

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Postow MA, Callahan MK, Barker CA, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. *N Engl J Med* 2012;366:925-31.

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Supplementary Appendix

Supplement to: Postow M, et al. Immunologic Correlates of the Abscopal Effect in a Patient with Melanoma

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Supplementary Table 1:

Gene Symbol	Antigen	Ratio Pre/Post RT*
C11orf66	Chromosome 11 open reading frame 66	+5.7
COPB2	Coatomer Protein complex, subunit beta 2 (beta prime)	-5.1
GTPBP10	GTP-binding protein 10	+5.6
LRRC8E	Leucine-rich repeat- containing protein 8E	+9.1
MED6	Mediator complex subunit 6	+6.9
OCRL	Inositol polyphosphate 5- phosphatase	+6.7
PCLO	Presynaptic Cytomatrix Protein (piccolo)	+12.2
PTK2	Focal Adhesion Kinase 1	+10.9
RNF113A	Ring finger protein 113A	+6.8
RNF113B	Ring finger protein 113B	+6.3
UBE2O	Ubiquitin- Conjugating Enzyme E2O	+12.2
USP5	Ubiquitin Specific Peptidase 5	-9.0

* Positive numbers reflect a fold increase in antibody responses after RT. Negative numbers reflect a fold decrease.

Supplementary Table 1: Seromic analysis showing the 10 antigenic targets that were associated with increased (and the two with decreased) antibody responses following RT. Samples were obtained pre-RT (November 2010) and post-RT (April 2011), and optical density values were normalized and transformed per methods described in Gnjjatic 2009.¹¹ Ratios of values were calculated for each antigen and considered

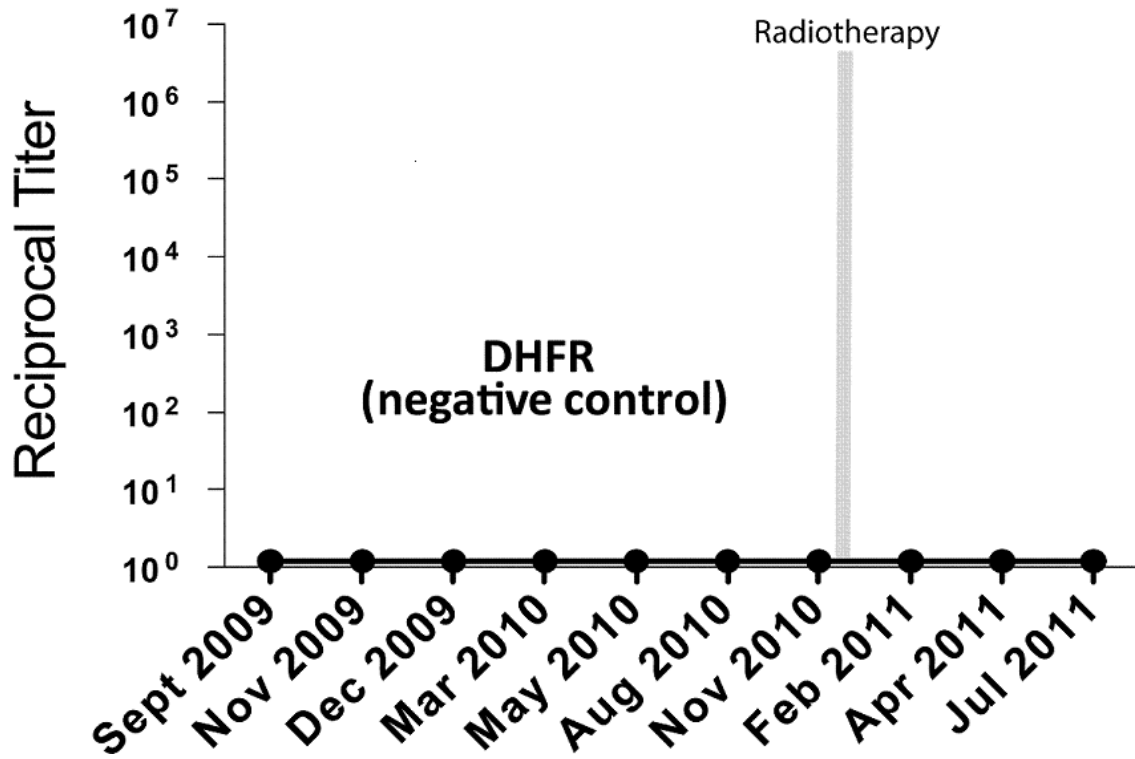
significant if they exceeded a 5-fold difference as shown in the "Ratio Pre/Post RT" column.

