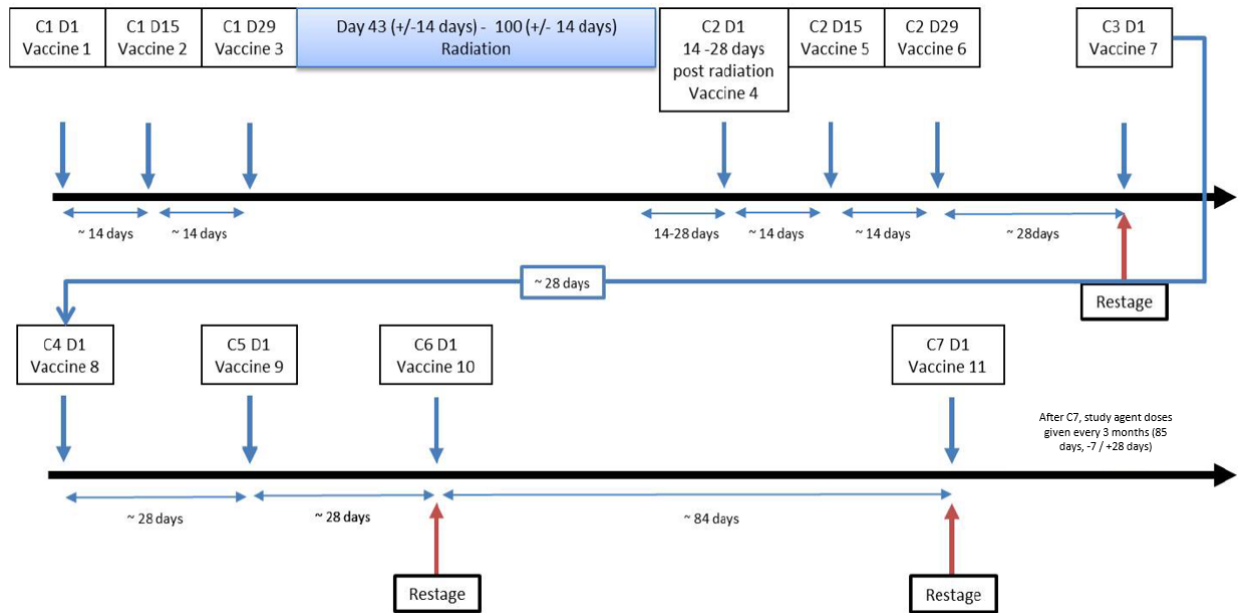


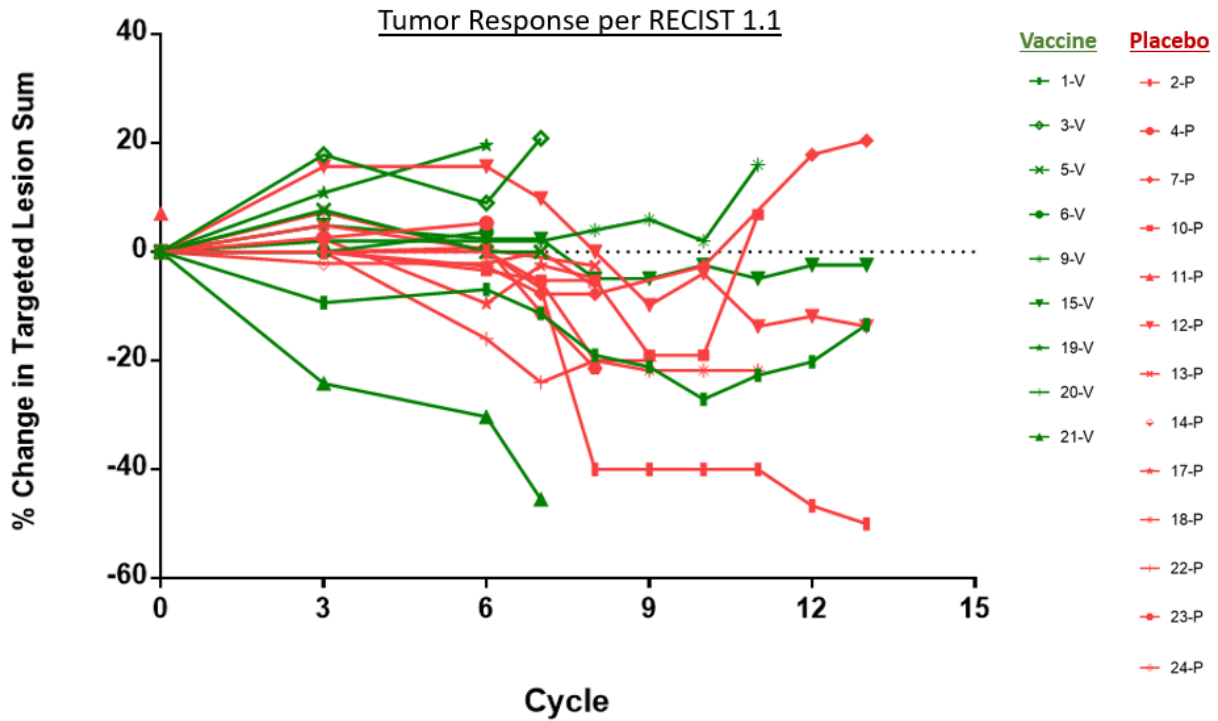
Supplemental Figures for:
Phase II Study of Yeast-Brachyury Vaccine (GI-6301) in Combination with Standard-of-Care Radiotherapy in Locally Advanced, Unresectable Chordoma
James Gulley et al.

