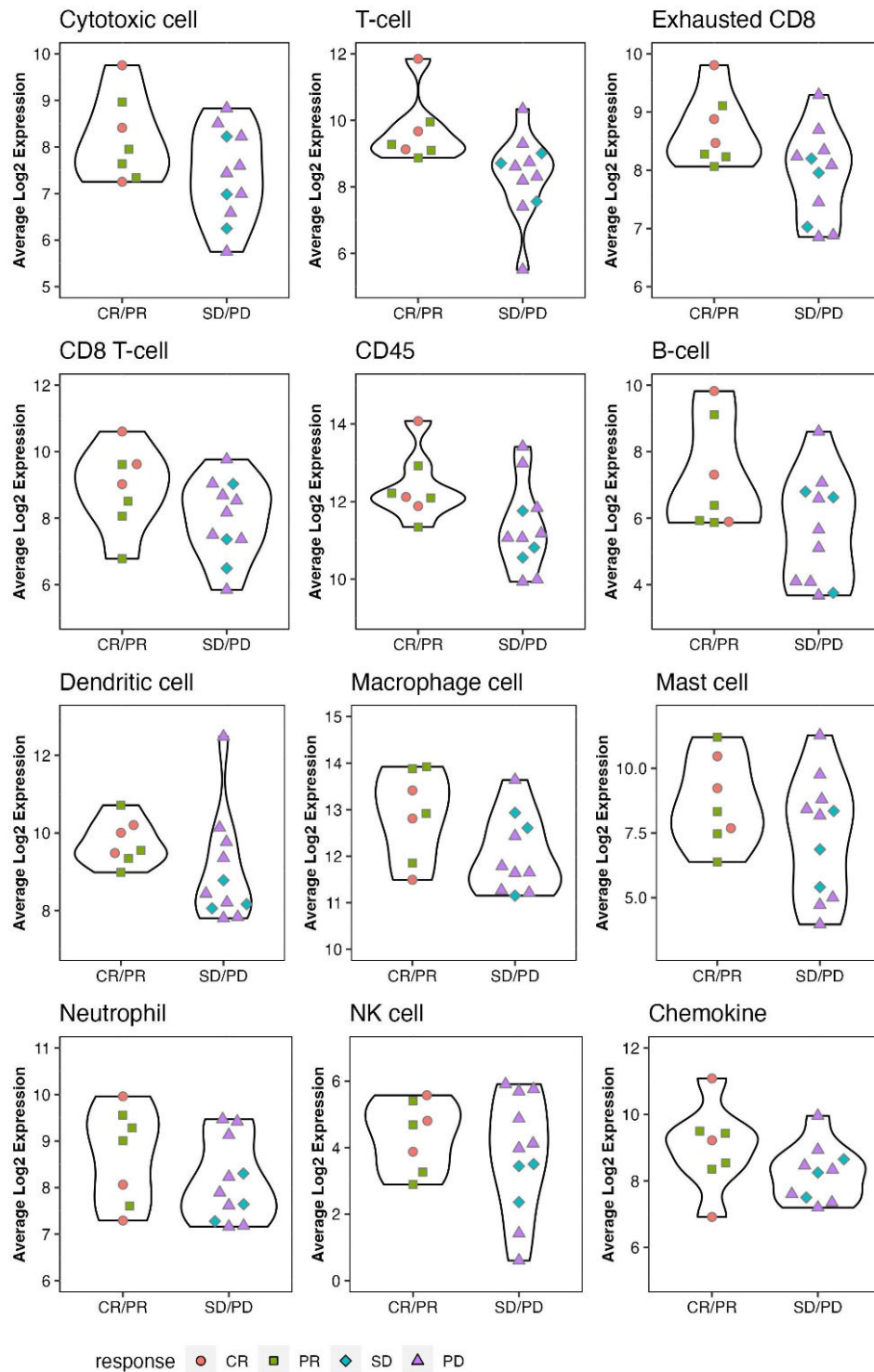
Supplementary figure 6: Baseline peripheral T-cell diversity metrics according to response



Caption: Distribution of previously-described T-cell receptor sequencing metrics according to clinical response. Means are illustrated by the horizontal line.

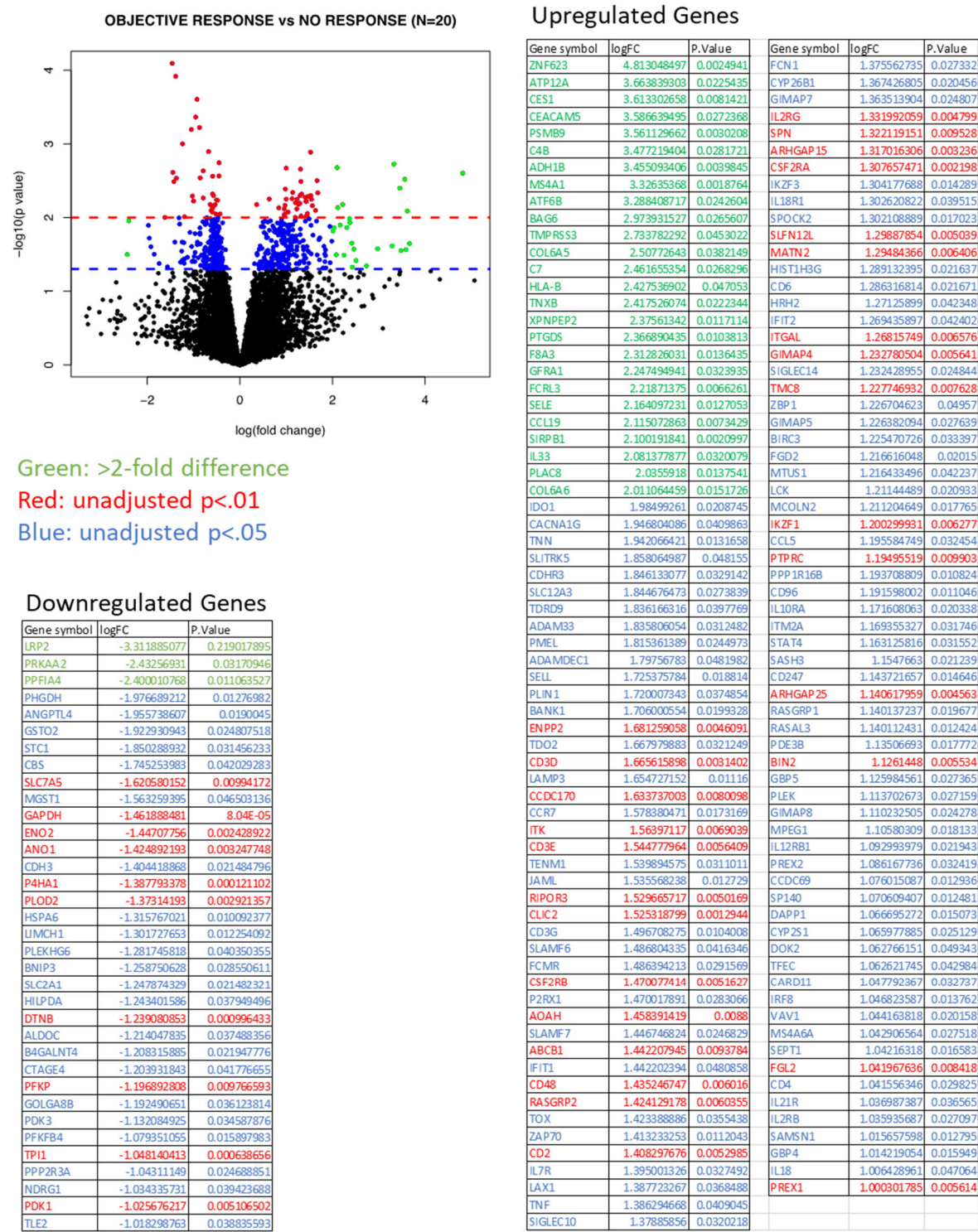
Legend: CR: complete response; PR: partial response; SD: stable disease; PD: progression of disease; TCR: T-cell receptor

