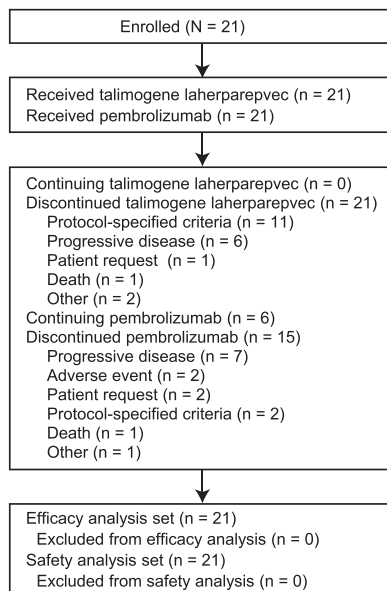


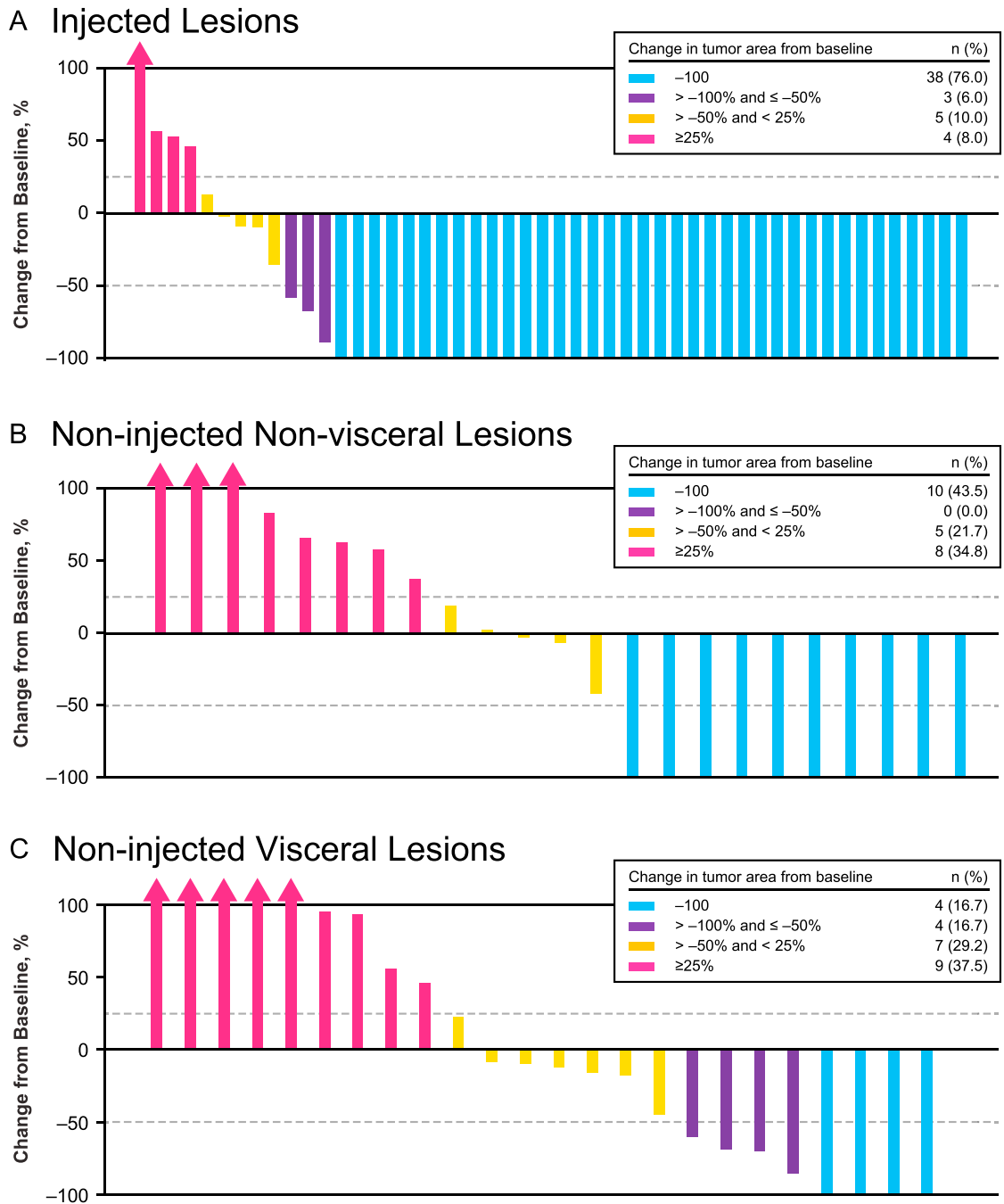
A Disposition of patients enrolled in the study



