Histological subtype	Total	RT alone	RT + Chemotherapy			
			AIM	VDC/IE	Ifosfamide	Gemcytabine/ docetaxel
UPS	14	2	11	0	0	1
Ewing	3	0	0	3	0	0
Leiomyosarcoma	2	2	0	0	0	0
Myxoid	3	1	2	0	0	0
Synovial	3	0	2	0	1	0
Other	7	6	1	0	0	0
Myxofibrosarcoma	2	2	0	0	0	0
Fibroblastic-myofibroblastic sarcoma	1	1	0	0	0	0
Sarcoma with epithelioid features	1	0	1	0	0	0
Solitary fibrous tumor	1	1	0	0	0	0
Sclerosing epithelioid fibrosarcoma	1	1	0	0	0	0
Fibromyxoid sarcoma	1	1	0	0	0	0

Supplemental Table S1. Distribution of number of patients receiving radiotherapy (RT) alone or chemotherapy and RT organized by histological subtype and type of chemotherapy. AIM: anthracycline, ifosfamide and mesna; VDC/IE: vincristine, doxorubicin and cyclophosphamide/ ifosfamide and etoposide.

Panel 1

Position	Antibody	Clone / Host	Company / Item	Concentration	OPAL Fluor
1	CD3	SP7 / Rabbit	Thermo / RM-9107	0.06 ug/ml	520
2	FoxP3	236A/E7 / Mouse	eBioscience / 14-4777-82	5 ug/ml	690
3	PD-1	EH33 / Mouse	Cell Signaling / 43248	0.19 ug/ml	570
4	VISTA	D1L2G / Rabbit	Cell Signaling / 64953S	0.19 ug/ml	620
5	CD206	CLO387 / Mouse	Novus / NBP2-52927	1 ug/ml	570
6	CD163 + CD68	EP324/Rabbit + PG-M1/Mouse	BioSB/BSB3276 + DAKO/M0876	0.125 ug/ml + 0.04 ug/ml	690
Secondary	Opal Polymer HRP Ms+Rb		Akoya Biosciences / ARH1001EA	RTU	

Panel 2

Position	Antibody	Clone / Host	Company / Item	Concentration	OPAL Fluor
1	CD8	C8/144B / Mouse	DAKO / M7103	0.05 ug/ml	520
2	CD66b	G10F5 / Mouse	BD Pharm. / 555723	2.4 ug/ml	540
3	CD33	RBT-CD33 / Rabbit	Bio SB / BSB3451	1.0 ug/ml	620
4	HLA-DR	EP96 / Rabbit	Bio SB / 6797	0.13 ug/ml	570
5	CD4	EP204 / Rabbit	Epitomics / AC0173A	0.08 ug/ml	690
6	Ki67	MIB1 / Mouse	DAKO / M7240	0.23 ug/ml	650
Secondary	Opal Polymer HRP Ms+Rb		Akoya Biosciences / ARH1001EA	RTU	

Supplemental Table S2. Multiplex immunohistochemistry antibodies.

	Timepoint	Mean	SD	Lower 95% CI	Upper 95% CI	n	P value	Significant
T-cells (% of total cells)	Pre-RT	3.83	4.09	2.18	5.48	26	0.979	ns
	Post-RT	3.80	3.91	2.22	5.38	26		
CD4 T-cells (% of total cells)	Pre-RT	3.02	3.52	1.60	4.44	26	0.605	ns
	Post-RT	3.57	3.80	2.04	5.11	26		
Treg T-cells (% of total cells)	Pre-RT	0.19	0.55	-0.03	0.41	26	0.350	ns
	Post-RT	0.07	0.35	-0.07	0.21	26		
CD8 T-cells (% of total cells)	Pre-RT	0.62	1.84	-0.12	1.36	26	0.245	ns
	Post-RT	0.16	0.57	-0.07	0.39	26		
Monocytes (% of total cells)	Pre-RT	6.74	5.01	4.72	8.77	26	**0.002**	**
	Post-RT	14.06	11.48	9.43	18.70	26		
M1 macrophages (% of total cells)	Pre-RT	0.65	0.88	0.30	1.01	26	0.504	ns
	Post-RT	0.81	0.67	0.54	1.08	26		
M2 macrophages (% of total cells)	Pre-RT	3.18	3.63	1.72	4.65	26	0.426	ns
	Post-RT	4.01	3.09	2.77	5.26	26		
B-cells (% of total cells)	Pre-RT	8.60	6.23	6.09	11.12	26	**0.006**	**
	Post-RT	15.41	12.13	10.51	20.31	26		
NK-cells (% of total cells)	Pre-RT	1.06	1.57	0.43	1.70	26	**0.010**	*
	Post-RT	2.21	2.07	1.37	3.04	26		

Supplemental Table S3. Percentage of cells assessed by the CoFactor ImmunoPrism assay, pre- and post-neoadjuvant therapy. SD, standard deviation; CI, confidence interval. Statistical differences were tested using two-tailed paired t-tests. * p-value <0.05; **<0.01.

