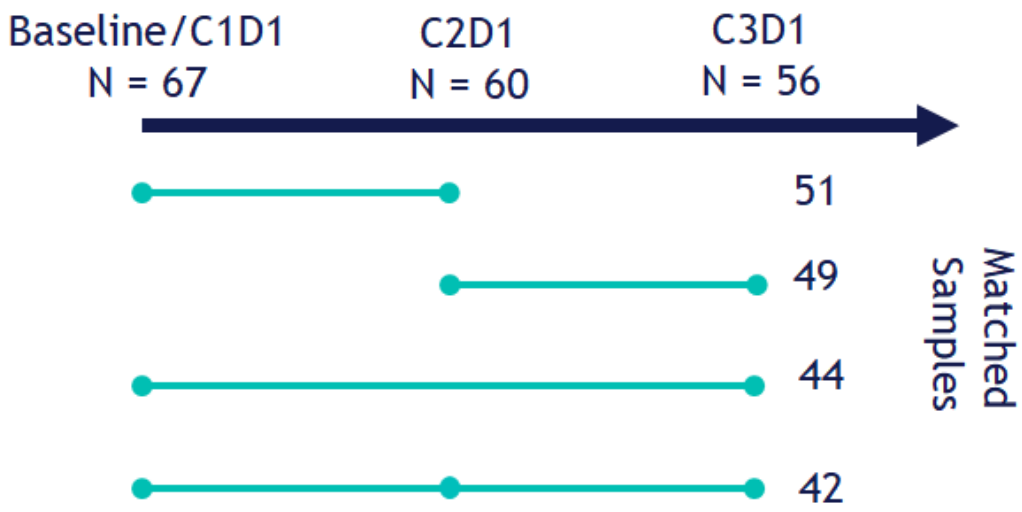
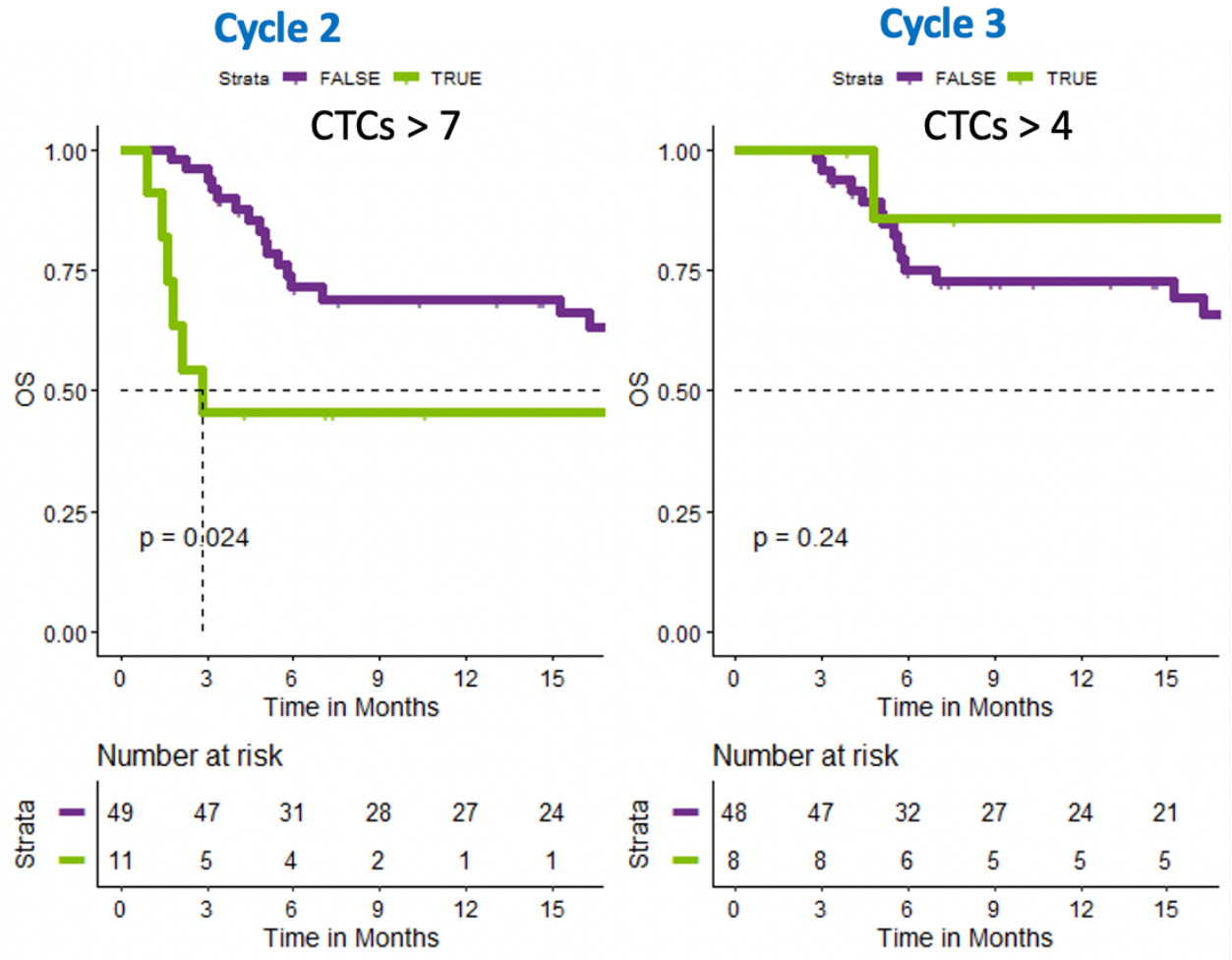
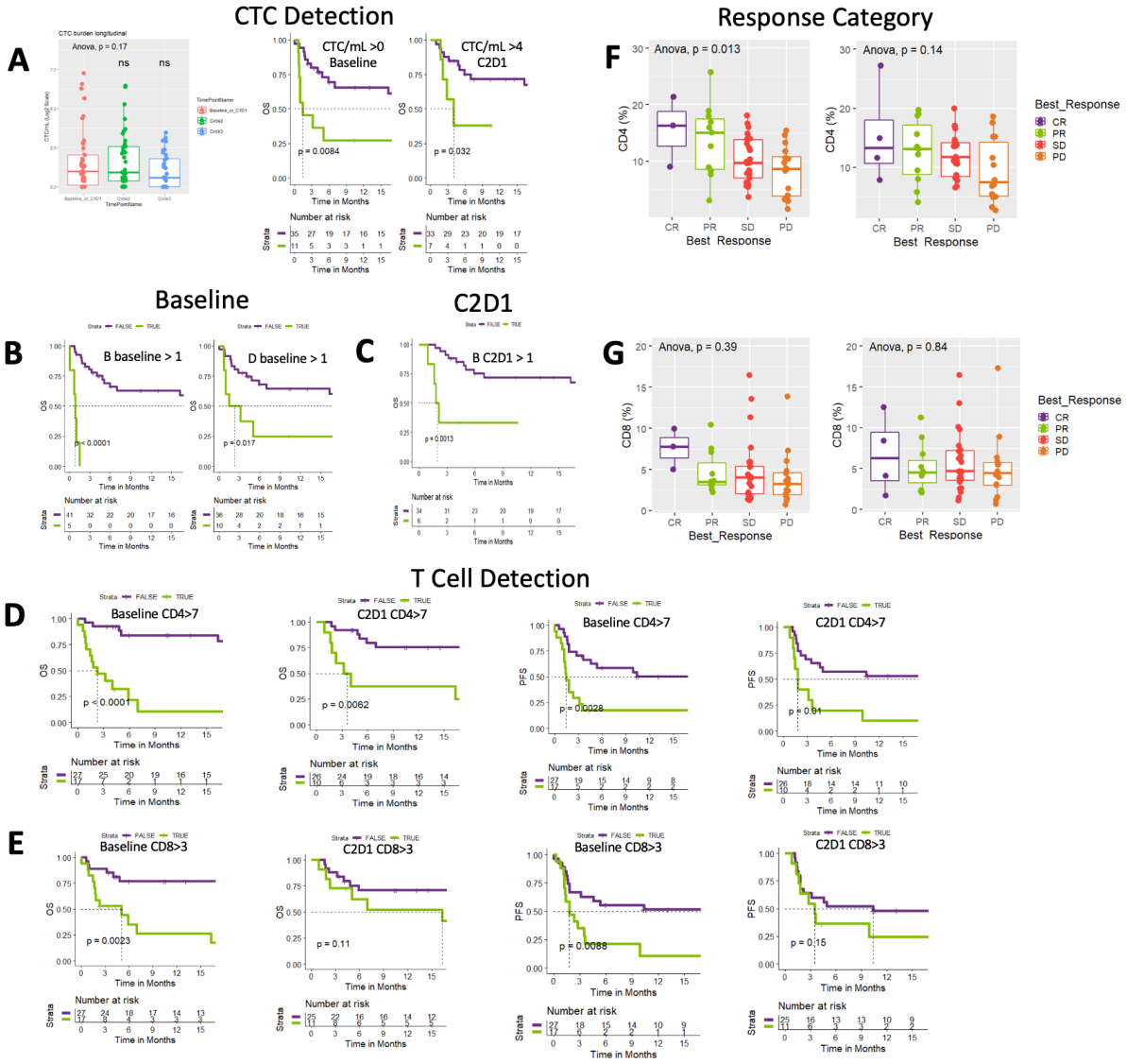
Supplementary Figure 1. Schematic of total number of samples at 3 time points and breakdown of longitudinal matching