B Number of patients for which biomarker results were available

Assay	Week -5 Baseline	Week 0 Injected	Week 0 Non-injected	Week 24 Injected	Week 24 Non-injected
IHC PD-L1	23	10	4	6	1
IHC CD8, GZMB					
Total	19	15	5	4	1
Tumor	18	10	5	0	0
Cancer-cell depleted	1	5	0	4	1
Multi-Omyx	13	13	4	3	0
NanoString	18	9	6	0	0

Figure S1. Disposition of Patients Enrolled in the Study and Biopsy Availability for Biomarker Testing, Related to Figure 1



Includes index or new measurable lesion.

Evaluable indicates at least 2 assessments with bi-dimensional measurements.

Safety analysis set includes all subjects who received at least 1 dose of talimogene laherparepvec or pembrolizumab.

Figure S2. Changes in Tumor Burden at Lesion Level, Related to Figure 1

(A) Injected lesion response, (B) noninjected, nonvisceral lesion response, and (C) noninjected visceral lesion response.

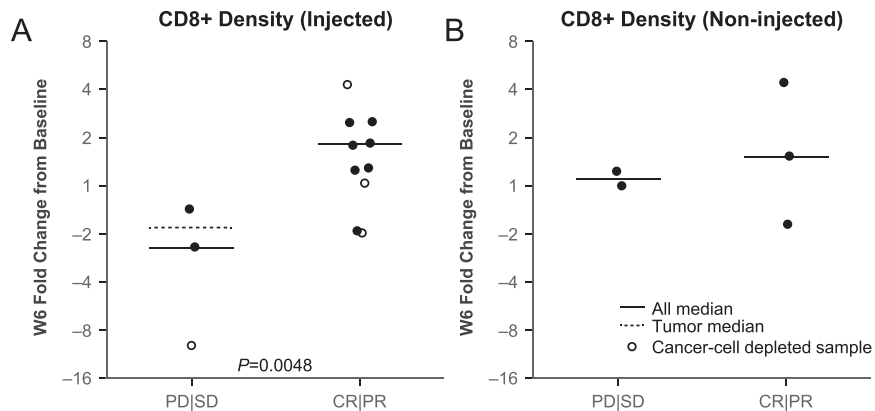


Figure S3. Responders Had CD8 Density Increases in Injected Lesions after Talimogene Laherparepvec, Related to Figure 3

Changes from baseline (week 1) in CD8 density as measured by IHC after talimogene laherparepvec treatment but before the start of combination therapy (W6) are plotted for responders and nonresponders separately. (A) Changes in injected lesions and (B) noninjected lesions are shown on a fold change scale. Response was defined as best overall response per investigator of CR or PR and nonresponse as PD or SD. Cancer cell-depleted samples are indicated with open circles. Median fold change is indicated with a horizontal line (solid for all samples, dashed for only those with tumor present). BL = baseline; CR = complete response; IHC = immunohistochemistry; PR = partial response; SD = stable disease. Of note, the all median and tumor median for the CR/PR (CR = complete response; PR = partial response) group were the same. Therefore, the dotted line and the solid line for the CR/PR group in Figure S3A were overlapping.