Figure S1. Study schema. Following randomization, blinded vaccine was administered on cycle 1 days 1, 15, and 29. After completing the first cycle of treatment, proton or photon RT was given at the discretion of the patient’s local radiation oncologist. Post-RT, patients returned to the NIH Clinical Center where they continued to receive blinded vaccine until progression.



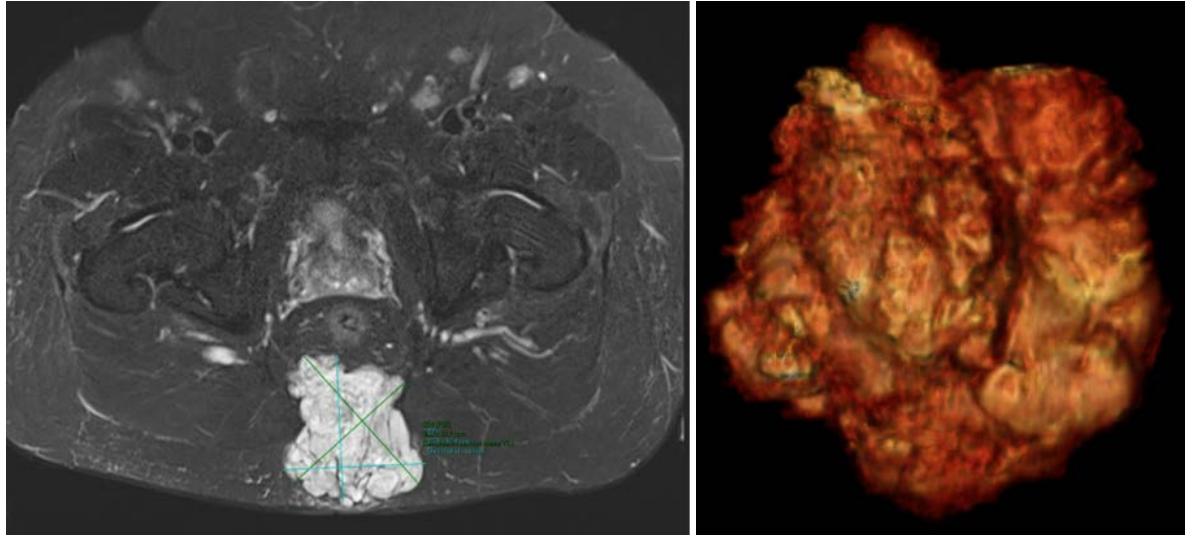
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 James Gulley et al.

Figure S2. Tumor Response per RECIST 1.1. This spider plot of RECIST v1.1 measurement data shows percentage change in the targeted lesion sum over time, represented by cycle number. Patients on the vaccine arm are shown in green; patients on the placebo arm are shown in red. No observable trend is identified.