Supplementary Figure 2. CTC Burden and Survival Analysis with Exploratory Cutoffs

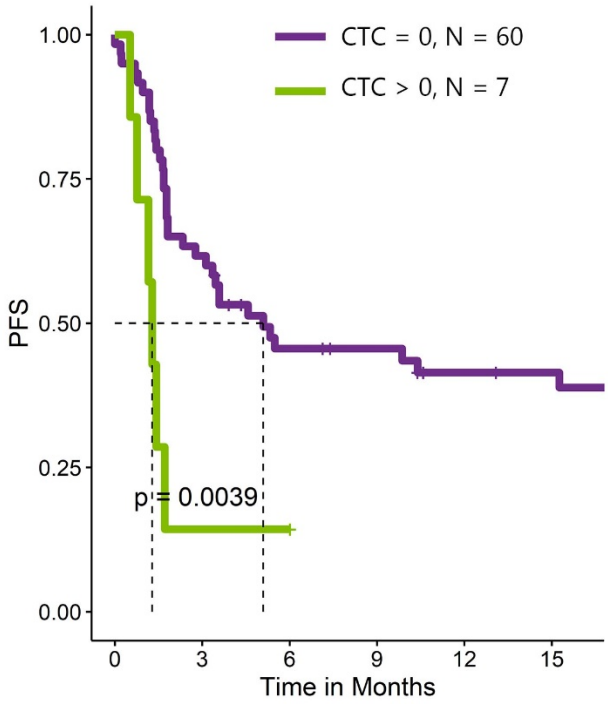


Supplementary Figure 3. Sub-Analysis Limited to Urothelial Histology

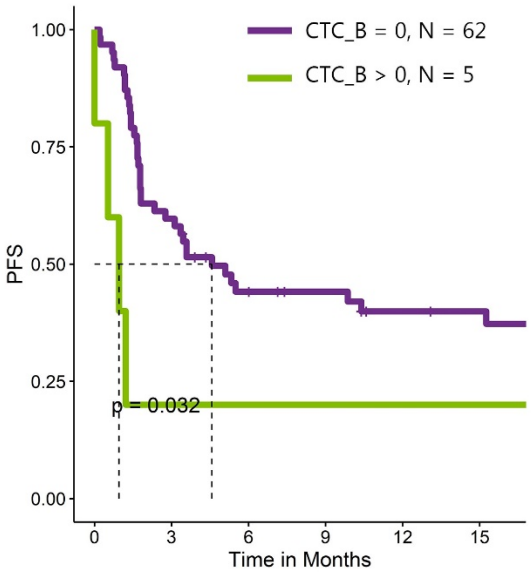


A) CTC detection across time points, Kaplan-Meier (KM) curves for OS at baseline with a cutoff of 0 and C2D1 with a cutoff of 4 CTCs. **B)** KM curves for OS for detection of subtypes B and D at baseline. **C)** KM curve for OS for detection of subtype B and C2D1. **D)** OS and PFS at baseline and C2D1 for white blood cells positive for CD4 with cutoff of 7%. **E)** OS and PFS at baseline and C2D1 for white blood cells positive for CD8 with cutoff of 3%. **F,G)** Analysis of variance for CD4+ and CD8+ T-cell populations across the different categories of treatment response (**F** CD4+, **G** CD8+).

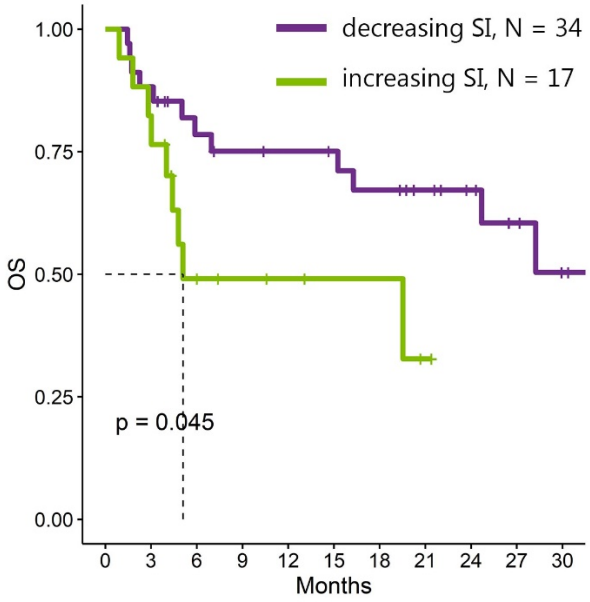
Supplementary Figure 4. Kaplan-Meier curve for PFS at baseline with PD-L1+ CTC cutoff of 0