Supplementary figure 7: Immune cell type score between patients with and without clinical benefit.



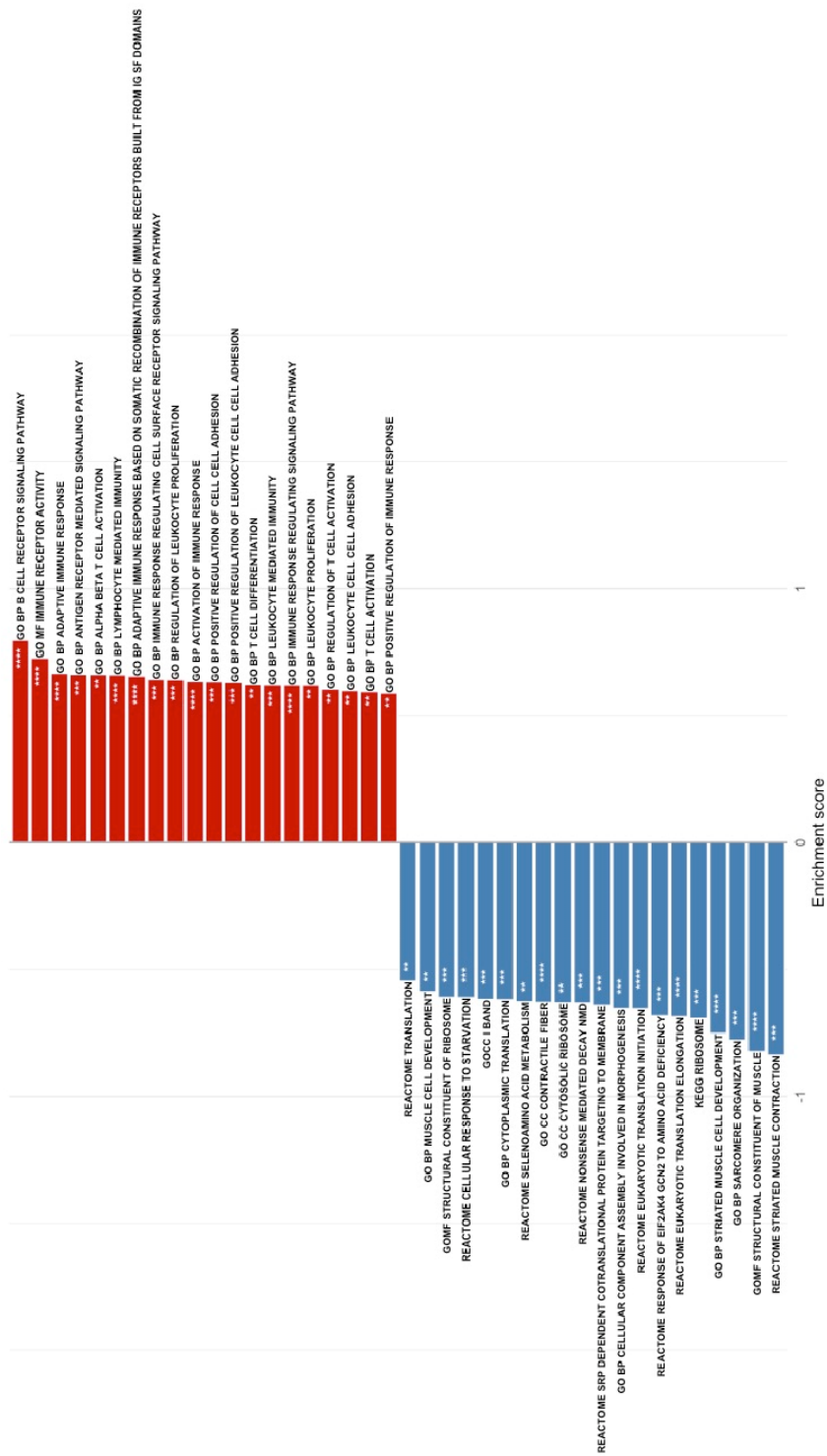
Legend: Distributions of previously described baseline RNA cell signatures according to clinical response. Genes used to compute immune cell type score is defined previously.⁵⁶ CB : clinical benefit (clinical response or stable disease), NO_CB : no clinical benefit, CR – complete response, PR – partial response, PD – progressive disease, SD – stable disease.

