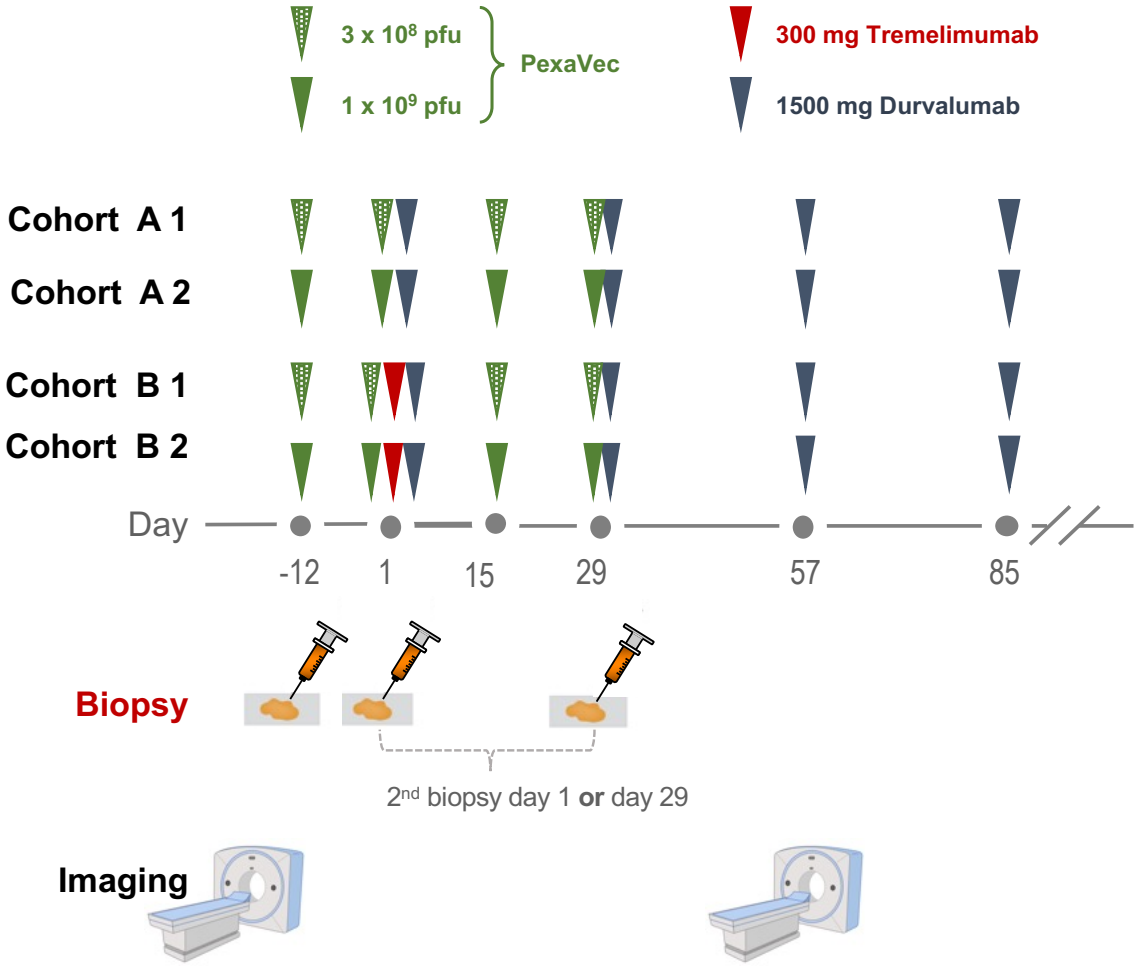
Supplementary Table 1: Patient characteristics