Supplementary Figure 5. Kaplan-Meier curve for PFS at baseline with CTC subtype B cutoff of 0

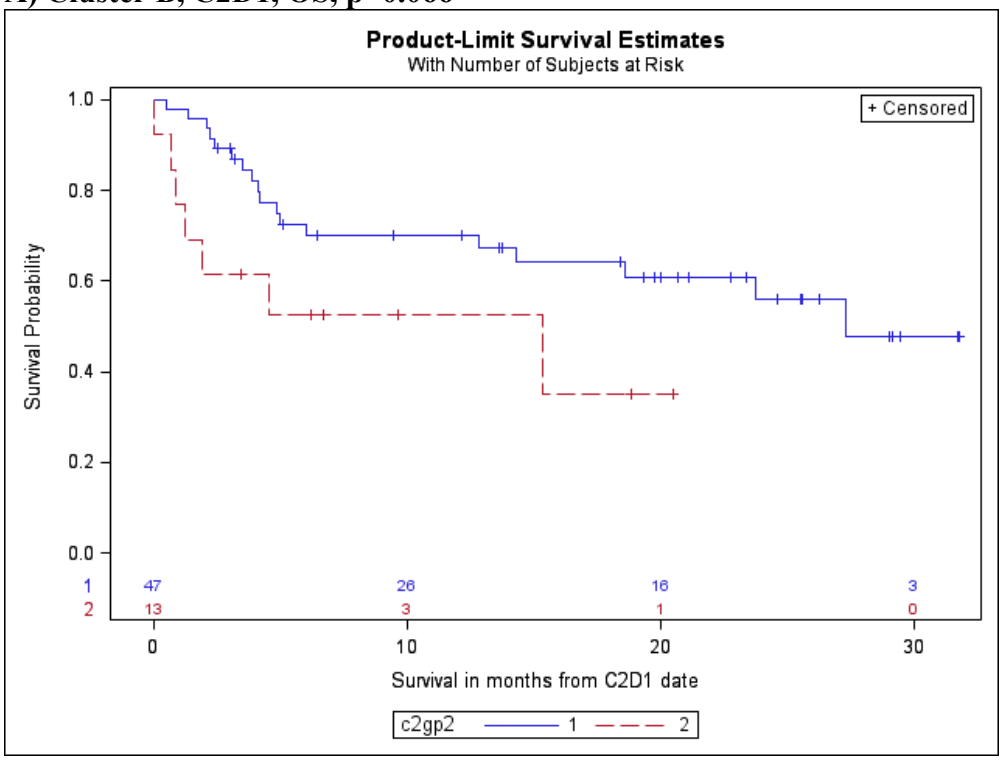


Supplementary Figure 6. Kaplan-Meier curve for OS with increasing and decreasing Shannon Index between baseline and C2D1

