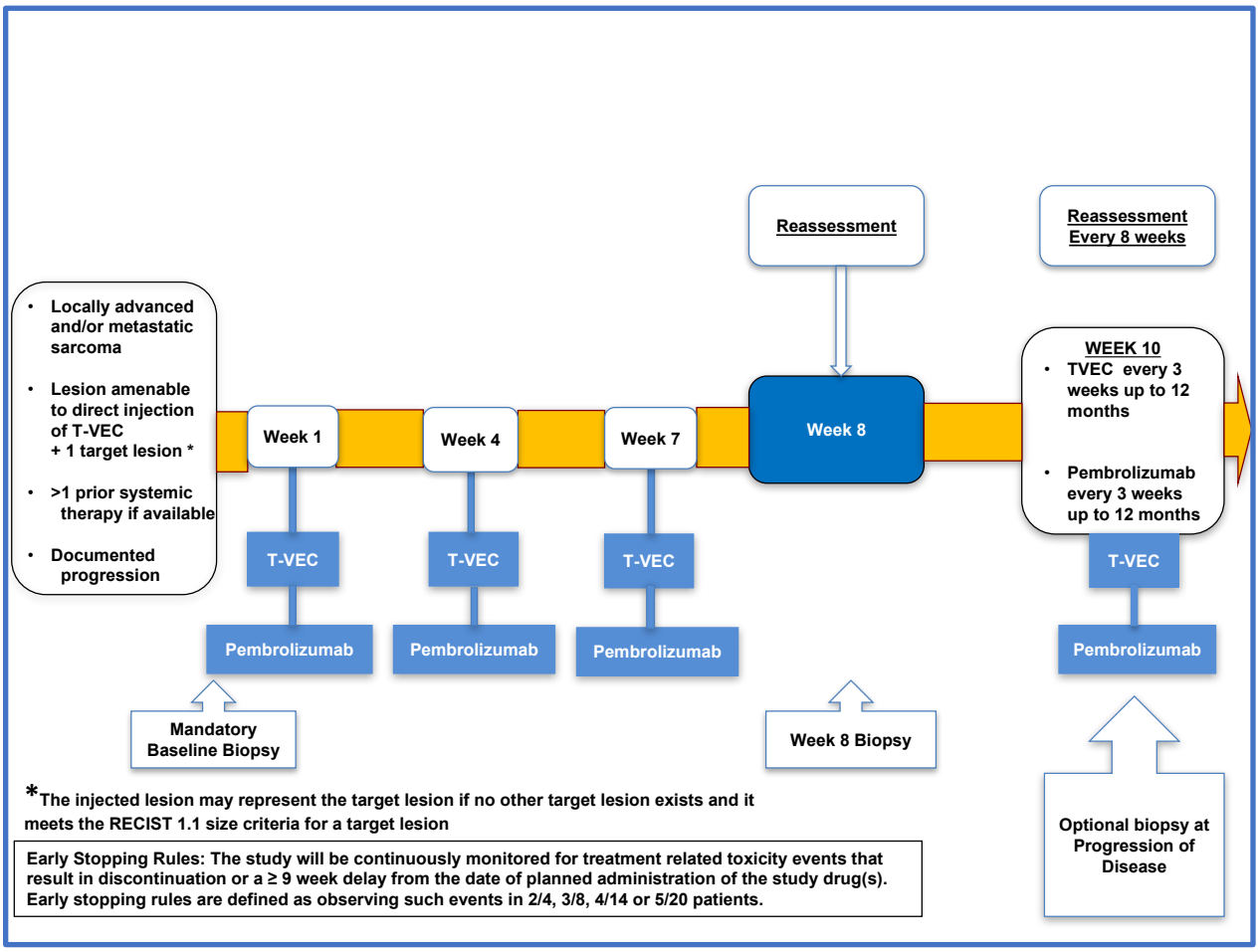
Supplementary Online Content

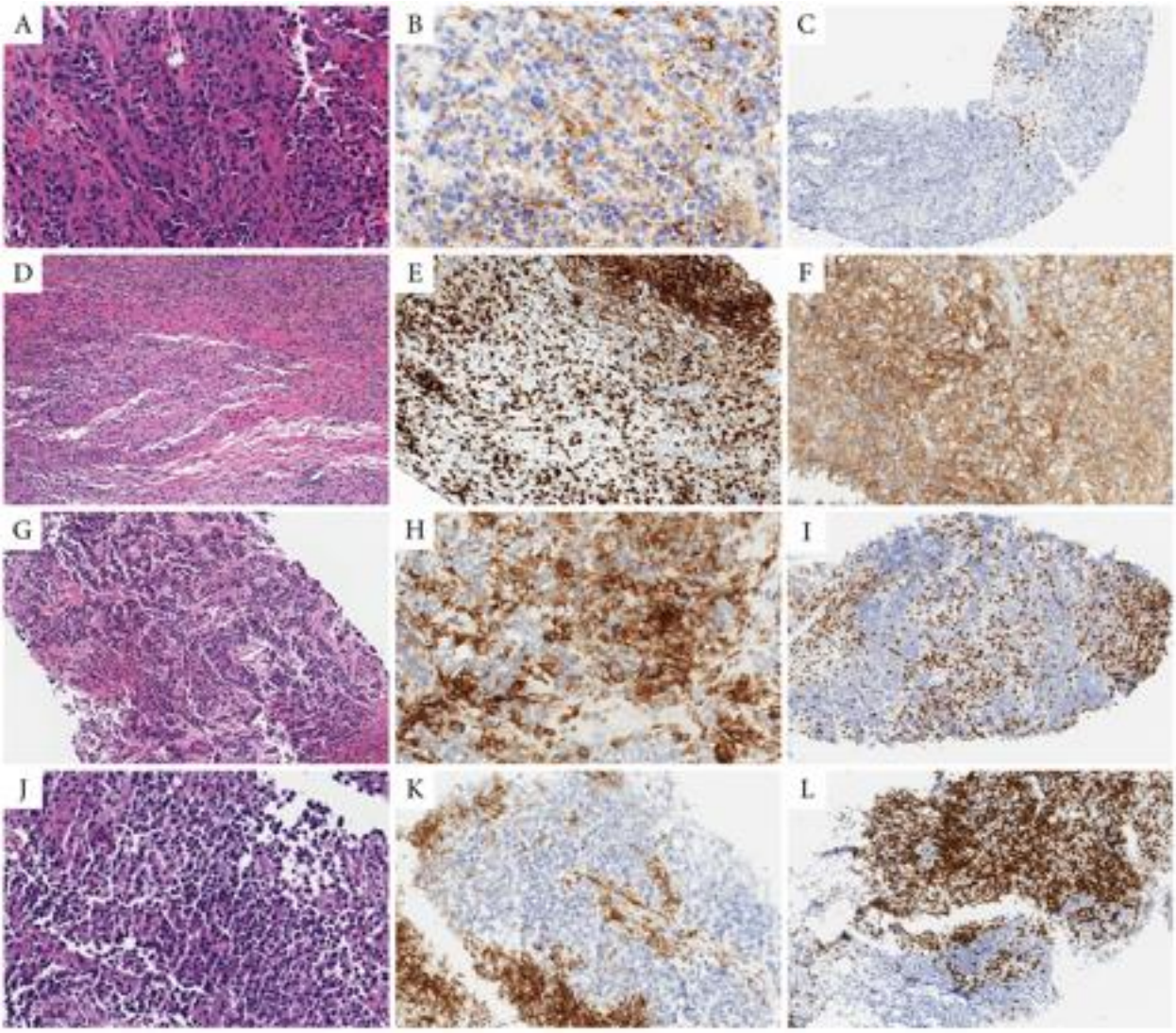
Kelly CM, Antonescu CR, Bowler T, et al. Objective response rate among patients with locally advanced or metastatic sarcoma treated with talimogene laherparepvec in combination with pembrolizumab: a phase 2 clinical trial. *JAMA Oncol.* Published online January 23, 2020. doi:10.1001/jamaoncol.2019.6152