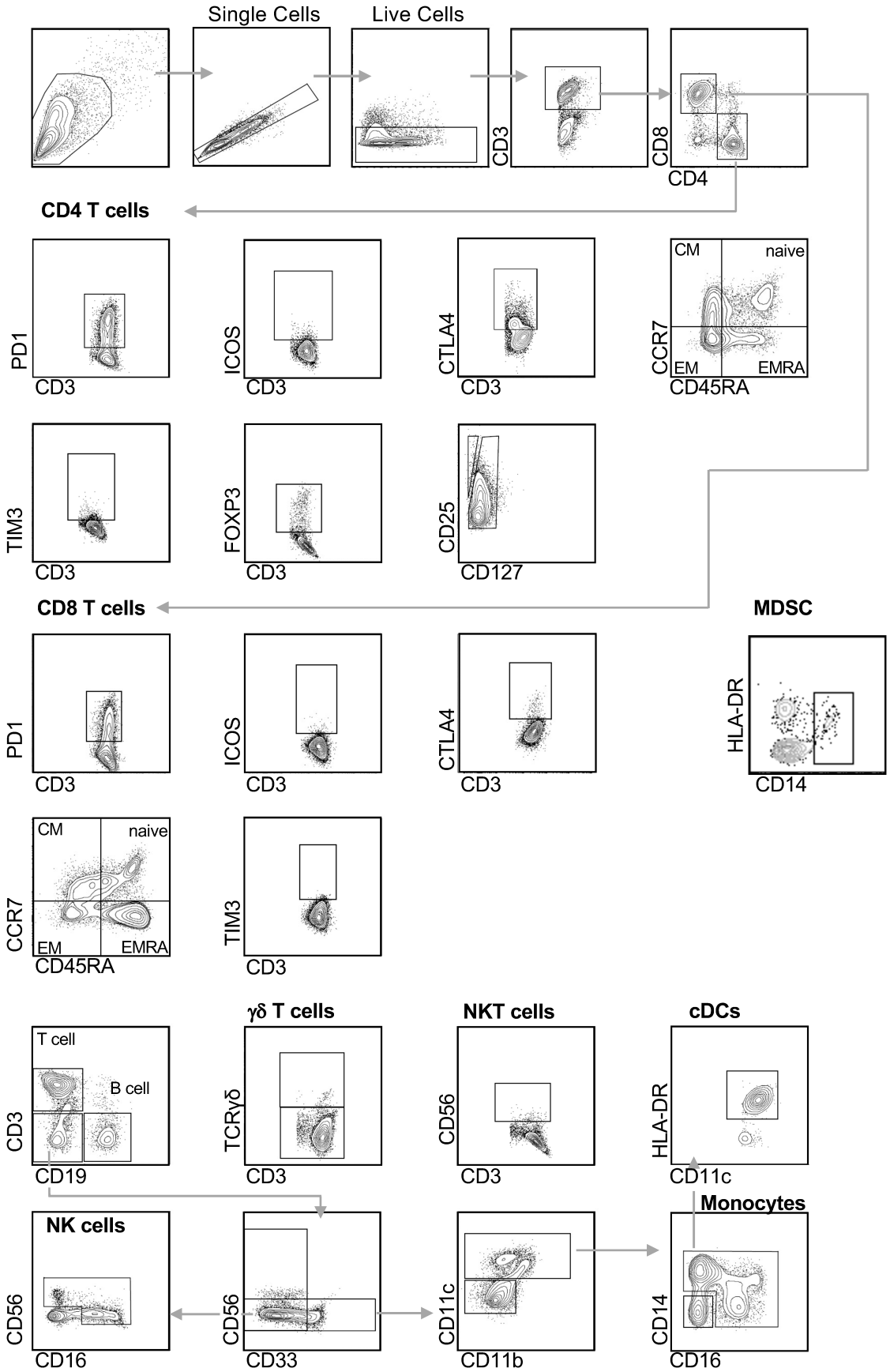
	PexaVec / durvalumab (N=16)	PexaVec / tremelimumab / durvalumab (N=18)
Age		
Median	53.5	56.5
Range	28-69	28-76
Sex -- no. (%)		
Female	10 (63)	10 (56)
Male	6 (37)	8 (44)
Race -- no. (%)		
Asian	2 (13)	2 (11)
Black	4 (25)	4 (22)
White	10 (63)	12 (67)
ECOG -- no/ (%)		
0	11 (69)	16 (89)
1	5 (31)	2 (11)
Primary site -- no. (%)		
Right colon	3 (19)	5 (28)
Left colon	11 (69)	10 (56)
Rectum	2 (13)	3(17)
Site of disease at enrollment -- no. (%)		
Liver	12 (75)	14 (78)
Lung	12 (75)	13 (72)
Distant lymph nodes	9 (56)	12 (67)
Bone	0 (0)	2 (11)
Peritoneum	3 (19)	3 (17)
KRAS mutant—no. (%)		
Yes	56 (9)	44(8)
No	44 (7)	56(10)
Previous adjuvant chemotherapy –no. (%)		
Yes	6 (37)	7 (39)
No	10 (63)	11 (61)
Number of prior chemotherapies for advanced or metastatic disease -- no (%)		
<= 2 lines	4 (25)	5 (28)
> 2 lines	12 (75)	13 (72)

Supplementary Table 2: Best response and survival.