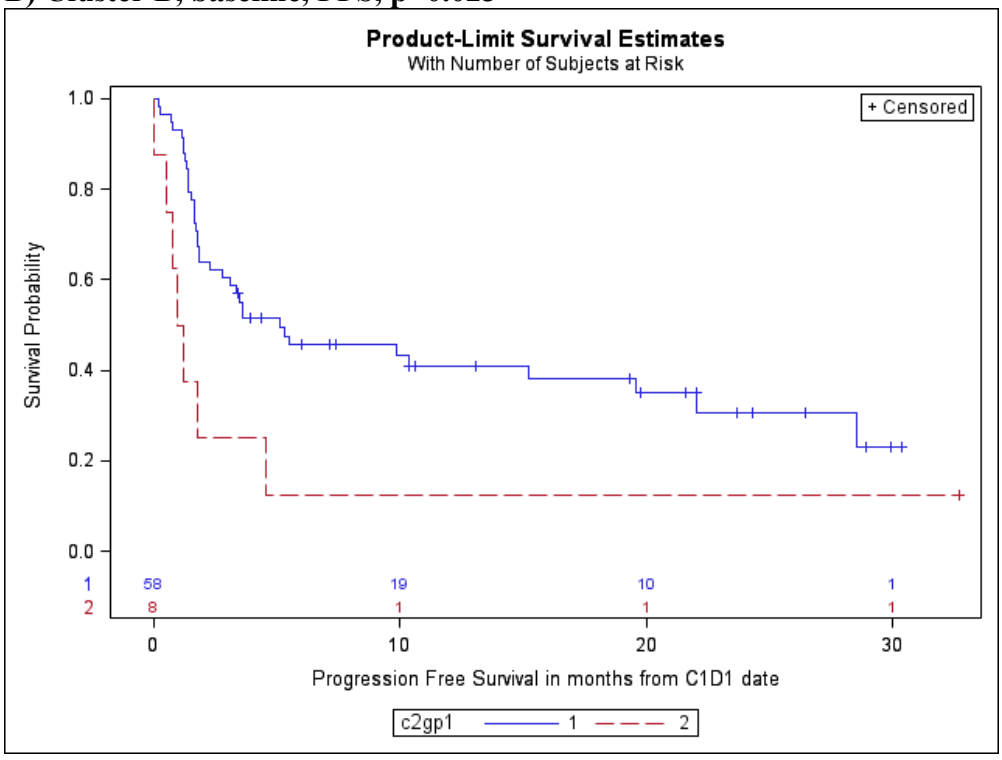


Supplementary Figure 7. Internal analysis of morphology-based CTC subtypes and progression-free and overall survival

