

Supplementary appendix

Cytokine analysis

Analyte	BeadRegion	Standard Curve(pg/mL)	Serum	Plasma	CellCultur	Sensitivity(pg/mL)
Angiopoietin-like Protein 4/ANGPTL4	73	1,804 - 438,380	1:02	1:02	1:02	86
CCL2/JE/MCP-1	62	33-8,017	1:02	1:02	1:02	9.9
CCL3/MIP-1 alpha	35	101.2-24,580	1:02	1:02	1:02	16.2
CCL4/MIP-1 beta	37	150.2 - 36,493	1:02	1:02	1:02	5.8
CCL7/MCP-3/MARC	19	35.3 - 8,570	1:02	1:02	1:02	3.2
CCL11/Eotaxin	51	60.7 - 14,750	1:02	1:02	1:02	14.6
CCL22/MDC	36	48.8 - 11,870	1:02	1:02	1:02	8.5
CD40 Ligand/TNFSF5	74	212 - 51,430	1:02	1:02	1:02	34.7
CX3CL1/Fractalkine	46	1,114 - 270,610	1:02	1:02	1:02	64.8
CXCL1/GRO alpha/KC/CINC-1	44	151 - 12,200	1:02	1:02	1:02	5.3
CXCL2/GRO beta/MIP-2/CINC-3	27	49.2 - 11,960	1:02	1:02	1:02	7.86
CXCL10/IP-10/CRG-2	21	2.8 - 690	1:02	1:02	1:02	1.18
EGF	25	18.7 - 4,550	1:02	1:02	1:02	8.9
FGF basic/FGF2/bFGF	47	1.81-440	1:02	1:02	1:02	6.5
Flt-3 Ligand	77	14.9 - 3,610	1:02	1:02	1:02	1.5
G-CSF	54	24-5,840	1:02	1:02	1:02	4.1
GM-CSF	66	12.2-2,970	1:02	1:02	1:02	4.1
IFN-gamma	29	58.5-14,209	1:02	1:02	1:02	0.4
IL-1 alpha/IL-1F1	38	5.2-1,270	1:02	1:02	1:02	0.9
IL-1 beta/IL-1F2	28	19.5-4,744	1:02	1:02	1:02	0.8
IL-1ra/IL-1F3	30	28-6,793	1:02	1:02	1:02	18
IL-2	43	29.6-7,200	1:02	1:02	1:02	1.8
IL-3	15	84.5 - 20,530	1:02	1:02	1:02	11.6
IL-4	39	14.6-3,550	1:02	1:02	1:02	9.3
IL-5	53	6.6-1,610	1:02	1:02	1:02	0.5
IL-6	13	4.8-1,154	1:02	1:02	1:02	1.7
IL-7	20	4.5 - 1,100	1:02	1:02	1:02	0.41
IL-8/CXCL8	18	5.2-1,255	1:02	1:02	1:02	1.8
IL-10	22	4.8-1,162	1:02	1:02	1:02	1.6
IL-12 p70	56	137-33,340	1:02	1:02	1:02	20.2
IL-15	52	6.3 - 1,540	1:02	1:02	1:02	1.01
IL-17/IL-17A	42	12.8-3,110	1:02	1:02	1:02	1.8
Lymphotoxin-alpha/TNF-beta	45	6.21-1,510	1:02	1:02	1:02	1.52
TGF-alpha	63	18.6 - 4,510	1:02	1:02	1:02	0.82
TNF-alpha	12	9.7-2,359	1:02	1:02	1:02	1.2
VEGF	26	8.4-2,051	1:02	1:02	1:02	2.1

Table S1: To determine serological correlates with TVEC treatment, we performed multiplexing of 36 cytokines using serum samples at five different time points from 27 patients.