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

eFigure 1. Study Schema

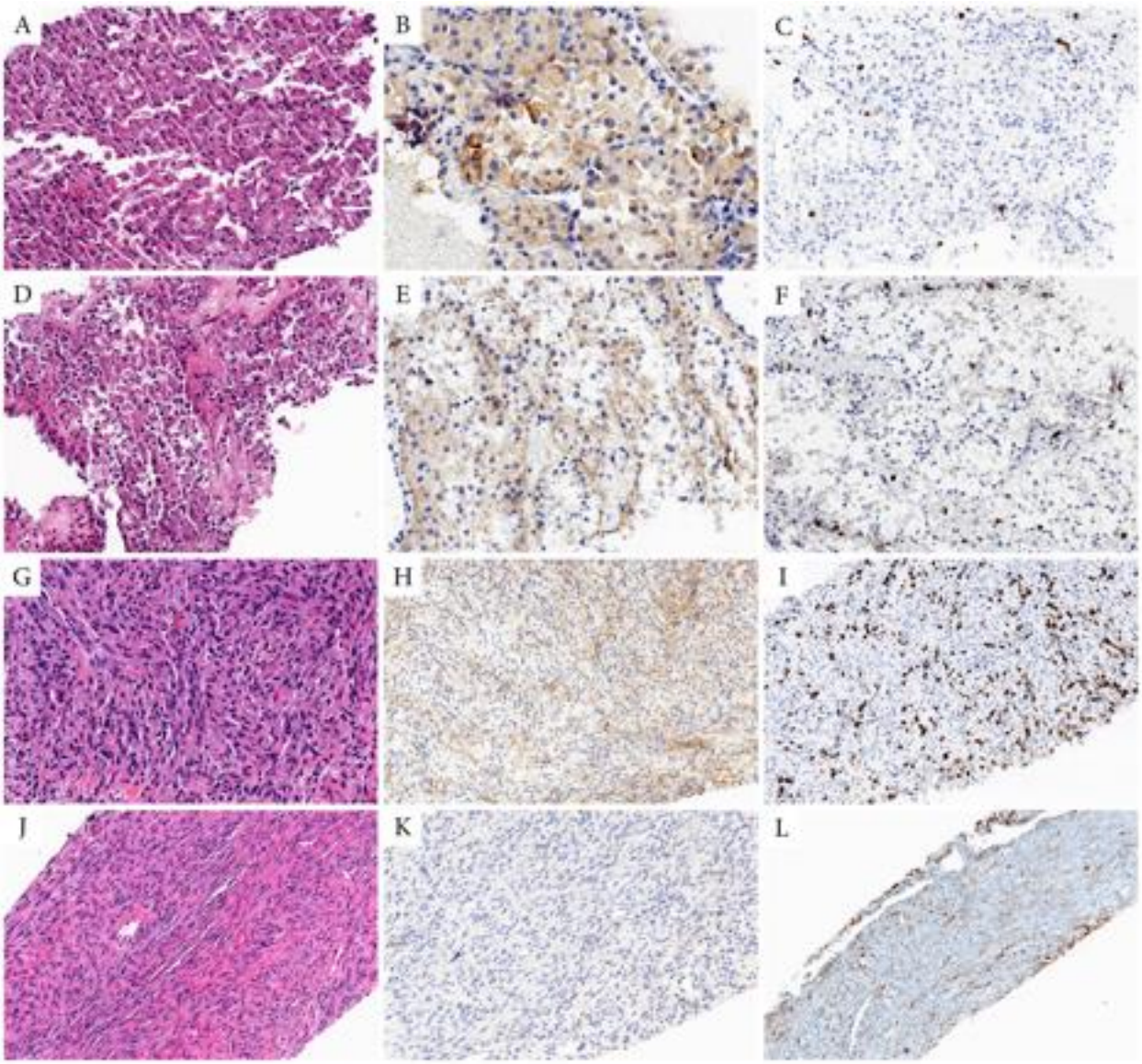


eFigure 2A. Morphologic Appearance and Immunohistochemical Results of Responders



A-C. Pre-therapy biopsy of a patient with scalp angiosarcoma showing solid growth of highly atypical epithelioid tumor cells (A). Immunohistochemistry showing focal PDL1 positivity in the tumor cells (B) and low power view showing clusters of CD3-positive TILs at the peripheral advancing edge of the tumor, with very rare positive cells been seen intermixed (C). D-F. Post-therapy biopsy of the same patient with angiosarcoma showing no residual viable disease; sample shows sheets of foamy histiocytes, hemosiderin deposition and inflammatory cells (D), immunohistochemistry highlights increased number of CD8-positive lymphocytes (E) and PD1-positive histiocytes (F). G-I. Pre-therapy biopsy of a patient with epithelioid sarcoma showing a high-grade malignancy composed of nests of epithelioid cells divided by thin fibrous septa (G). Immunohistochemistry shows positivity for PDL1 (H) and increased number of CD3-positive TILs mainly at the peripheral edge of the tumor and around the fibrovascular septa, and to a lesser degree admixed within the tumor (I). J-L. Post-therapy biopsy showing residual viable sarcoma (J), while PDL1 expression is mainly noted in the inflammatory cells at the periphery of the tumor (K) and marked increase in CD3 TILs arranged in confluent sheets at the advancing edge of the tumor (L). TIL, tumor-infiltrating lymphocytes.

eFigure 2B. Morphologic Appearance and Immunohistochemical Results of Nonresponders



A-F. Pre- and post-therapy biopsies from an alveolar soft part sarcoma patient showing no significant differences on cell viability or immune infiltrate. On H&E, tumor was viable before (A) and after (D) therapy, while immunohistochemistry showed some weak non-specific PDL1 (B, E) and rare CD3-positive TILs (C, F), without any cluster formation at the advancing edge of the tumor. G-L. Pre- and post-therapy from a patient with high-grade spindle cell sarcoma, NOS, showing no evidence of tumor necrosis on H&E (G, J). On immunohistochemistry, the pre-therapy sample showed weak focal PDL1 staining in the inflammatory cells (H) and scattered CD8-positive TILs admixed within the tumor, without forming clusters at the periphery (I). The post-therapy biopsy showed very rare (<1%) PDL1 positive inflammatory cells (K) and very rare CD4-positive TILs (L). TIL, tumor-infiltrating lymphocytes.