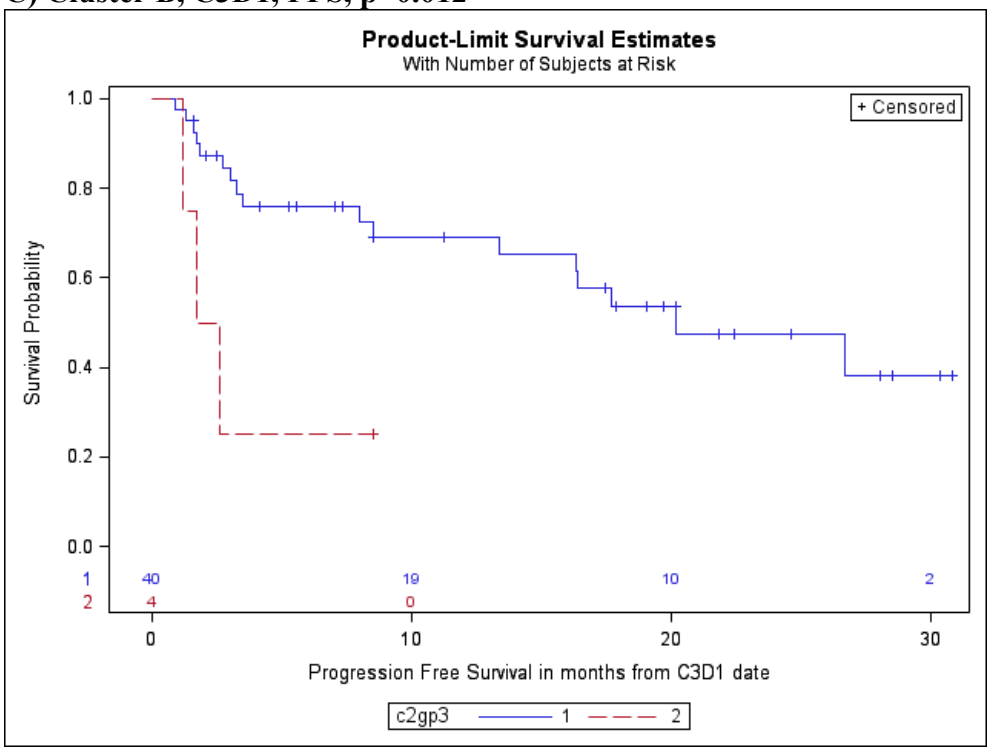
A) Cluster B, C2D1, OS, p=0.066



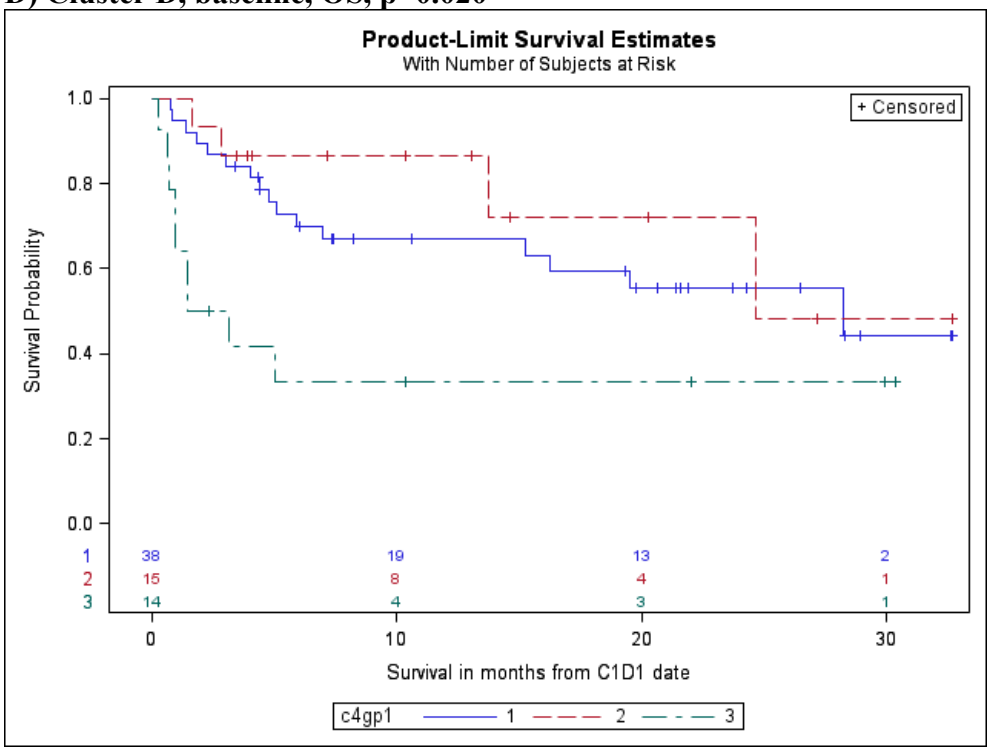
B) Cluster B, baseline, PFS, p=0.023



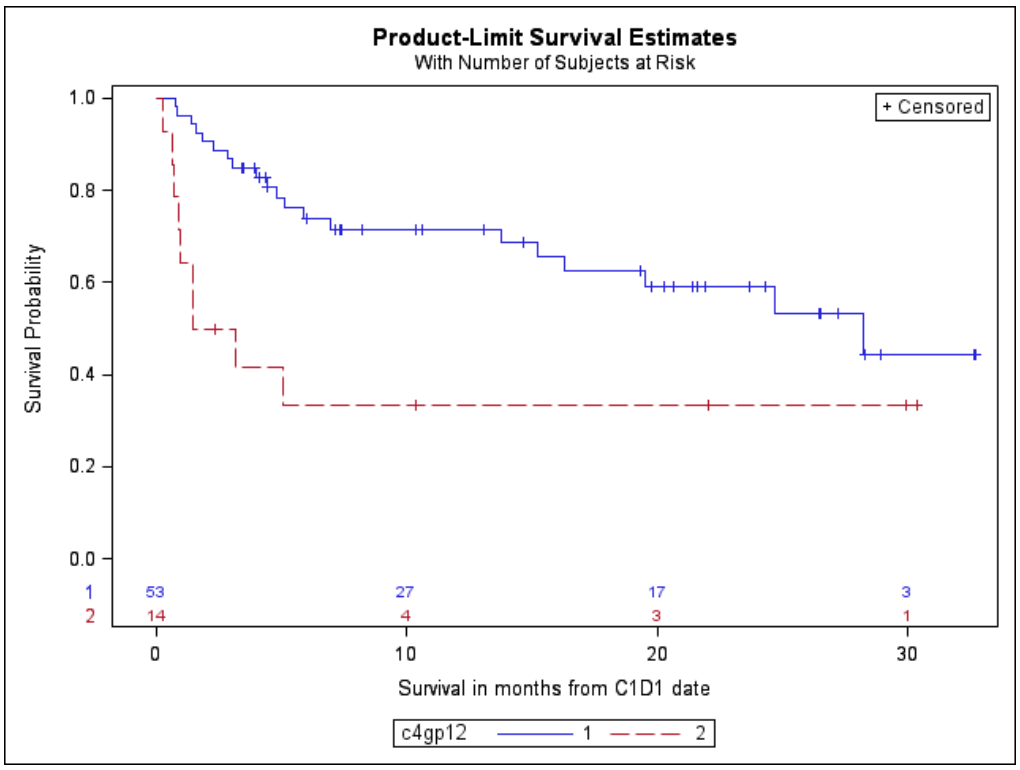
C) Cluster B, C3D1, PFS, p=0.012



D) Cluster D, baseline, OS, p=0.020

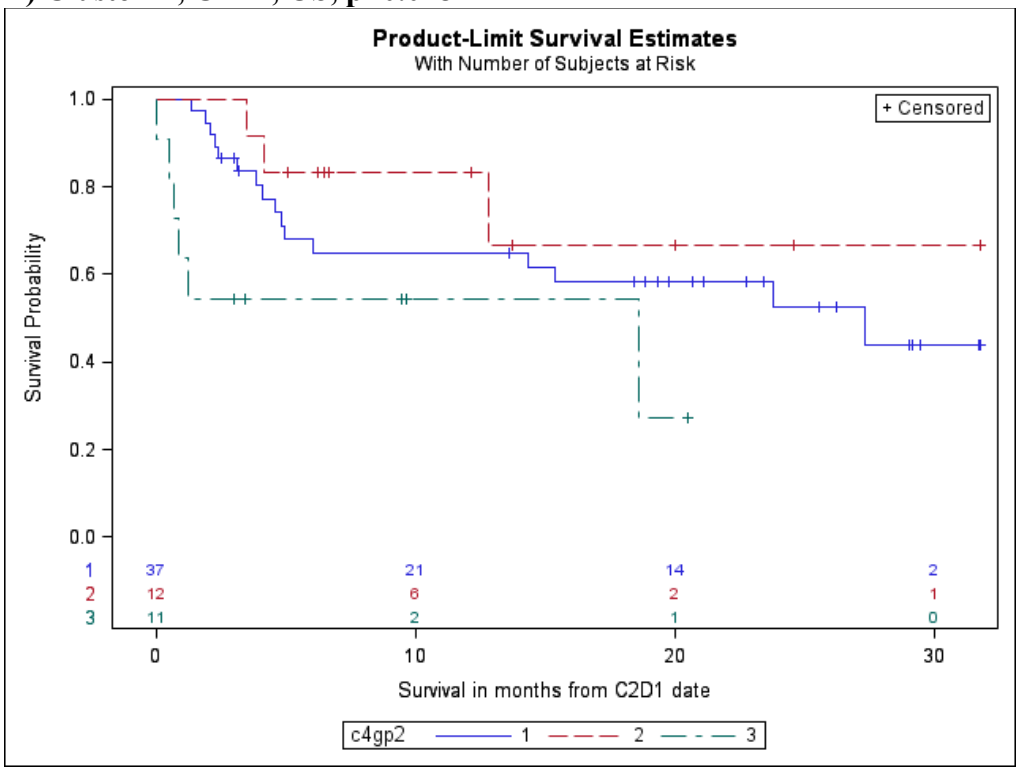