Supplementary figure 8: Volcano plot and table showing enriched genes among patients with clinical benefit versus not



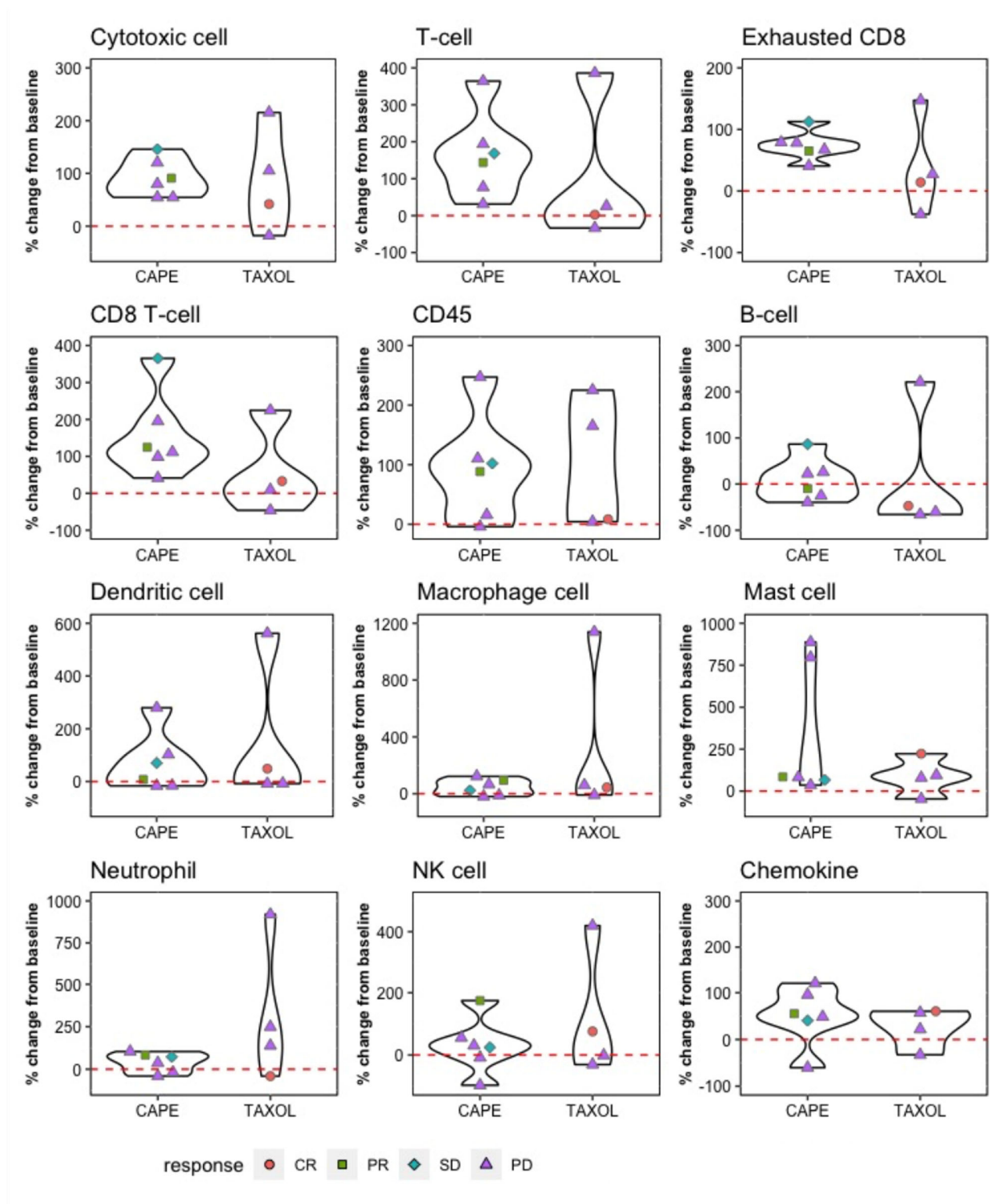
Legend: All genes with greater than 10-fold differential are listed. Genes with an estimated greater than 100-fold differential are illustrated in green. Genes that are associated with unadjusted p values <.01 or .05 are illustrated in red and blue, respectively. logFC: log fold change; P.Value: unadjusted P-values.

Supplementary figure 9: Gene set enrichment analysis



Legend: Top 20 significant gene sets enriched by genes that are up regulated in responders (red) and non-responders (blue). ****: false discovery rate (FDR) $q\text{-value} < .00001$; ***: FDR $q\text{-value} < .0001$; **: FDR $q\text{-value} < .001$

Supplementary figure 10: Changes in immune cell RNA signatures related to capecitabine versus paclitaxel.

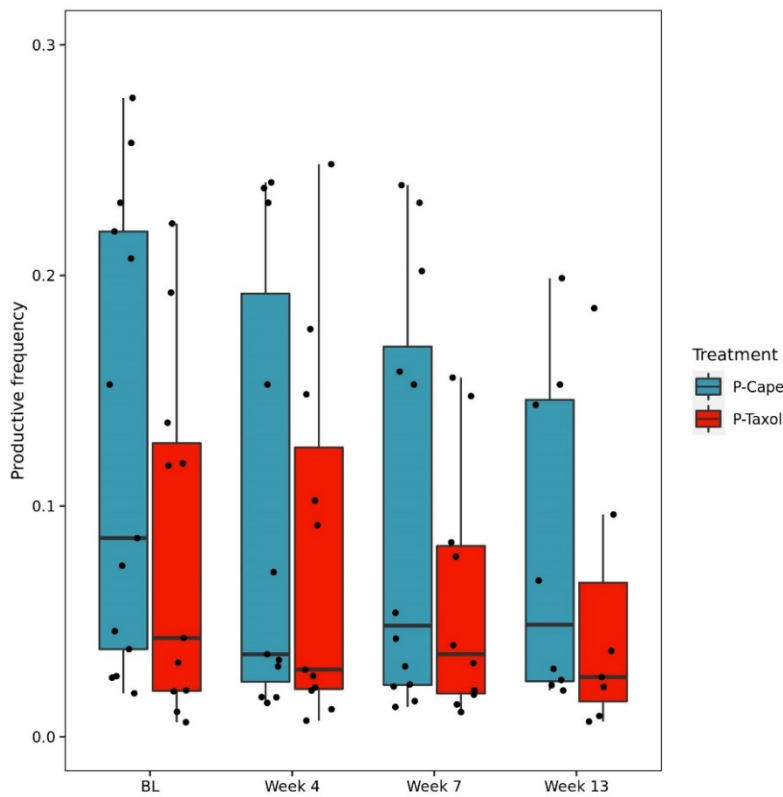


Legend: Distribution of fold-changes RNA signatures following therapy with either capecitabine or paclitaxel. Genes used to compute immune cell type score are defined previously.