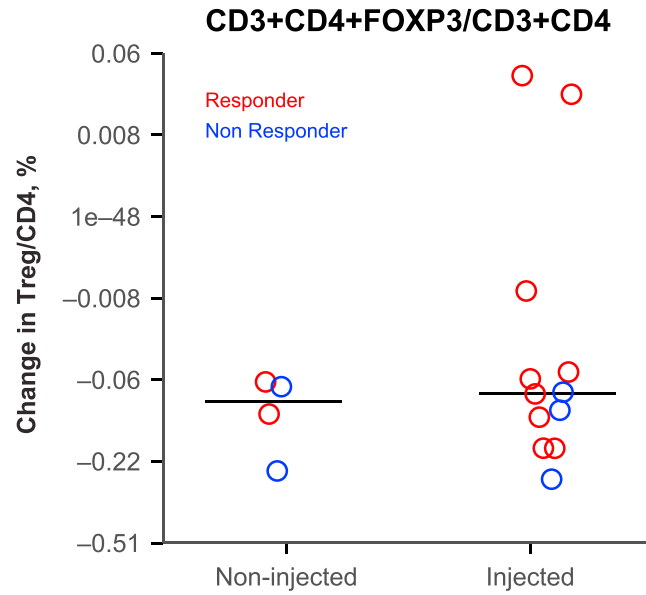


Figure S4. Talimogene Laherparepvec Decreases Treg Cell Fraction of CD4⁺ T Cells in Tumors, Related to Figure 4

Twelve-color immunofluorescence staining was performed on a single slide from paired tumor biopsies at pre- and post-talimogene laherparepvec from each of 13 patients. Markers evaluated included S100 (as melanoma segmentation marker), CD3, CD4, CD8, PD-1, PD-L1, CTLA-4, CD45RO, Foxp3, CD56, CD68, and CD20. Changes from baseline in Treg fraction of CD4⁺ T cells at week 6 are graphed for noninjected samples (left) and injected samples (right). Median change for each subset is shown with a horizontal line. Response is color-coded for best overall response per investigator: complete or partial response in red and nonresponse in blue. CTLA-4 = cytotoxic T cell-associated antigen 4; PD-1 = programmed death protein 1; PD-L1 = programmed death ligand 1; Treg = regulatory T cell.

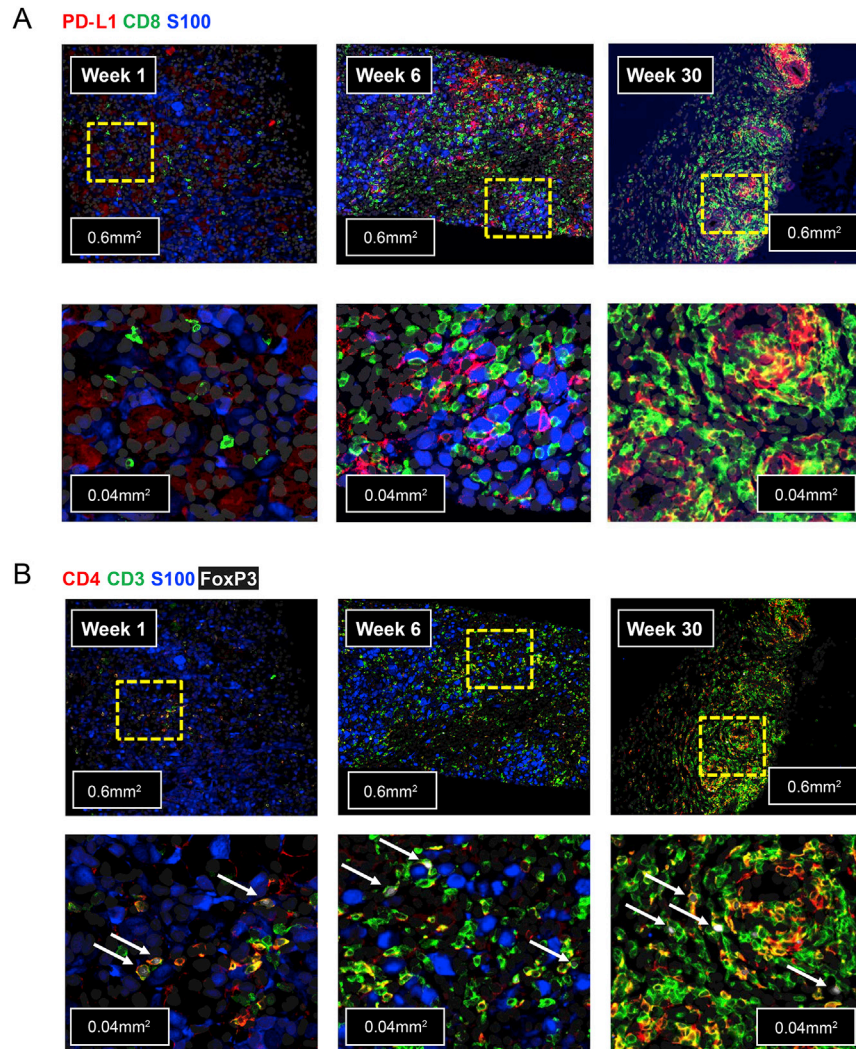


Figure S5. Additional Multiparameter Imaging (MultiOmyx platform) Examples from a Patient with a Partial Response to Therapy, Related to Figure 4