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James Gulley et al.

Figure S3. 2D RECIST vs 3D Volumetric Measurement of Chordomas. Comparison of 2-dimensional (RECIST) and 3-dimensional volumetric tumor measurement. Images of sacrococcygeal chordoma from patient who developed partial response on vaccine arm.



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James Gulley et al.

Figure S4. Immune analysis of patient level antigen-specific data. Absolute number of CD4+ or CD8+ T cells producing cytokine (IFN γ , TNF α , IL-2) or positive for CD107a per 1×10^6 PBMCs plated at the start of the in vitro stimulation assay. Numbers in bold are positive responses after vs before therapy and following subtraction of background [TAA after therapy – HLA after therapy] – [TAA before therapy – HLA before therapy] >250 per 1×10^6 PBMCs. Grey rows indicate time points at which PBMCs were insufficient for analysis. In the current study, antigen-specific responses fluctuated in many patients over time. This phenomenon has been observed in numerous cancer vaccine trials [41-45] and may be due to T-cell trafficking into and out of the tumor, as well as homeostatic regulation by regulatory T cells to suppress an immune response against self-antigens.

Treatment	BOR	Post # of vaccines or placebo (vs pre)	Immune Responses to Brachyury								Immune Responses to MUC1								Immune Responses to CEA								
			CD4 CD107a	CD4 IFN γ	CD4 IL-2	CD4 TNF	CD8 CD107a	CD8 IFN γ	CD8 IL-2	CD8 TNF	CD4 CD107a	CD4 IFN γ	CD4 IL-2	CD4 TNF	CD8 CD107a	CD8 IFN γ	CD8 IL-2	CD8 TNF	CD4 CD107a	CD4 IFN γ	CD4 IL-2	CD4 TNF	CD8 CD107a	CD8 IFN γ	CD8 IL-2	CD8 TNF	
GI-6301	PR	2	0	0	1089	979	0	0	0	0	0	0	0	0	0	0	0	0	0	0	150	352	609	374	0	0	
		3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
GI-6301	SD	2	0	0	356	515	472	564	35	1852	0	2077	1726	2598	1119	859	52	777	0	0	0	0	0	0	0	0	
		3	0	1531	1365	1206	1169	195	0	1386	357	0	0	0	0	0	720	0	1467	0	0	0	0	0	270	0	0
		9	3926	205	1268	6159	751	0	0	3393	545	0	0	0	3848	198	141	3052	954	0	0	0	0	0	0	0	
GI-6301	SD	2	0	0	296	432	0	0	0	1236	0	0	0	0	0	0	0	613	664	650	0	0	0	0	101	515	
		3	0	201	1094	1138	4696	1363	0	0	0	0	692	80	0	0	0	0	0	2289	3606	3881	0	0	0	0	
		9	0	0	0	294	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
GI-6301	SD	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	
		3	203	754	0	0	0	147	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		9	0	0	150	312	0	0	0	0	0	0	21	0	0	0	0	34	0	0	0	0	0	0	0	0	
GI-6301	PD	2	0	408	0	0	0	1066	0	617	0	514	445	617	0	145	0	0	0	0	0	0	0	0	38	1120	
		3	0	828	0	0	0	171	0	0	0	0	0	0	0	0	0	0	0	0	379	324	0	0	24	0	
		9	0	204	0	0	0	306	41	0	0	277	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
GI-6301	PD	2	0	0	0	0	0	0	0	0	0	31	0	0	0	0	0	456	1112	2321	342	1412	3347	9541	14	2308	
		3	0	775	1613	2595	11418	5253	6	5668	0	0	0	0	4798	4255	0	4462	0	1753	2623	2441	0	0	0	2962	
		9	0	0	0	0	0	0	0	0	308	282	0	104	0	466	0	581	0	1092	293	829	8659	10999	52	6468	
GI-6301	PD	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	383	
		3	0	0	0	0	0	0	10	0	394	47	0	1298	0	0	0	0	0	0	0	0	0	0	0	0	
		9	0	0	667	0	0	0	4	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	7	