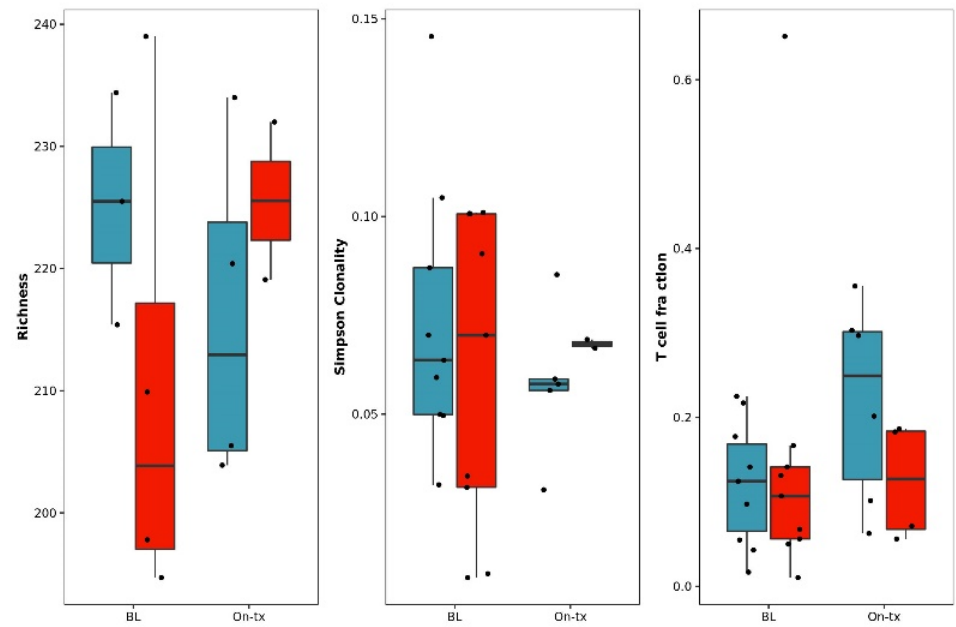
Cape: capecitabine; CR – complete response, PR – partial response, PD – progressive disease, SD – stable disease