1 vs. 2: p = 0.93, 1 vs. 3: p = 0.072, 2 vs. 3: p = 0.021. Groups 1 and 2 can be pooled and the combination tested against 3.

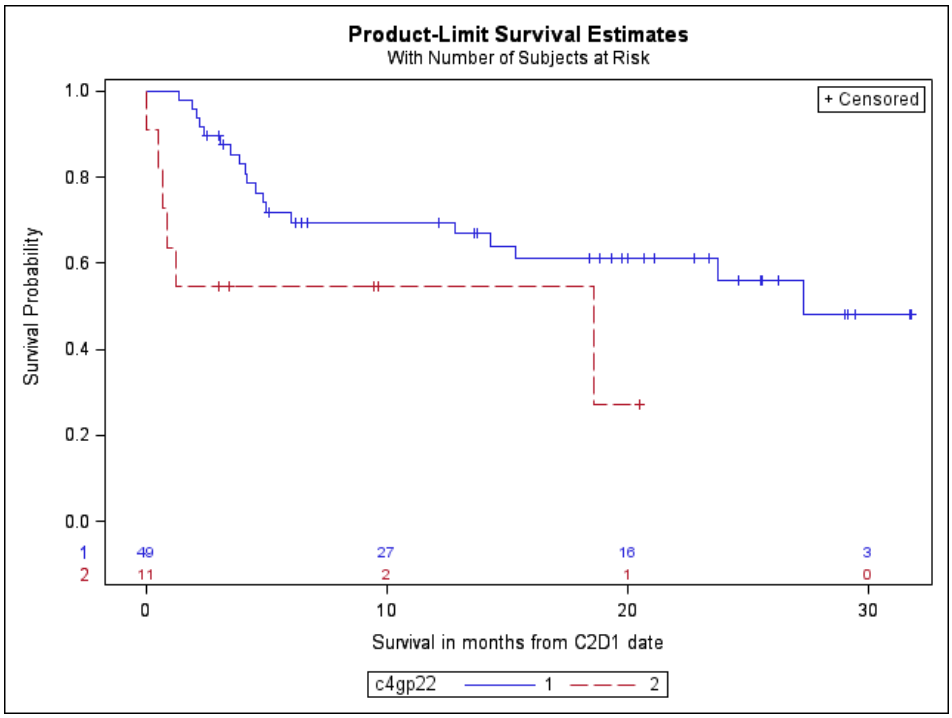


Unadjusted p = 0.0098; adjusted p = 0.020 (after adjusting for 2 possible combinations and reporting the p value for the greater difference between groups formed).

