

Supplementary Table S1 Pre-treatment anti-Gal titers, α -gal glycolipids dose and volume

Patient ID	Pre-treatment anti-Gal titers	Dose level (mg)	Treatment volume α -gal glycolipids (ml)
UW001	1:400	0.1	1.0
UW002	1:200	0.1	1.0
UW003	1:200	0.1	1.0
UW004	1:80	1.0	1.0
UW005	1:320	1.0	1.0
UW006	1:80	1.0	1.0, 0.5 ^a
UW007	1:640	10.0	1.0
UW008	1:80	10.0	1.0
UW009	1:160	10.0	1.0

^a Volume of first and second dose was 1 ml and 0.5 ml, respectively. Per protocol, concentrated doses were used for subjects whose target lesion could not accommodate a full 1 ml dose volume.

Supplementary Table S1 Pre-treatment anti-Gal titers, α -gal glycolipids dose and volume. Data are presented for the pre-treatment sera anti-Gal titers, and the dose and volume of α -gal glycolipids delivered to each patient.

Supplementary Table S2a Tumor biopsy PD-L1 expression: Inflammatory cells^a

Patient ID	Pre-treatment		2 wk. post 1 st injection		4 wk. post 2 nd injection	
	Target	Non-target	Target	Non-target	Target	Non-target
UW001	N/A	N/A	Neg	ND	++	ND
UW002	+	ND	+	ND	++	ND
UW003	+	+	+++	ND	+++	++
UW004	+	+	+	ND	+	++
UW005	Neg	ND	+	ND	Neg	+
UW006	Neg	Neg	Neg	ND	++	ND
UW007	Neg	ND	+	ND	Neg	ND
UW008	++	ND	++	ND	++	ND
UW009	+++	ND	+++	ND	+++	ND

^a Exploratory analyses, no formal statistical analysis.

Abbreviations: N/A, Inadequate tissue for staining; ND, biopsy not done; Neg, Negative; +, Rare and dim staining; ++, Moderate staining of most cells; +++, Strong staining of most cells

Supplementary Table S2a Tumor biopsy PD-L1 expression: Inflammatory cells. Data is presented for the level of PD-L1 staining on dendritic-like and macrophage-like cells from biopsies collected at pre-treatment, 2 weeks after the 1st injection, and 4 weeks after the 2nd injection for both injected (target) lesion and non-injected (non-target) lesion.

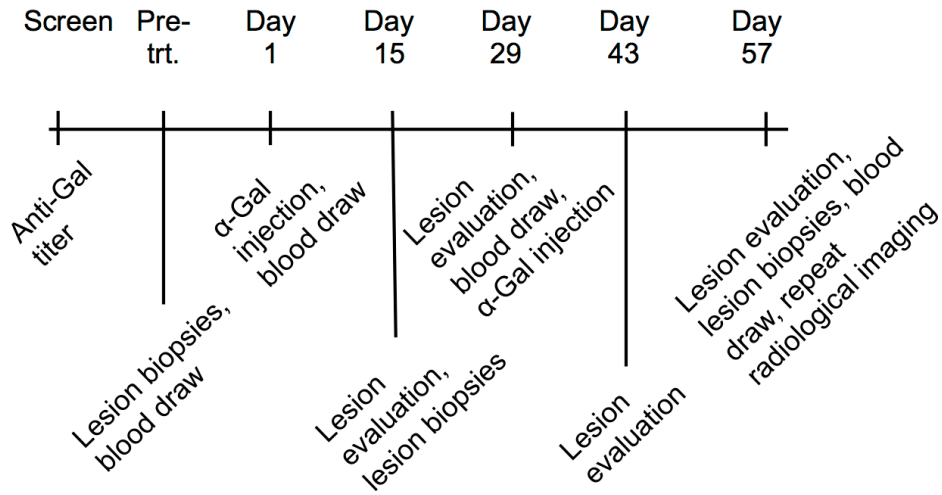
Supplementary Table S2b Tumor biopsy PD-L1 expression: Melanoma cells^a

Patient ID	Pre-treatment		2 wk. post 1 st injection		4 wk. post 2 nd injection	
	Target	Non-target	Target	Non-target	Target	Non-target
UW001	N/A	N/A	+	ND	Neg	Neg
UW002	Neg	ND	Neg	ND	Neg	ND
UW003	Neg	+	+	ND	+	+++
UW004	Neg	Neg	Neg	ND	Neg	Neg
UW005	Neg	ND	+	ND	Neg	Neg
UW006	++	++	++	ND	++	ND
UW007	Neg	ND	Neg	ND	Neg	ND
UW008	Neg	ND	Neg	ND	Neg	ND
UW009	+	ND	++	ND	++	ND