Supplementary figure 11: Effect of therapy on T cell diversity metrics

a

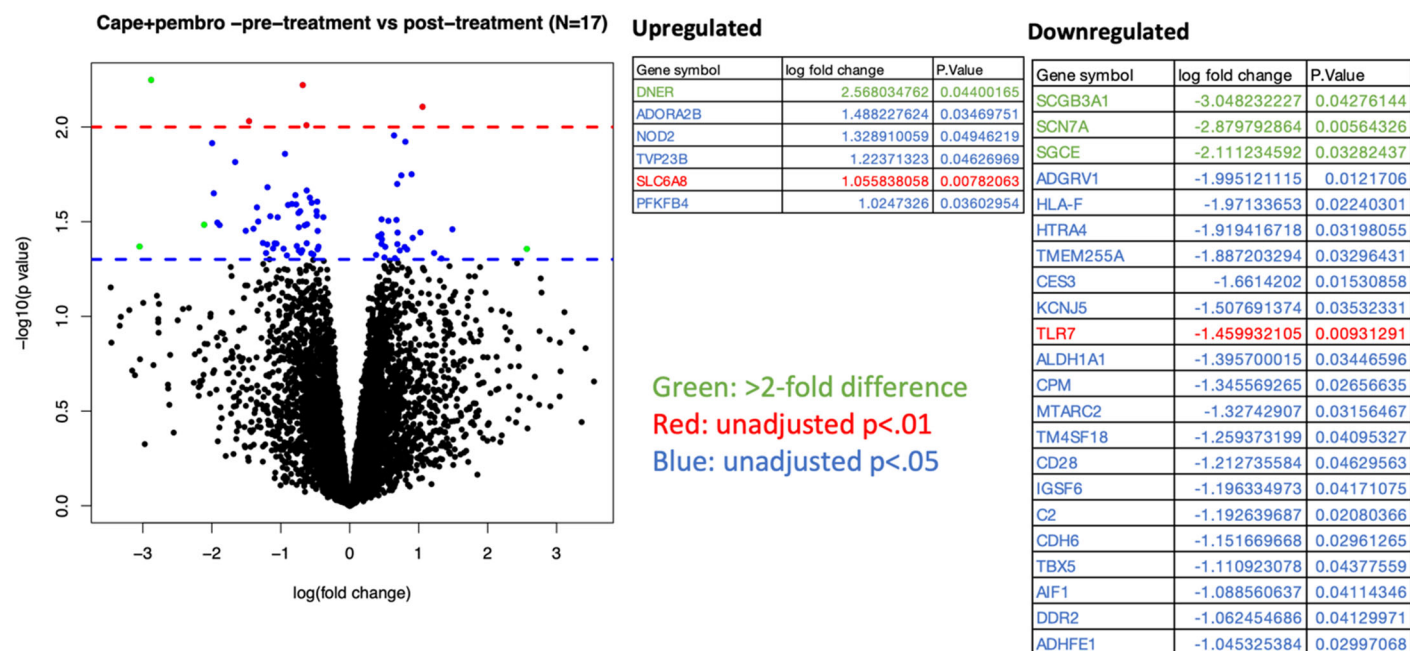


b



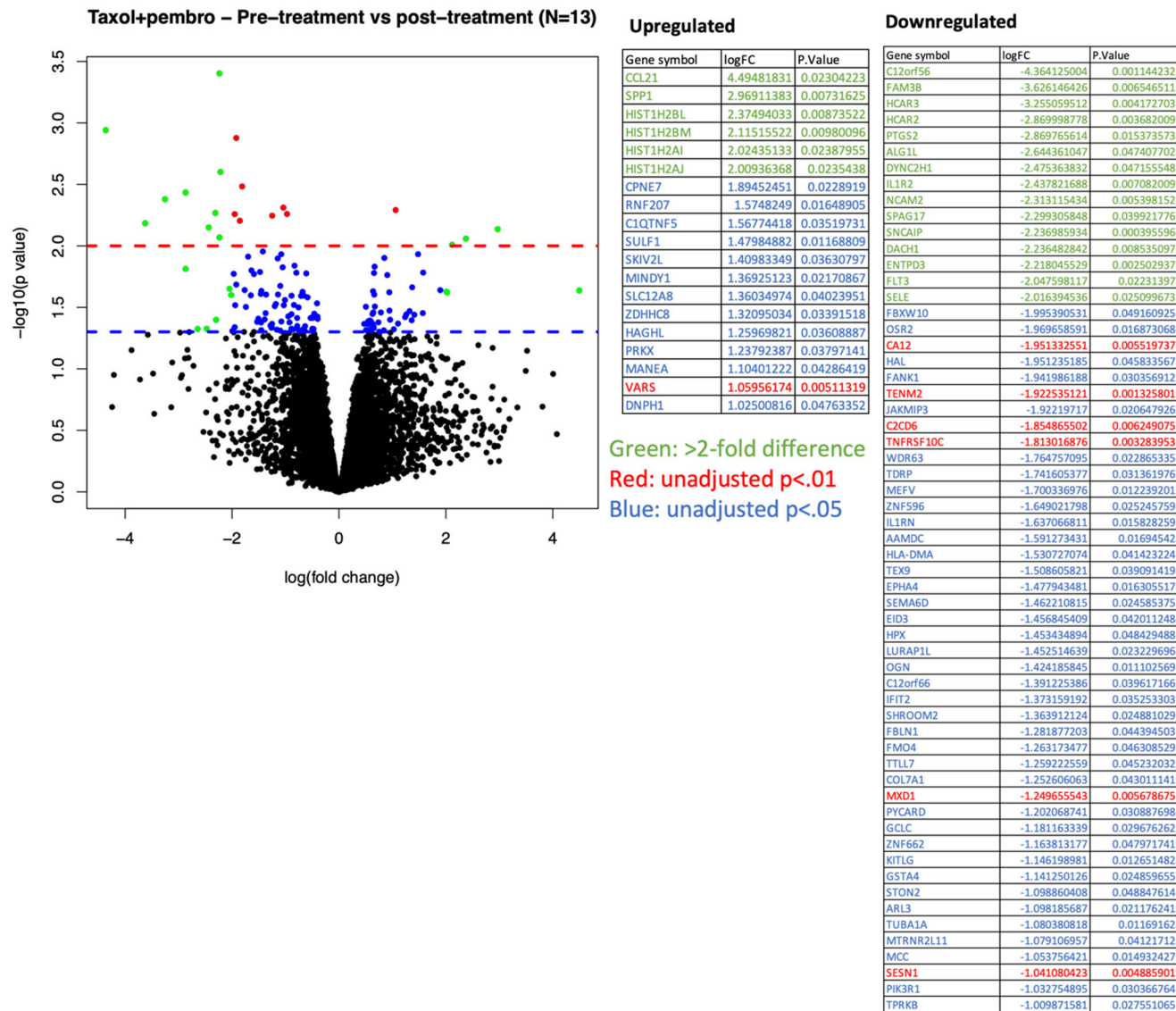
Legend: A. Change in productive frequency of top 10 clones during treatment. B. Change in T-cell diversity metrics during treatment. P-Cape: pembrolizumab + capecitabine; P-Taxol: pembrolizumab + paclitaxel; BL: baseline.

Supplementary figure 12: Volcano plot and table showing changes in genes following capecitabine/pembrolizumab



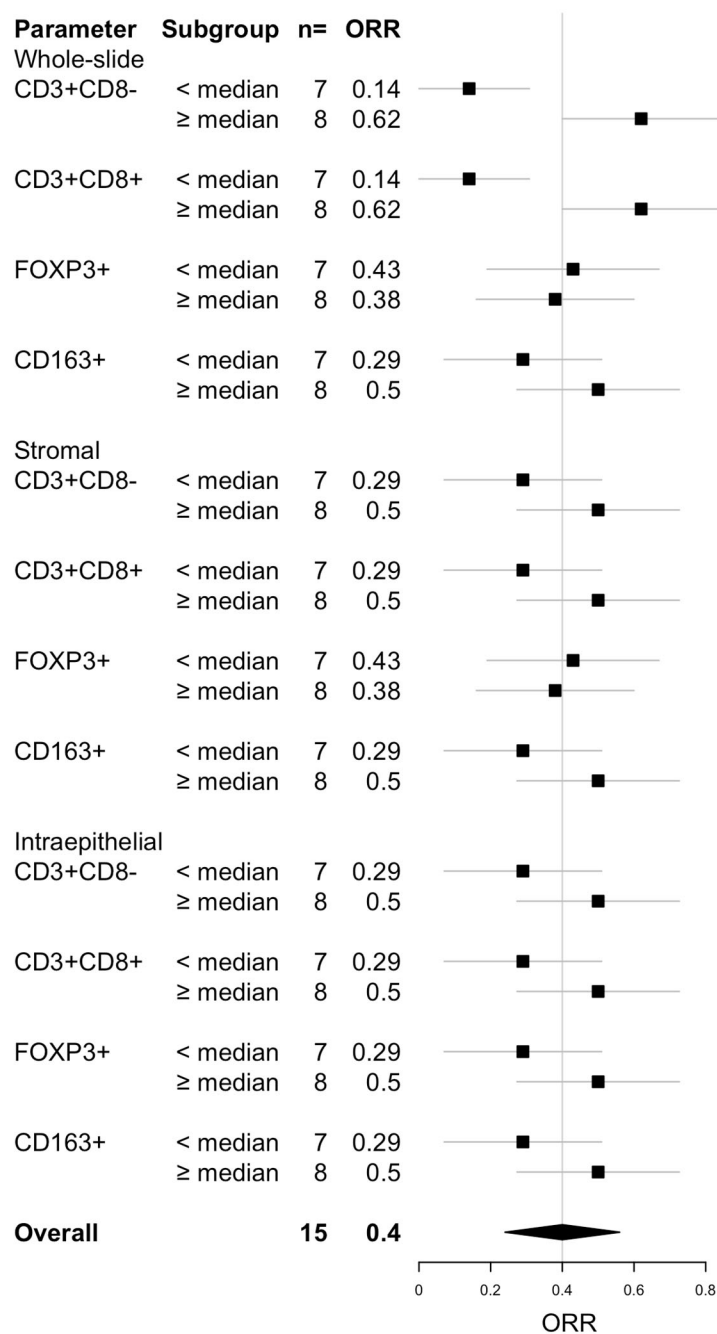
Legend: All genes with greater than 10-fold differential are listed. Genes with an estimated greater than 100-fold differential are illustrated in green. Genes that are associated with unadjusted p values <.01 or .05 are illustrated in red and blue, respectively. logFC: log fold change; P.Value: unadjusted P-values.