	PexaVec/Durva (N=16)	PexaVec/Durva/Treme (N=18)
Best response		
CR	0	0
PR	1 (6.3)	0
SD	1 (6.3)	3 (16.7)
PD	12 (75)	8 (44.4)
Unevaluable	2 (12.5)	7 (38.9)
Objective response rate (CR or PR)	1 (6.3)	0
Disease control rate (CR, PR, or SD)	2 (12.5)	3 (16.7)
Median PFS months (95% CI)	2.3 (2.2-3.2)	2.1 (1.7-2.8)
Median OS months (95% CI)	7.5 (4.9-10.3)	5.2 (4.3-10.2))

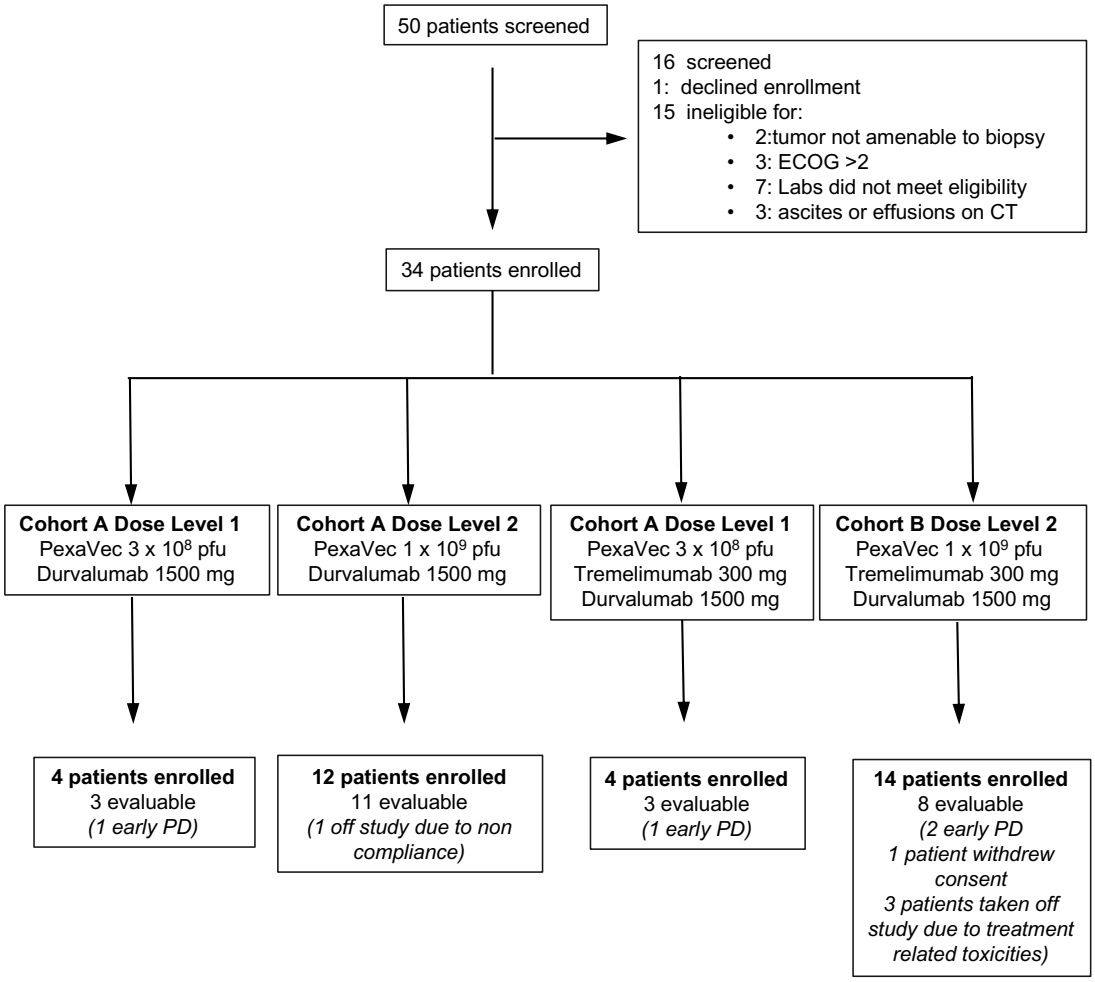


Supplementary Figure 1: Study Design of "A Phase I/II Study of PexaVec in Combination with Immune Checkpoint Inhibition in Refractory Metastatic Colorectal Cancer".