(A) The combination of S100, CD8, and PD-L1 (0.6- and 0.04-mm² image area) at baseline (week 1), week 6 after injection of talimogene laherparepvec, and at week 30 after long-term treatment with the combination of talimogene laherparepvec and pembrolizumab. (B) S100, CD3, CD4, and Foxp3 staining are shown (0.6- and 0.04-mm² image area). In the 0.04 mm² images, white arrows indicate some of the FoxP3 expressing cells. PD-L1 = programmed death ligand 1.

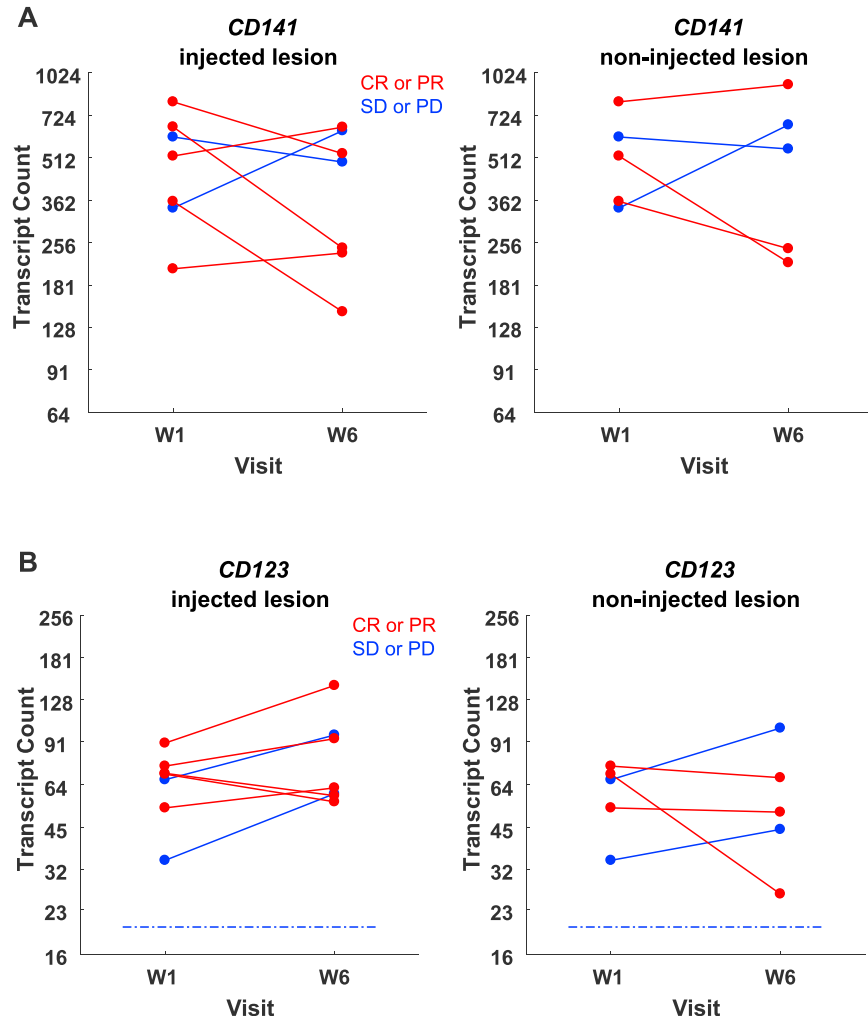


Figure S6. *CD141* and *CD123* mRNA Levels before and after Talimogene Laherparepvec Treatment in Injected and Noninjected Lesions, Related to Figure 4

mRNA levels of (A) *CD141* (*THBD*, *BDCA-3*) and (B) *CD123* (*IL3RA*) before and after talimogene laherparepvec treatment in injected and noninjected lesions. No significant changes from baseline, adjusting for total RNA and baseline expression, or association with response were observed. Blue dashed line indicates limit of blank.