Supplementary figure 13: Volcano plot and table showing changes in genes following paclitaxel/pembrolizumab



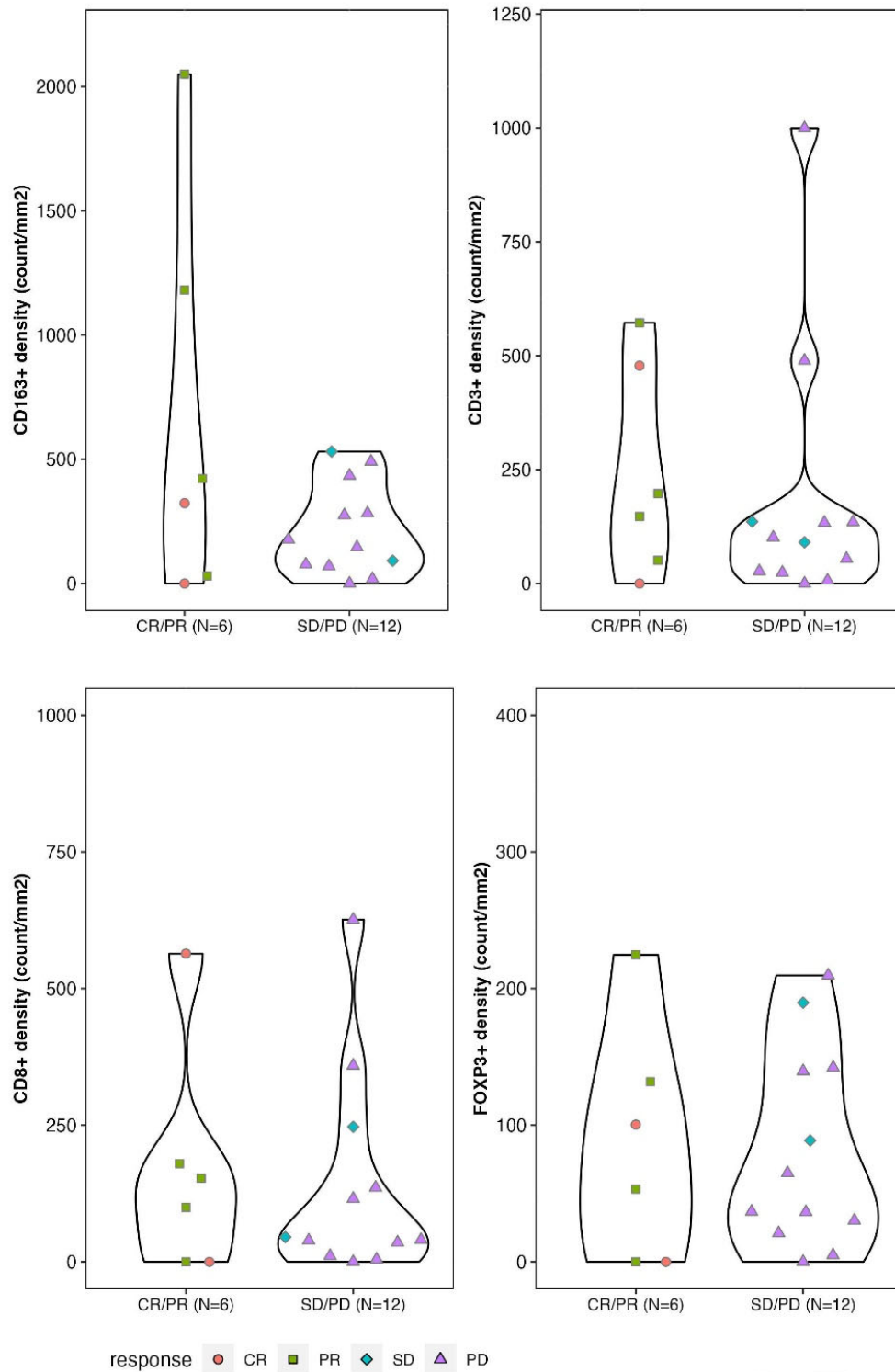
Legend: All genes with greater than 10-fold differential are listed. Genes with an estimated greater than 100-fold differential are illustrated in green. Genes that are associated with unadjusted p values <.01 or .05 are illustrated in red and blue, respectively. logFC: log fold change; P.Value: unadjusted P-values.

Supplementary figure 14: Associations of response with mIF cell density.