eTable 1. Talimogene Laherparepvec Injection Volume Guideline Based on Tumor Size

Tumor Size (longest dimension)	Maximum Injection Volume
>5.0 cm	4.0 mL
>2.5 cm to 5.0 cm	2.0 mL
>1.5 cm to 2.5 cm	1.0 mL
>0.5 cm to 1.5 cm	0.5 mL
≤0.5 cm	0.1 mL

eTable 2. Demographic and Disease Characteristics of Responders (n=7)

Histologic subtype	Clinical status at study entry	Prior lines of therapy	Prior Immunotherapy	Time to response (wks)
Cutaneous angiosarcoma	Recurrent locally advanced	3	Yes (atezolizumab & TIGIT antibody)	32
Cutaneous angiosarcoma	Locally advanced	1	No	16
Epithelioid sarcoma	Stage IV[a]	2	Yes (nivolumab & ipilimumab)	8
Unclassified sarcoma	Stage IV[a]	1	No	16
UPS[b]	Stage IV	1	No	8
UPS	Recurrent locally advanced	1	No	16
Myxofibrosarcoma (low grade)	Recurrent locally advanced	0	No	24

[a] Inoperable, locally advanced disease with local regional lymph node involvement.
[b] Patient had a diagnosis of Li Fraumeni syndrome.

eTable 3. Incidence of Treatment-Related Adverse Events

AEs	**N=20 (100%)**	
Grade ≥3 AEs	4 (20)	
Fatal AEs	0	
AEs leading to discontinuation of talimogene laherparepvec	0	
AEs leading to discontinuation of pembrolizumab	2 (10)	
AEs occurring in ≥15% of patients		
	All grades	Grade 3/4
• Fatigue	16 (80)	0
• Chills	9 (45)	0
• Fever	9 (45)	1 (5)
• Nausea	6 (30)	0
• Anemia	5 (25)	1 (5)
• Vomiting	4 (20)	0
• AST increase	4 (20)	0
• Pruritus	4 (20)	0
• Amylase Increased	3 (15)	0
• Cough	3 (15)	0
AEs of special interest		
	All grades	Grades 3/4
• Pneumonitis	2 (10)	1 (5)
• Optic neuritis	1[a] (5)	0
• Uveitis	1[a] (5)	0
• Hypothyroidism	4 (20)	0
• Thrombocytopenia	1 (5)	0

[a] Represents AEs that occurred in the same individual.

Abbreviations: AEs, adverse events; AST, aspartate aminotransferase.

AEs were recorded using the NCI CTCAE v4.03. AEs of special interest include immune-related AEs. All AEs of special interest were deemed to be related to pembrolizumab.

eTable 4. Tumor Membranous PD-L1 Expression and Characterization of Tumor-Infiltrating Lymphocytes

Best Objective Response	PD-L1 status (MPS)(Percent tumor)		TIL Score		Histology
	Pre-Treatment	Post-Treatment	Pre-Treatment	Post-Treatment	
PR	N/A	+ve (10)(5)	N/A	3	UPS
PR	N/A	+ve (60)(75)	N/A	3	UPS
PR	N/A	N/A	N/A	N/A	Myxofibrosarcoma
PR	-ve	+ve (5)(5)	1	3	Angiosarcoma
PR	-ve	+ve (60)(60)	3	3	Angiosarcoma
PR	+ve (15)(15)	N/A	3	N/A	Epithelioid Sarcoma
PR	-ve	N/A	3	N/A	Sarcoma NOS
SD	-ve	+ve (5)(5)	1	3	LMS
SD	-ve	-ve	1	1	ESMC
SD	-ve	-ve	2	1	LMS
SD	-ve	-ve (5)(20)	3	3	LMS
SD	-ve	+ve (90)(65)	2	3	LMS
SD	N/A	+ve (25)(90)	N/A	3	Sarcoma NOS MSI High
SD	-ve	-ve	2	0	ASPS
PD	-ve	N/A	2	N/A	Synovial Sarcoma
PD	-ve	-ve	0	0	Chondrosarcoma
PD	-ve	N/A	3	N/A	Angiosarcoma
PD	-ve	N/A	2	N/A	Sarcoma NOS
PD	-ve	+ve (5)(30)	1	3	MPNST
PD	-ve	-ve	2	3	LMS

Abbreviations: ASPS, alveolar soft-part sarcoma; ESMC, extraskeletal myxoid chondrosarcoma; LMS, leiomyosarcoma; MPS, modified proportion score; MPNST, malignant peripheral nerve sheath tumor; N/A, not available; NOS, not otherwise specified; PD, progression of disease; PR, partial response; SD, stable disease; TIL, tumor-infiltrating lymphocytes; UPS, undifferentiated pleomorphic sarcoma; +ve, positive; -ve, negative.