

Additional File 1 for

Split Tolerance Permits Safe Ad5-GUCY2C-PADRE Vaccine-Induced T-cell Responses in Colon Cancer Patients

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Supplementary Table 1. Baseline characteristics of CRC patients treated with Ad5-GUCY2C-PADRE

Baseline Characteristic	(n = 10)
Median age (range)	65 (49-76)
Male (%)	5 (50%)
Race	
Caucasian (%)	8 (80%)
African American (%)	2(20%)
TNM Staging	
Stage I (%)	9 (90%)
Stage II (%)	1 (10%)

Supplementary Table 2. Summary of immune responses to Ad5-GUCY2C-PADRE

Patient	Peak GUCY2C Antibody Titer ^a	Antigen-Specific T-Cell Response ^b at Day 30, Significance ^c				Ad5 NAb Titer ^{a,d}	Ad5 NAb Status ^d
		GUCY2C		PADRE			
1001	20	0.00	<i>P</i> = NS	0.00	<i>P</i> = NS	1074	High
1002	ND ^e	8.67	<i>P</i> = NS	0.42	<i>P</i> = NS	15951	High
1003	ND	2.00	<i>P</i> = NS	0.50	<i>P</i> = NS	4379	High
1004	ND	18.00	<i>P</i> < 0.001	11.50	<i>P</i> < 0.001	60	Low
1005	ND	10.50	<i>P</i> = NS	0.00	<i>P</i> = NS	12	Low
1006	ND	6.00	<i>P</i> = NS	1.25	<i>P</i> = NS	27	Low
1007	640	43.25	<i>P</i> < 0.001	7.50	<i>P</i> < 0.001	10	Low
1008	ND	63.83	<i>P</i> < 0.001	0.00	<i>P</i> = NS	19	Low
1009	ND	2.17	<i>P</i> = NS	0.33	<i>P</i> = NS	1309	High
1010	20	8.17	<i>P</i> < 0.001	1.75	<i>P</i> = NS	3355	High

^a Reciprocal dilutions are shown for GUCY2C antibody and Ad5 NAb titers

^b SFCs/5x10⁵ PBMCs

^c mDFR(2x) compared to background and day 0.

^d Ad5 NAb titers and status prior to Ad5-GUCY2C-PADRE administration are shown

^e ND = not detected

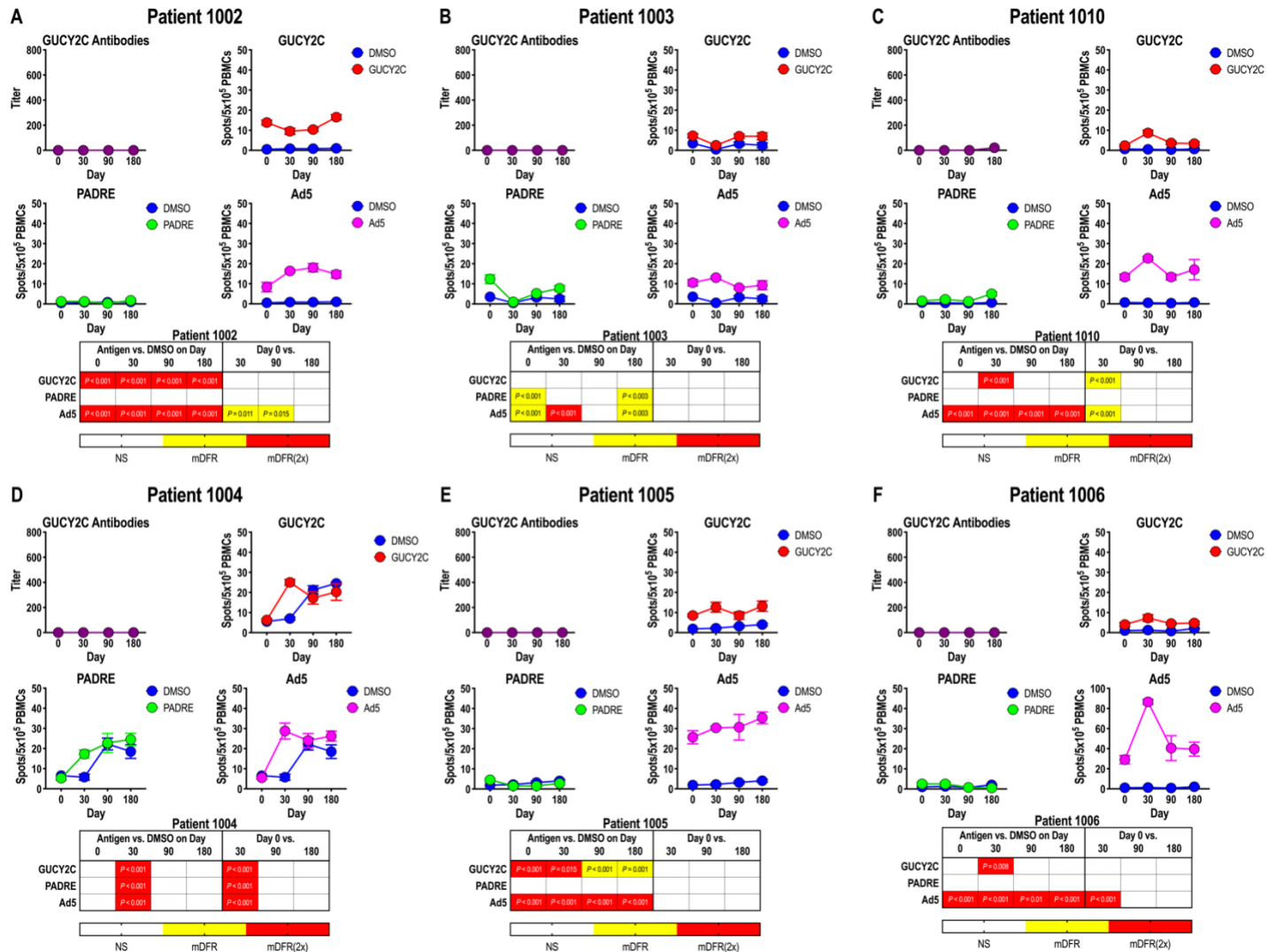


Figure 1. Ad5-GUCY2C-PADRE-induced immune responses. Patient blood samples were collected before (day 0) and 30, 90 and 180 days after Ad5-GUCY2C-PADRE immunization. GUCY2C-specific antibody titers were quantified by ELISA and GUCY2C, PADRE, and Ad5 -specific T-cell responses were quantified by IFN γ -ELISpot. ELISpot assays employed DMSO as an antigen-negative control. The statistical significance for T-cell responses at each time point (compared to DMSO) was determined by modified DFR(eq) or DFR(2x) after Westfall–Young max-T correction, and p-values <5% are shown in yellow [mDFR(eq)] or red [mDFR(2x)], respectively. The statistical significance of T-cell responses obtained for each post-vaccination time point (compared to day 0) were determined by a similar modified DFR-like permutation method with Westfall–Young max-T correction. Representative GUCY2C non-responders and responders are shown in **Figure 2**. All other patient responses are shown here.