E) Cluster D, C2D1, OS, p=0.048

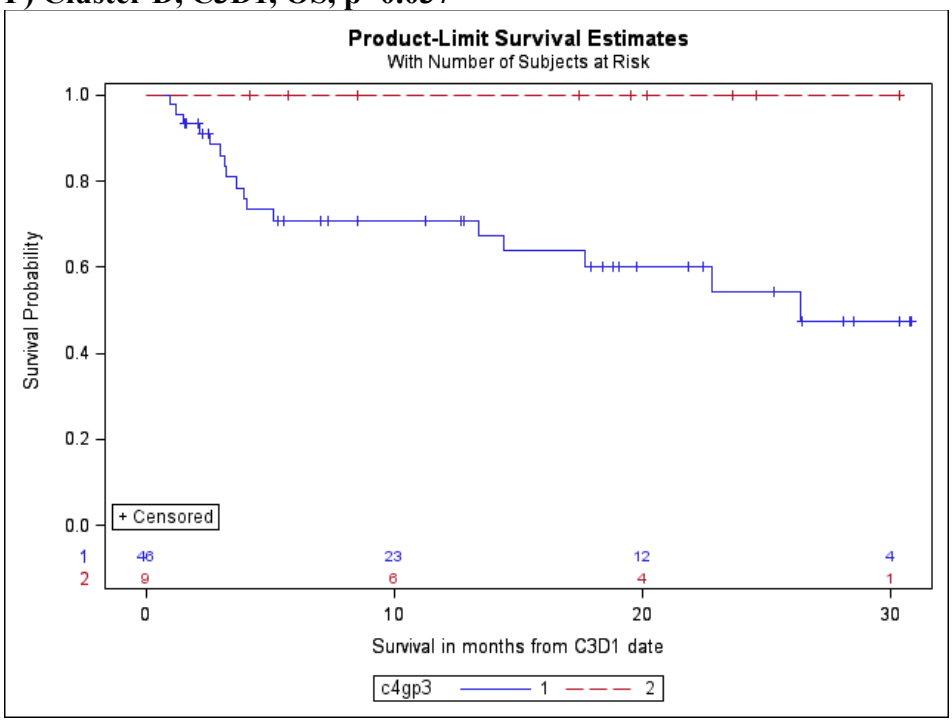


1 vs. 2: p = 0.72, 1 vs. 3: p = 0.19, 2 vs. 3: p = 0.035. 1 and 2 can be pooled and the combination tested against 3.



Unadjusted p = 0.024; adjusted p = 0.048 (after adjusting for 2 possible combinations and reporting the p value for the greater difference between groups formed).

F) Cluster D, C3D1, OS, p=0.037



Supplementary Table 1. Enrichment analysis for each CTC subtype (urothelial vs. other patients)

Baseline	Subtype A	Other subtypes	Subtype B	Other subtypes	Subtype C	Other subtypes	Subtype D	Other subtypes	Subtype E	Other subtypes
Urothelial	13	33	5	41	9	37	10	36	0	46
Other	7	14	0	21	3	18	4	17	2	19
Chi.sqr	0.177		2.46		0.27		0.063		4.51	
pval	0.67		0.11		0.6		0.8		0.03	

Baseline	Subtype A	Other subtypes	Subtype B	Other subtypes	Subtype C	Other subtypes	Subtype D	Other subtypes	Subtype E	Other subtypes
Urothelial	13	27	6	36	10	30	11	29	1	39
Other	6	14	0	20	2	18	2	18	0	20
Chi.sqr	0.038		3.16		1.875		2.4		0.508	
pval	0.84		0.075		0.17		0.12		0.47	

Baseline	Subtype A	Other subtypes	Subtype B	Other subtypes	Subtype C	Other subtypes	Subtype D	Other subtypes	Subtype E	Other subtypes
Urothelial	9	25	0	34	7	27	4	30	1	33
Other	5	17	0	22	3	19	0	22	0	22
Chi.sqr	0.099		NaN		0.44		2.78		0.65	
pval	0.75		NaN		0.507		0.095		0.41	

Supplementary Table 2. Prevalence of CTC subtypes at each time point

	Baseline/C1D1		C2D1		C3D1	
Prevalence	N (%)	median (range)	N (%)	median (range)	N (%)	median (range)
Subtype A	36/67 (53.73)	0.44 (0,69.33)	36/60 (60.00)	0.473 (0,27.04)	32/56 (57.14)	0.393 (0,6.013)
Subtype B	9/67 (13.43)	0 (0,101.6)	13/60 (21.67)	0 (0,30.5)	7/56 (12.50)	0 (0,0.8)
Subtype C	24/67 (35.82)	0 (0,74.53)	25/60 (41.67)	0 (0,35.36)	19/56 (33.93)	0 (0,12)
Subtype D	29/67 (43.28)	0 (0,45.76)	23/60 (38.33)	0 (0,29.96)	9/56 (16.07)	0 (0,2.4)
Subtype E	9/67 (13.43)	0 (0,1.386)	9/60 (15.00)	0 (0,2.08)	3/56 (5.36)	0 (0,1.293)

Supplementary Table 3. Proportion of CTC subtypes at each time point

Proportion %	Baseline/C1D1	C2D1	C3D1
Subtype A	39	32	44
Subtype B	23	13	4
Subtype C	20	25	41
Subtype D	17	29	10
Subtype E	1	1	1