Supplementary Table 2:

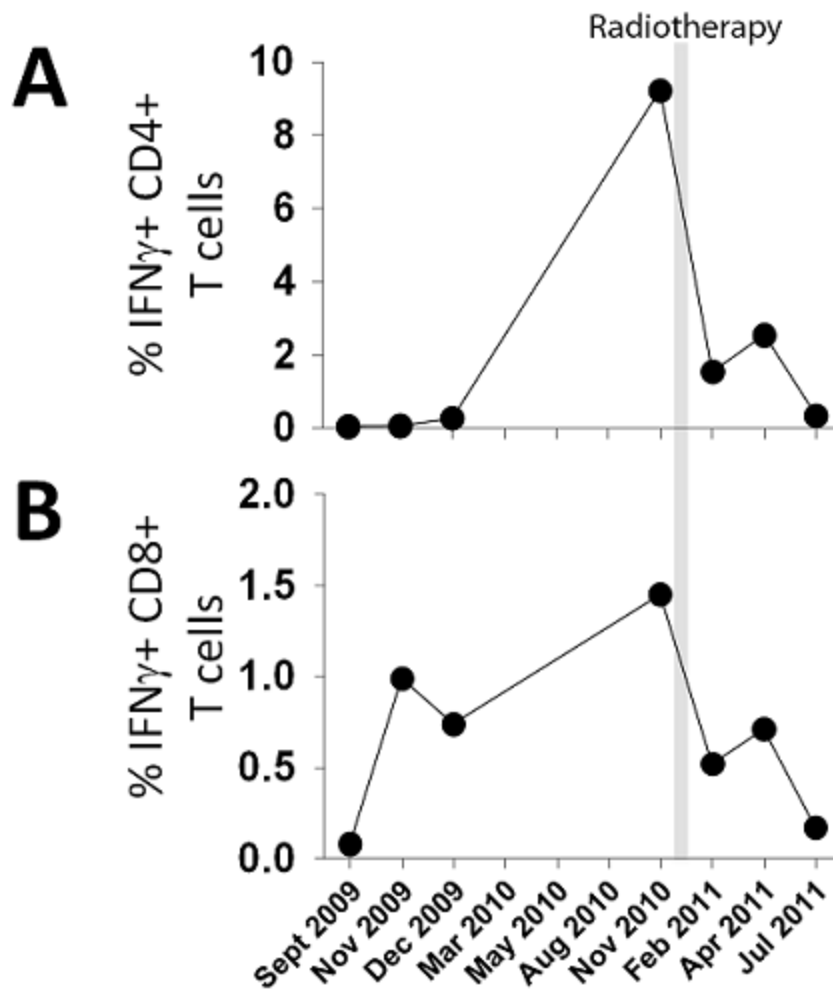
Date of CT	Timepoint	Paraspinal Mass	Right Hilar Lymphadenopathy	Splenic nodule
8/2009	A	1.3	1.79	0*
11/2010	B	18.86	3.66	0.32
1/2011	C	21.27	6.27	0.65
4/2011	D	10.23	1.88	0*
10/2011	E	7.3	1.16	0*

*Nodule was not apparent on axial imaging at these time points.

Supplementary Table 2: Three-dimensional assessment of tumor volumes as indicated in cm³. Systemic disease response occurred sometime after January 2011 and before April 2011, consistent with the images shown in Figure 1. The tumor volume in the spleen was calculated by assessing the largest apparent lesion in the spleen.



Supplemental Figure 1: Dihydrofolate reductase (DHFR) was used as a negative control for the ELISA NY-ESO-1 antibody titer experiments. Titers to DHFR remained negative at all evaluated timepoints.



Supplemental Figure 2: Frequencies of IFN- γ -producing CD4+ and IFN- γ -producing CD8+ cells as assessed by intracellular cytokine staining following NY-ESO-1 overlapping peptide stimulation. Though there was no clear trend of a CD8+ T cell response (B), with ongoing ipilimumab therapy and disease progression, the frequency of IFN- γ -producing CD4+ T cells increased (A). Despite this increase, it was not until radiation occurred that tumor regression was seen, suggesting that the tumor was still somehow resistant to T cell mediated antitumor immunity until radiation occurred.