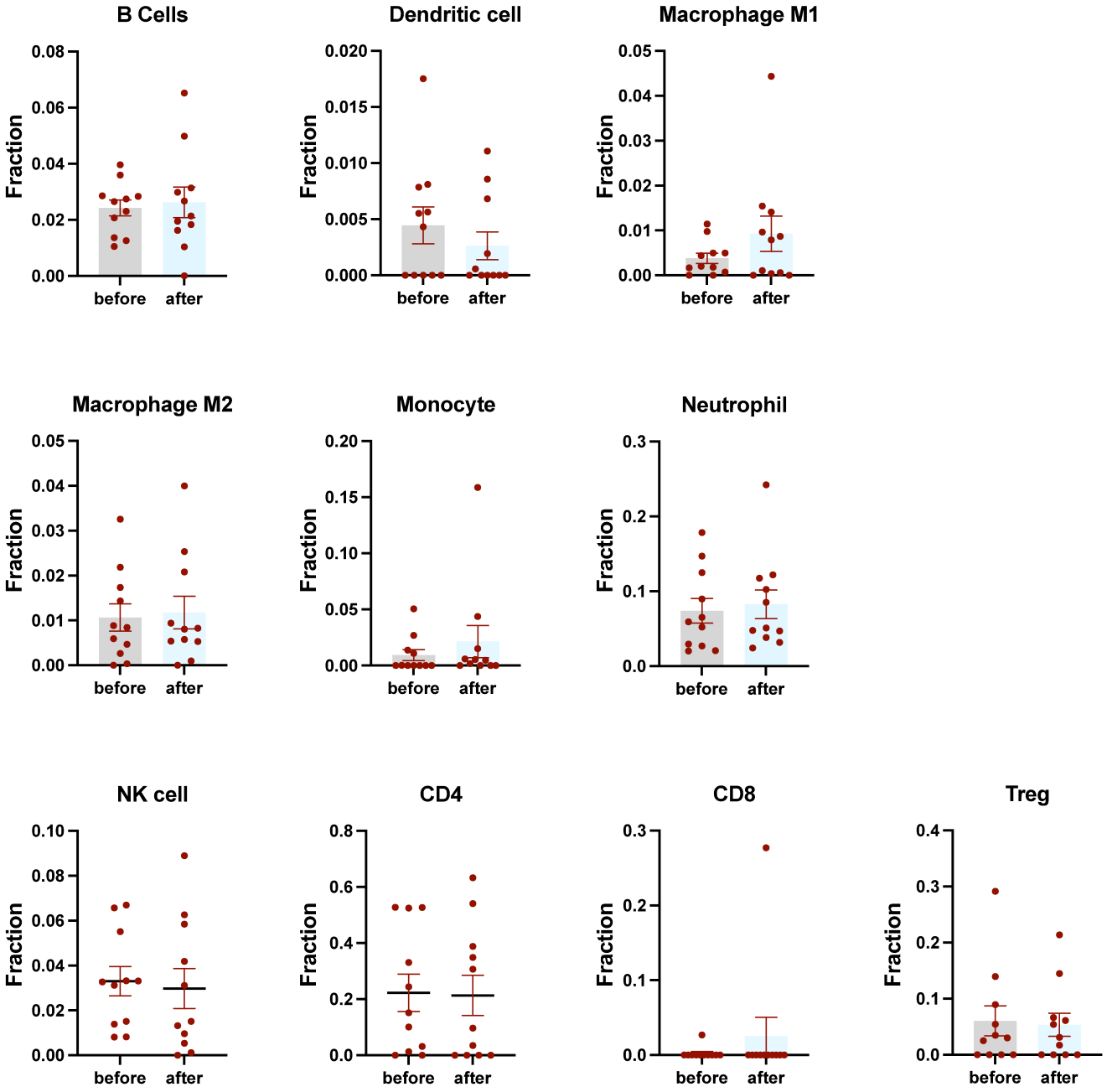


Supplementary Figure 2

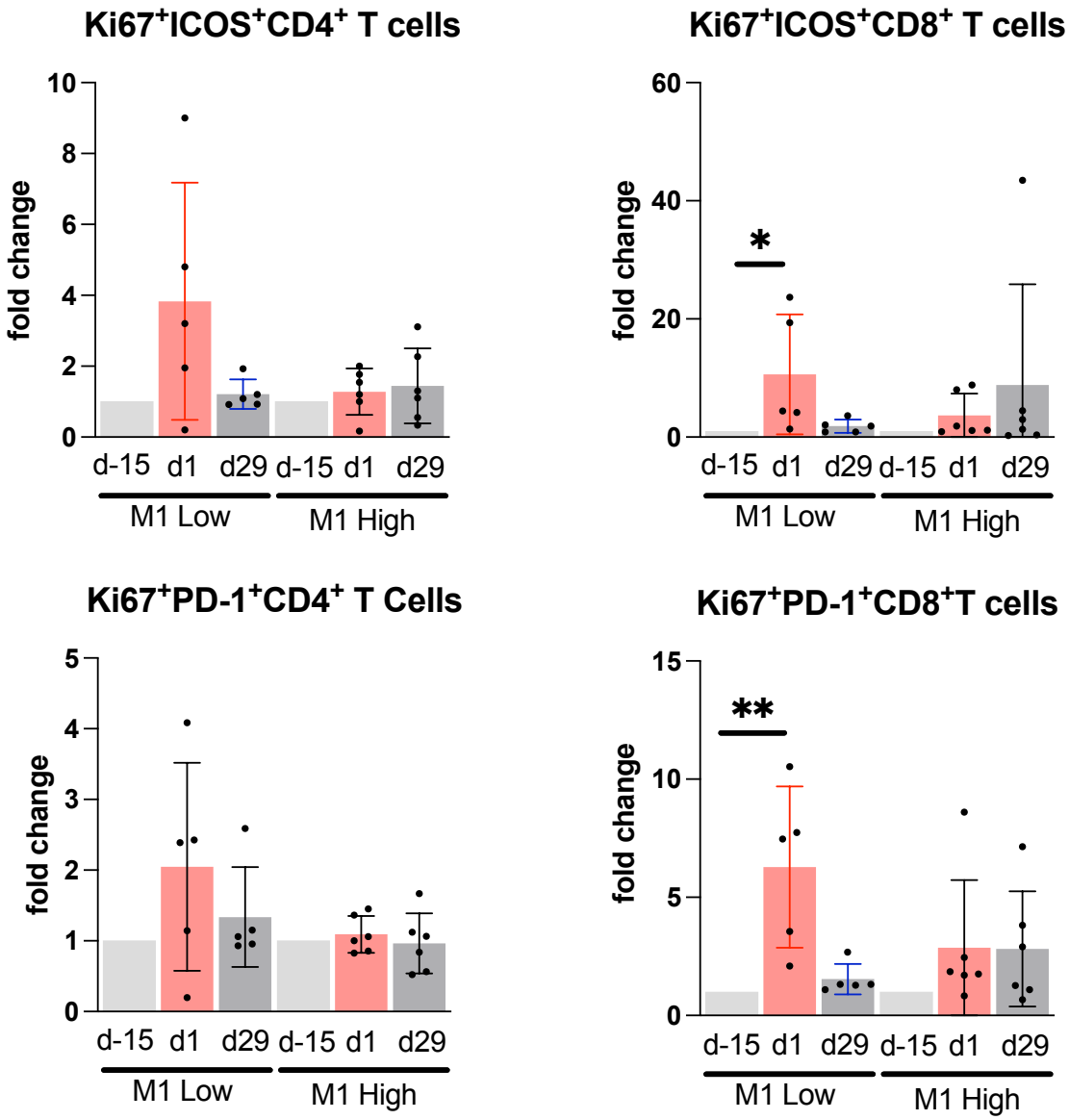
Supplementary Figure 2: Gating strategy for analysis of different immune cell populations.