GI-6301	PD	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		3	0	0	0	583	0	0	0	0	0	0	3518	2768	0	0	0	0	0	0	0	288	0	0	0	0	
		9	0	0	753	763	0	0	0	693	0	1573	2506	2316	2091	0	0	0	0	0	0	188	318	0	0	0	
Placebo	PR	2	492	101	228	127	0	202	0	0	0	0	63	101	0	0	37	502	2149	0	116	278	1061	0	50	359	
		3	429	1028	0	155	0	422	0	0	0	2083	360	488	0	590	21	425	369	334	0	163	105	24	15	234	
		9	0	0	0	0	0	8	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	
Placebo	SD	2	839	121	369	67	240	59	0	490	1449	0	378	68	0	0	0	0	0	0	31	260	0	0	0	14	
		3	555	580	0	501	1475	183	0	609	1833	0	611	0	0	0	30	0	574	0	0	369	0	0	0	0	
		9	452	0	0	0	0	2	0	0	331	0	0	0	0	0	4	0	0	234	0	0	0	0	0	6	
Placebo	SD	2	0	1217	1867	10114	5806	1325	102	1270	2053	0	0	0	726	63	0	0	0	2052	0	1584	280	6	88	0	
		3	0	0	0	0	127	0	30	0	0	0	0	0	0	0	0	0	23	271	0	0	0	70	0	0	
		9	0	618	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0	81	0	0	129	
Placebo	SD	2	2141	56677	4078	56974	3569	1510	127	2333	0	0	0	151	924	53	0	861	0	0	0	0	0	0	0	33	
		3	2320	15357	951	14457	0	165	0	337	0	0	0	0	1268	0	518	0	0	473	0	3880	446	455	64	1108	
		9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	1470	182	0	369	0	0	24	0	
Placebo	SD	2	0	0	73	262	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	
		3	0	1926	576	1686	2734	886	24	4750	0	0	0	0	896	0	11	0	0	0	0	0	0	0	5	0	
		9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Placebo	PD	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		3	0	0	0	301	13033	0	0	6313	957	0	0	212	16207	0	0	8505	0	2513	0	1968	20415	12563	0	10362	
		9	21453	369	0	578	47998	15912	0	23782	0	0	0	0	0	0	0	0	0	26	0	917	0	0	0	0	
Placebo	PD	2	0	2192	0	728	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		3	0	1274	1957	1338	0	0	0	1391	0	0	1943	740	0	0	0	0	117	1158	770	1192	0	0	0	0	
		9	0	1375	0	1162	7	0	0	1475	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Placebo	PD	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		3	1148	0	0	0	0	0	49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37	0	
		9	0	1058	0	626	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Placebo	PD	2	961	0	1347	1648	3082	0	0	0	0	0	0	0	799	0	1239	0	267	0	0	0	474	0	0	0	
		3	640	0	0	0	0	0	0	0	1333	928	305	546	1500	583	0	2238	834	110	459	0	1874	553	92	1631	
		9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Placebo	PD	2	181	0	0	0	0	0	0	45	0	0	0	0	0	0	0	0	388	100	0	0	133	30	0	0	
		3	1505	0	0	0	0	0	0	858	0	0	0	0	0	0	0	0	2032	53	0	0	994	112	0	758	
		9	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	0	0	0	0	0	0	0	2369	
Placebo	PD	2	0	1910	342	0	0	0	0	0	5024	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		3	0	0	0	0	0	0	0	0	0	258	0	0	0	2336	40	0	0	0	0	0	0	0	36	0	
		9	0	0	0	0	0	338	1	0	0	0	0	0	0	0	62	0	0	0	0	0	0	0	0	0	
Placebo	PD	2	0	1919	1423	5146	0	0	0	0	0	0	147	0	0	0	0	0	0	0	0	0	0	0	35	0	
		3	0	0	0	0	0	0	0	0	0	794	345	0	0	0	0	0	0	0	0	0	0	0	0	0	
		9	0	0	0	0	0	0	0	0	2758	2476	6398	3209	4340	772	0	740	0	0	0	0	0	0	0	0	
Placebo	PD	2	0	0	0	0	310	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		3	0	486	0	0	0	170	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		9	0	59	352	0	563	0	9	0	0	0	423	0	0	0	0	0	157454	97495	73173	100511	141687	57659	0	26854	