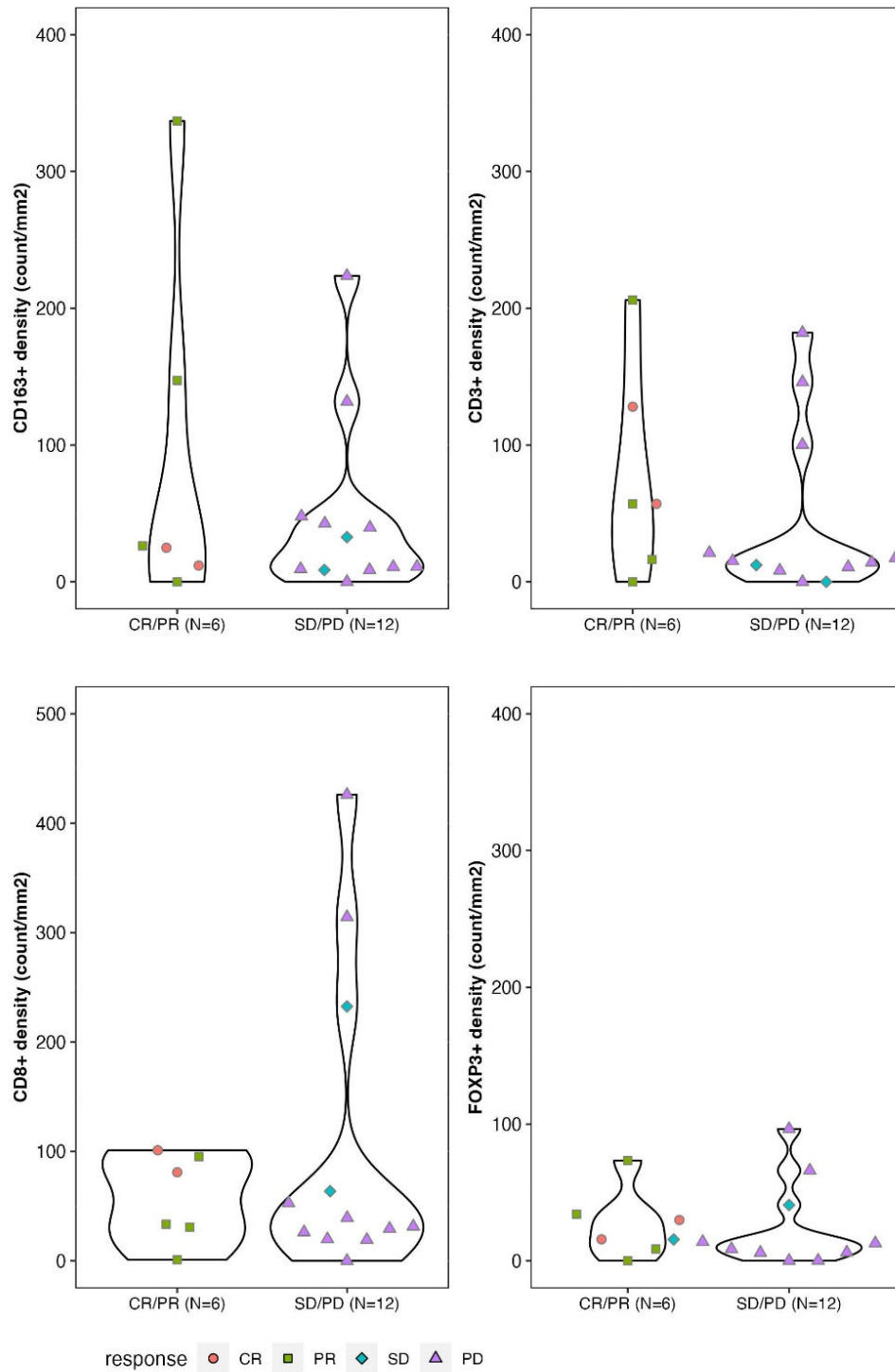
Legend: Intervals represent the 80% confidence interval of the point estimate of response rate. Statistical testing is not conducted given the exploratory nature of the assessment. ORR: overall response rate.

Supplementary figure 15: Baseline mIF stromal tumor infiltrates according to response



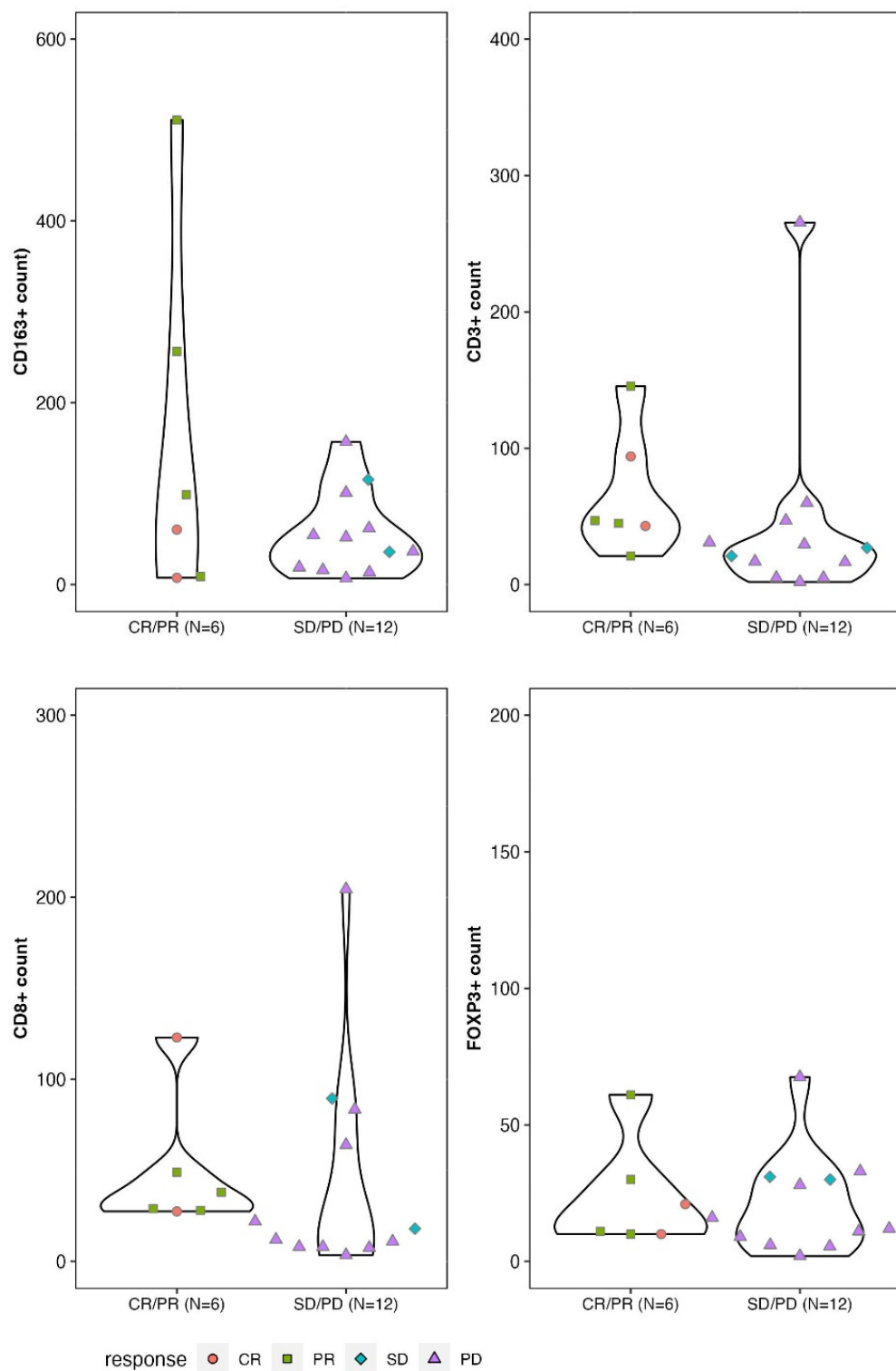
Legend: Distribution of baseline cell densities in the tumor stromal compartment are illustrated for patients with objective response versus not. CR: complete response; PR: partial response; SD: stable disease; PD: progression of disease.