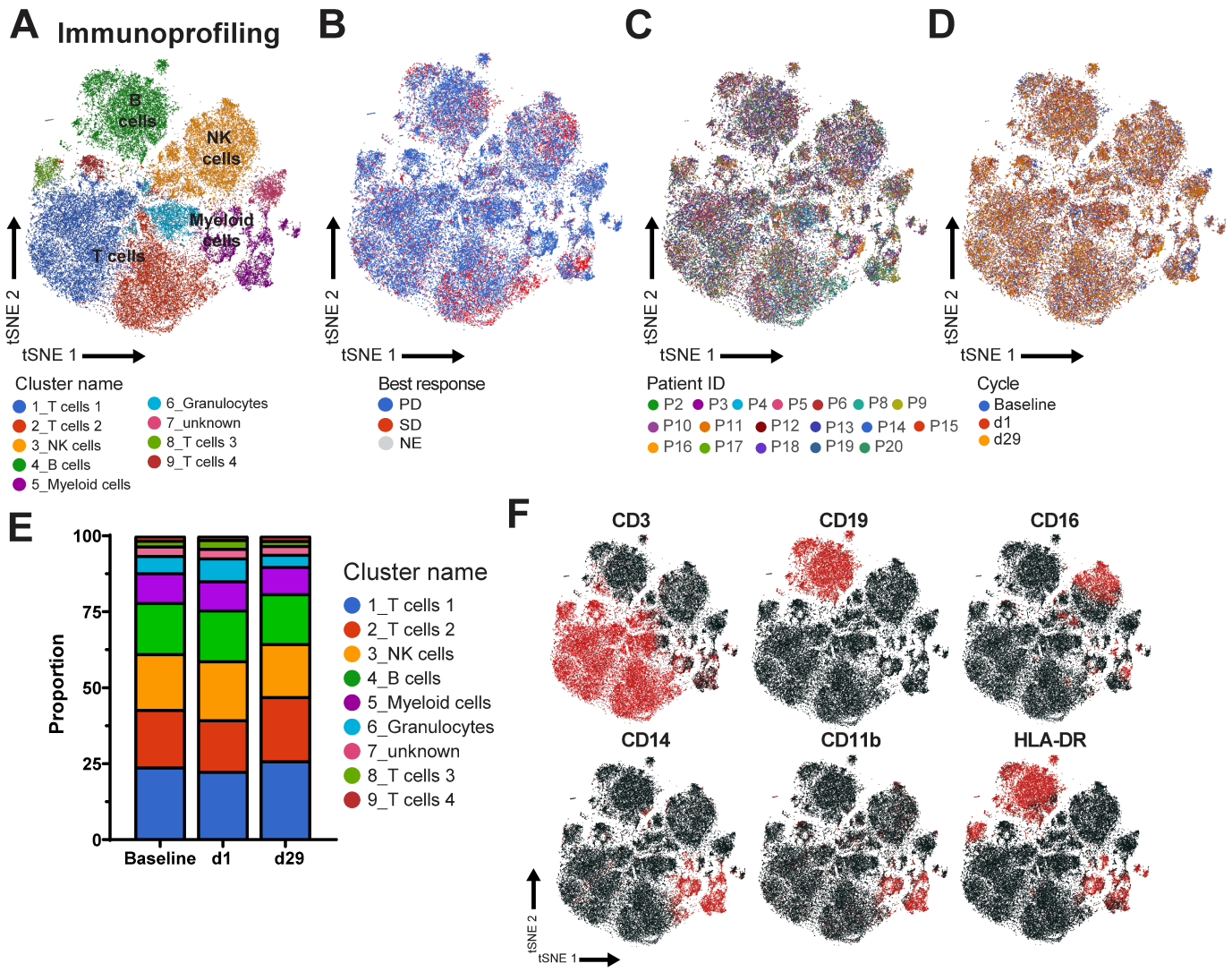
Supplementary Figure 3: CONSORT diagram. (PD, progressive disease)