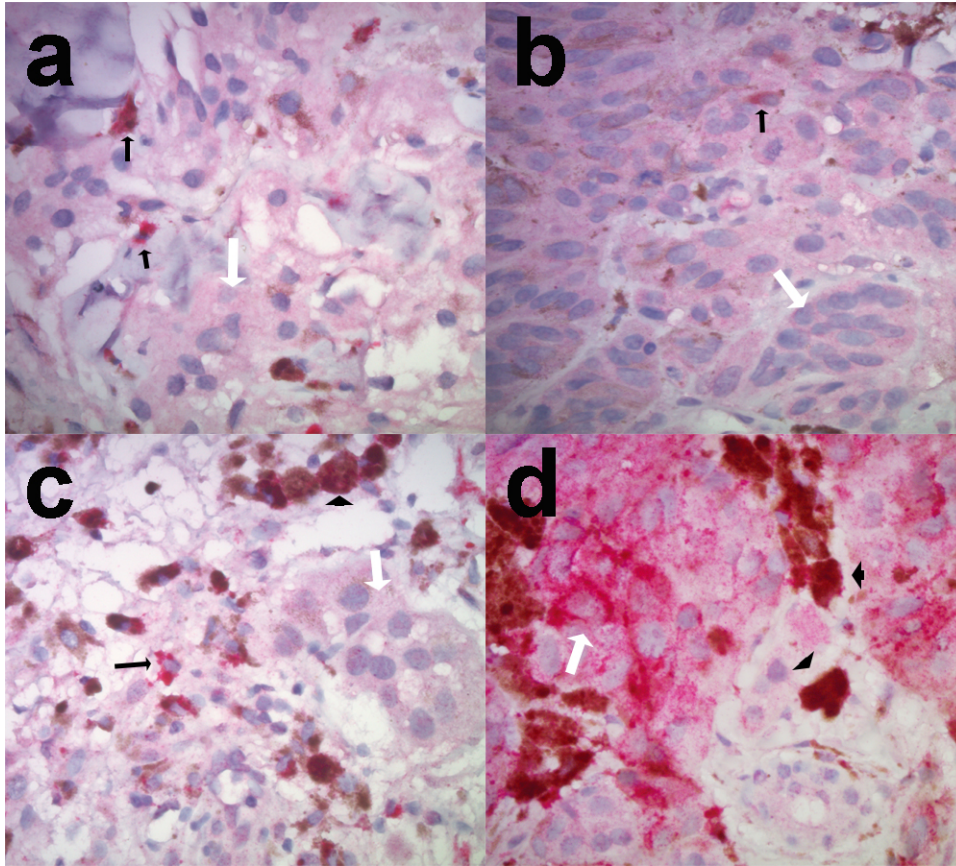
^a Exploratory analyses, no formal statistical analysis.

Abbreviations: N/A, Inadequate tissue for staining; ND, biopsy not done; Neg, Negative; +, Rare and dim staining; ++, Moderate staining of most cells; +++, Strong staining of most cells

Supplementary Table S2b Tumor biopsy PD-L1 expression: Melanoma cells. Data are presented for the level of PD-L1 staining on melanoma cells from biopsies collected at pre-treatment, 2 weeks after the 1st injection, and 4 weeks after the 2nd injection for both injected (target) lesion and non-injected (non-target) lesion.



Supplementary Fig. S1 Schematic of α -gal glycolipids treatment, blood and biopsy collection, and tumor evaluation time points. Time points are relative to initiation of treatment.



Supplementary Fig. S2 PD-L1 expression in tumor biopsies before and after α -gal glycolipids treatment. Melanoma biopsies from an injected (target) lesion (**a** and **c**) and non-injected (non-target) lesion (**b** and **d**) were stained for PD-L1 expression (red) prior to therapy (**a** and **b**) and four weeks following the 2nd injection (**c** and **d**). Initially, scattered macrophage-like cells (black arrows) show PD-L1 expression with the tumor cells showing negative or very light staining which was not substantially above background (white arrows). Following therapy in this patient, increased numbers of macrophages (and melanophages, black arrowheads) showed PD-L1 staining and in this patient, tumor cells also showed increased expression as compared to the negative staining of the small vessel at the lower right of panel **d**. Magnification shown is 40x on the images (400x total).