TVEC-induced	CXCL10
	CX3CL1
	<i>FLT3L</i>
	IL-6
	IL-7
	IL-17A
TVEC-Suppressed	CCL11
	EGF
TVEC-NR	ANGPTL4
	<i>CCL2</i>
	CCL3
	CCL4
	CCL22
	CXCL1
	CXCL2
	CD40L
	FGF
	IL-1RA
	IL-2
	IL-8
	VEGF
	TGFalpha

Table S2: 22 out of 36 cytokines exhibited measurable levels, among which 6 cytokines (CXCL10, CX3CL1, FLT3L, IL-6, IL-7, and IL-17A) were induced by the treatment protocol, with 2 suppressed (EGF and CCL11) and others unchanged (TVEC_NR)

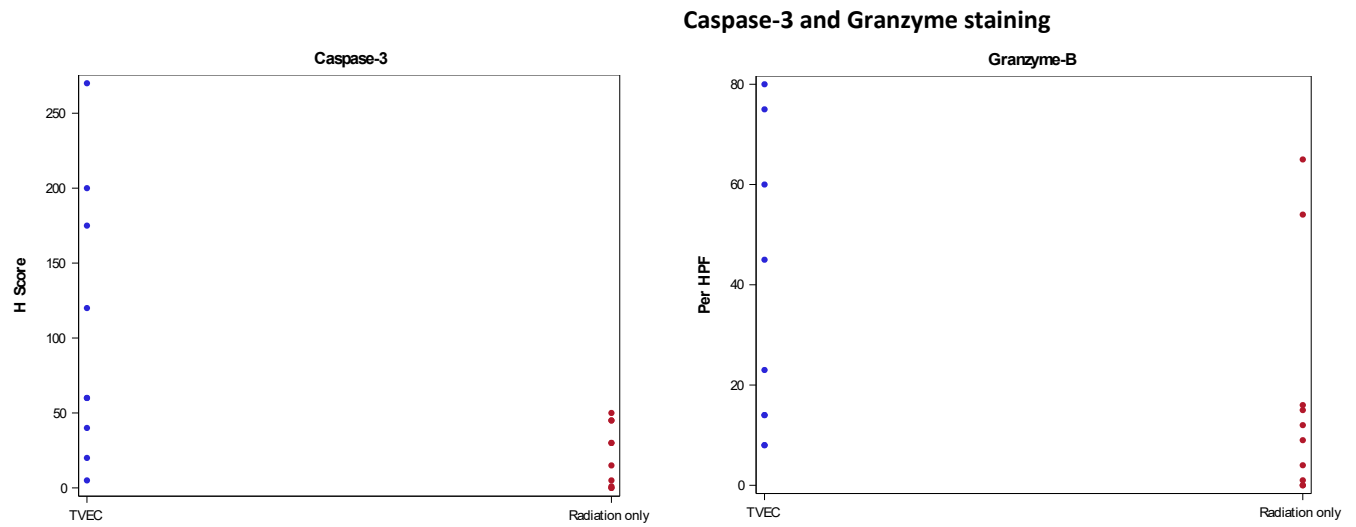


Figure S1 (A&B): H-score differences in Caspase 3 ($p=0.01$) and Granzyme B testing ($p=0.10$) between historical EBRT alone vs TVEC plus EBRT treated sarcoma tissue specimens

	Treatment		
	EBRT only	TVEC + EBRT	
	N=10	N=9	P-value
Caspase-3	22.5	60.0	0.01
	(0-50)	(5-270)	
Granzyme B	10.5	23.0	0.10
	(0-65)	(8-80)	

Table S2: H-scores between historical EBRT alone treated resected sarcoma specimens and TVEC plus EBRT treated resected tumor specimens

UPS	TVEC plus Radiation
Myxofibrosarcoma	
UPS	
Myxofibrosarcoma	
UPS	
Undifferentiated spindle cell sarcoma	
Myxoid liposarcoma	
Pleomorphic liposarcoma	
Myxoid liposarcoma	
Dedifferentiated Liposarcoma	Radiation only
Myxoid/round cell liposarcoma	
Pleomorphic liposarcoma	
Myxofibrosarcoma	
Undifferentiated spindle cell sarcoma	
UPS	
UPS	
Undifferentiated spindle cell	
Myxofibrosarcoma	
UPS	
Undifferentiated spindle cell sarcoma	

Table S3: Histological subtypes of resected radiation only treated tumor specimens used for comparison of Caspase-3 and Granzyme B testing