Supplementary figure 16: Baseline mIF intraepithelial tumor infiltrates according to response



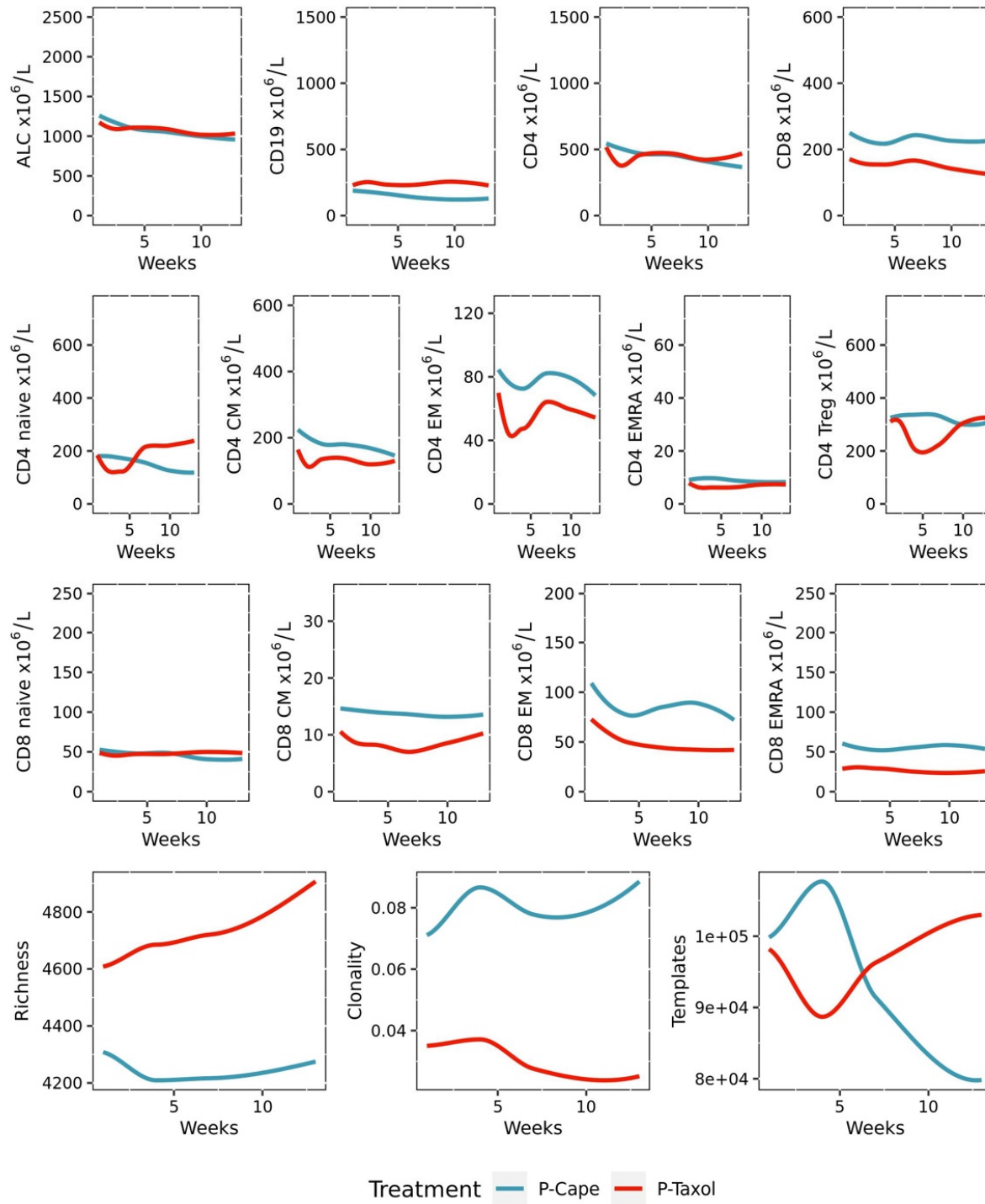
Legend: Distribution of baseline cell densities in the tumor intraepithelial compartment are illustrated for patients with objective response versus not. CR: complete response; PR: partial response; SD: stable disease; PD: progression of disease

Supplementary figure 17: Baseline mIF tumor infiltrates according to response (whole slide = stromal + intraepithelial).



Legend: Distribution of baseline whole-slide cell counts are illustrated for patients with objective response versus not. There appears to be general trend of increased immune infiltrates amongst patients with objective response. CR: complete response; PR: partial response; SD: stable disease; PD: progression of disease.

Supplementary Figure 18: Effect of therapy on peripheral blood mononuclear cells



Legend: On-treatment LOWESS curves of peripheral lymphocyte counts as measured by routine clinical testing (ALC), multi-parametric real-time flow cytometry, and T-cell receptor DNA deep sequencing (TCR Richness, clonality, template copy number). ALC: absolute lymphocyte count; P-Cape: pembrolizumab + capecitabine; P-Taxol: pembrolizumab + paclitaxel; metastatic treatment with pembrolizumab plus capecitabine; Taxol: metastatic treatment with pembrolizumab plus weekly paclitaxel; TCR: T-cell receptor. Units: ALC: 10^3 cells/uL; Richness: # productive templates/ T-cell counts: cells/uL