	Timepoint	Mean	SD	Lower 95% CI	Upper 95% CI	n	P value	Significant
CD3+ (% of Total cells)	Pre-RT	3.33	3.93	1.44	5.23	19	0.069	ns
	Post-RT	5.63	4.49	3.47	7.80			
CD4+ (% of Total cells)	Pre-RT	1.68	1.92	0.76	2.61	19	**0.015**	*
	Post-RT	4.80	4.97	2.40	7.19			
CD8+ (% of Total cells)	Pre-RT	1.33	1.60	0.56	2.10	19	0.175	ns
	Post-RT	2.24	2.19	1.19	3.30			
FoxP3+ (% of CD3+ cells)	Pre-RT	9.63	12.46	3.63	15.64	19	**0.023**	*
	Post-RT	4.17	5.12	1.71	6.64			
FoxP3- cells (% of CD3+ cells)	Pre-RT	90.14	12.45	84.14	96.14	19	**0.021**	*
	Post-RT	95.73	5.17	93.24	98.22			
CD68+ CD163+ (% of Total cells)	Pre-RT	7.05	11.56	1.48	12.62	19	0.055	ns
	Post-RT	12.87	13.92	6.17	19.58			
CD206+ (% of Total cells)	Pre-RT	4.85	10.46	-0.20	9.89	19	**0.031**	*
	Post-RT	9.84	13.51	3.33	16.35			
CD33+ CD66b- (% of Total cells)	Pre-RT	2.14	2.18	1.09	3.19	19	0.132	ns
	Post-RT	3.91	5.38	1.31	6.50			
CD33+ CD66b+ (% Total cells)	Pre-RT	0.07	0.27	-0.06	0.21	19	0.681	ns
	Post-RT	0.05	0.08	0.01	0.09			
CD33- CD66b+ (% Total cells)	Pre-RT	1.98	3.56	0.27	3.69	19	0.493	ns
	Post-RT	3.00	4.79	0.69	5.31			

Supplemental Table S4. Percentage of immune cells (T cells and myeloid) pre- and post- neoadjuvant therapy evaluated by mIHC. SD, standard deviation; CI, confidence interval Statistical differences were tested using two-tailed paired t-tests. * p-value <0.05.

	Timepoint	Mean	SD	Lower 95% CI	Upper 95% CI	n	P value	Significant
PD1+ (% of CD3+ cells)	Pre-RT	20.45	20.04	10.79	30.10	19	0.476	ns
	Post-RT	24.44	22.16	13.76	35.12	19		
HLA-DR+ (% of CD4+ cells)	Pre-RT	19.62	16.61	11.61	27.62	19	0.701	ns
	Post-RT	21.31	13.38	14.86	27.75	19		
ki67+ (% of CD4+ cells)	Pre-RT	7.02	9.11	2.62	11.41	19	0.972	ns
	Post-RT	7.13	9.04	2.78	11.49	19		
HLA-DR+ (% of CD8+ cells)	Pre-RT	5.01	4.67	2.69	7.33	18	0.096	ns
	Post-RT	9.01	7.82	4.99	13.03	17		
ki67+ (% of CD8+ cells)	Pre-RT	9.04	11.64	3.26	14.83	18	0.457	ns
	Post-RT	7.41	4.05	5.32	9.49	17		
HLA-DR+ CD4- CD8- (% of total cells)	Pre-RT	5.95	6.56	2.79	9.11	19	**0.003**	**
	Post-RT	14.54	11.39	9.05	20.03	19		
ki67+ CD4- CD8- HLA-DR- CD33- CD66b- (% of total cells)	Pre-RT	10.08	12.19	4.21	15.96	19	**0.010**	*
	Post-RT	2.30	2.50	1.09	3.50	19		
VISTA+ (% of CD3+ cells)	Pre-RT	13.86	10.64	8.73	18.99	19	0.632	ns
	Post-RT	12.48	11.65	6.87	18.09	19		
VISTA+ (% of CD68+ CD163+)	Pre-RT	35.12	33.39	19.02	51.21	19	0.551	ns
	Post-RT	29.91	24.23	18.23	41.58	19		

Supplemental Table S5. Percentage of cells expressing PD1, HLA-DR, ki67 and VISTA pre- and post-neoadjuvant therapy evaluated by mIHC. SD, standard deviation; CI, confidence interval. Statistical differences were tested using two-tailed paired t-tests. * p-value <0.05, ** p-value <0.01.