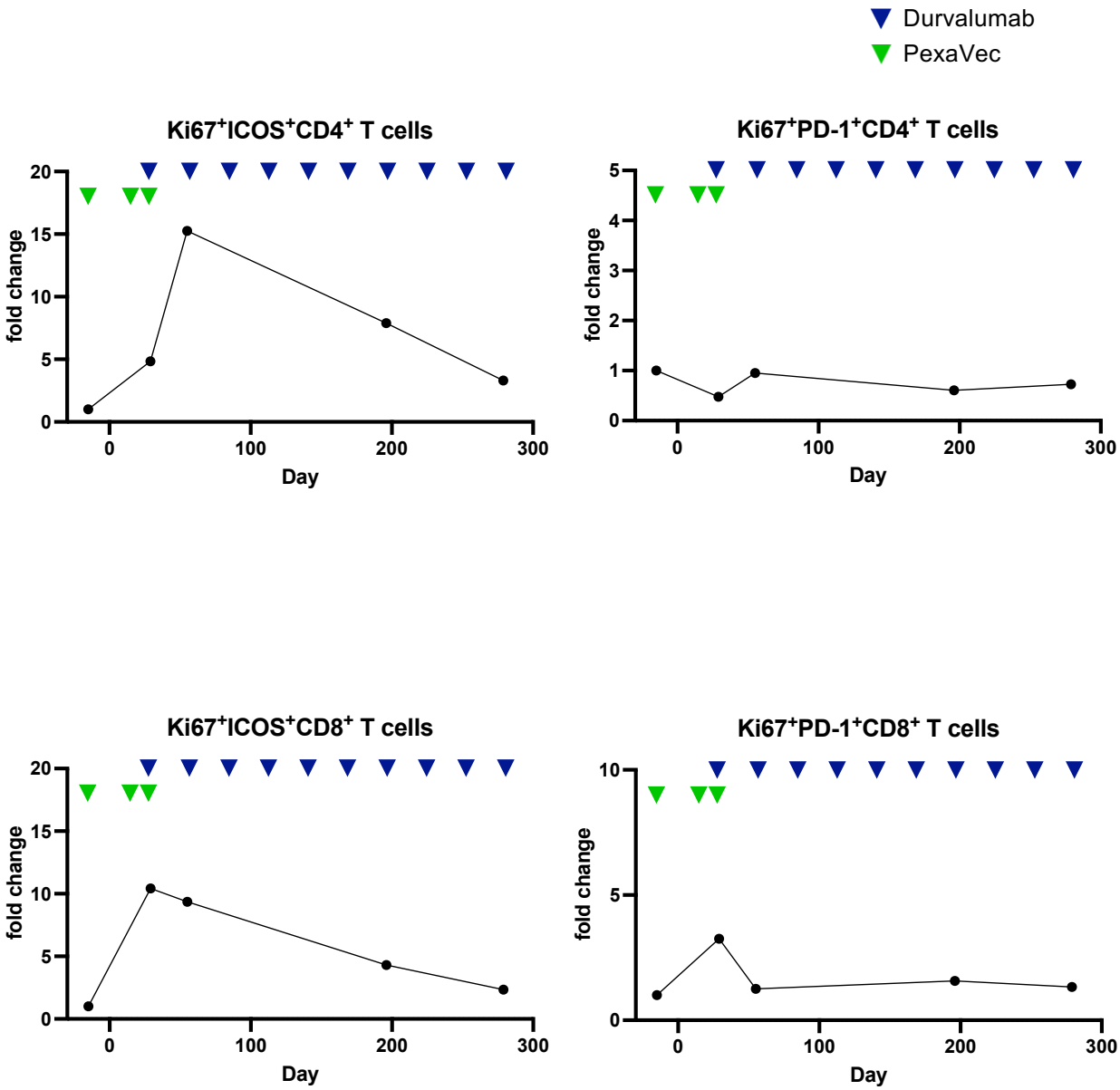
Supplementary Figure 4: : Immune Signature in patients after treatment. Scatter plot for immune cell fraction determined by QuanTIseq. Median ± SEM is shown.



Supplementary Figure 5: Stacked bar-graph show the frequency of distinct immune cell clusters across treatment cycles separated by high or low frequency of M1 macrophages in Supplementary Figure 4.



Supplementary Figure 6: Immunoprofiling of PBMCs. Peripheral blood mononuclear cells (PBMCs) were isolated from patients at baseline and the first day of first (d1) and second (d29) course. Analysis of the Immunoprofiling using high-dimensional flow cytometry is shown. (A) tSNE plot, showing clustering for immune cell subsets ($CD45^+$ gated) in CRC patient PBMC samples. (B)-(D) tSNE plots showing origin of single cells by best response (B), by patient (C, n=18) and treatment cycle (D; baseline, d1 & d 29). (E) Stacked bar-graph show the frequency of distinct immune cell clusters across treatment cycles. (F) tSNE projections showing expression of indicated surface markers used to identify distinct immune cell clusters (corresponding to A-E).



Supplementary Figure 7: Immune profile of Ki67+ICOS+CD4+ T cells, Ki67+ICOS+CD8+ T cells, Ki67+PD-1+CD4+ T cells and Ki67+PD-1+CD8+ T cells from patient P7 over time. Shown is the fold change in comparison to baseline of the relative number of different immune cell subsets in the CD4 and CD8 CD45 live gate.