SUPPLEMENTARY APPENDIX

Talimogene laherparepvec induces a systemic response with upregulation of immune-cell populations in non-injected lesions: findings from a phase II, multicenter, open-label study in patients with stage IIIB–IVM1c melanoma

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Supplementary Table 1 Full eligibility criteria

Inclusion criteria
Patient has provided informed consent prior to initiation of any study-specific activities/procedures
Male or female age ≥18 years at time of informed consent
Histologically confirmed diagnosis of melanoma
Patient with stage IIIB to IVM1c melanoma for whom surgery is not recommended
Patient who is treatment naïve or had received prior treatment for melanoma. Any systemic treatment for melanoma must have been completed ≥28 days prior to enrollment
Candidate for intralesional therapy (i.e., disease is appropriate for direct injection or through the use of ultrasound guidance) defined as one of the following: • ≥1 injectable cutaneous, subcutaneous, or nodal melanoma lesion ≥10 mm in longest diameter, or • multiple injectable melanoma lesions that in aggregate have a longest diameter of ≥10 mm
Measurable disease defined as one or more of the following: • ≥1 melanoma lesion that can be accurately and serially measured in ≥2 dimensions and for which the greatest diameter is ≥10 mm as measured by contrast-enhanced or spiral CT scan, MRI, or ultrasound for nodal/soft tissue disease (including lymph nodes) • ≥1 ≥10-mm superficial cutaneous or subcutaneous melanoma lesion as measured by calipers • multiple superficial melanoma lesions which in aggregate have a total diameter of ≥10 mm
Serum LDH levels ≤1.5× ULN within 28 days prior to enrollment
ECOG performance status of 0 or 1
Adequate organ function determined within 28 days prior to enrollment, defined as follows: • absolute neutrophil count ≥1500/mm^3 • platelet count ≥75,000/mm^3 • hemoglobin ≥ g/dL without need for hematopoietic growth factor or transfusion support • serum creatinine ≤1.5× ULN • serum bilirubin ≤1.5× ULN • AST ≤2.5× ULN • ALT ≤2.5× ULN • alkaline phosphatase ≤2.5× ULN • serum albumin ≥2.5 g/dL • PT ≤1.5× ULN (or INR ≤1.3)[a] • PTT ≤1.5× ULN* [a]Prolongation in INR, PT, and PTT when the result is from therapeutic anticoagulation treatment are permitted for patients whose injectable lesions are cutaneous and/or subcutaneous such that direct pressure could be applied in the event of excessive bleeding
Exclusion criteria
Clinically active cerebral metastases. Patients with up to 3 cerebral metastases may be enrolled, provided that all lesions have been adequately treated with stereotactic radiation therapy (including Gamma Knife) or resection, with no evidence of progression and have not required steroids for ≥2 months prior to enrollment
>3 visceral metastases (this does not include lung metastases or nodal metastases associated with visceral organs). For patients with ≤3 visceral metastases, no lesion >3 cm and liver lesions must be stable for ≥1 month prior to enrollment
Bone metastases
Primary ocular or mucosal melanoma
History or evidence of symptomatic autoimmune disease (eg, pneumonitis, glomerulonephritis, vasculitis, or other), or history of autoimmune disease that required systemic treatment (ie, use of corticosteroids, immunosuppressive drugs, or biological agents used for treatment of autoimmune diseases) in past 2 months prior to enrollment. Replacement therapy (eg, thyroxine for hypothyroidism, insulin for diabetes mellitus) is not considered a form of systemic treatment for autoimmune disease

Evidence of clinically significant immunosuppression such as the following: • primary immunodeficiency state such as Severe Combined Immunodeficiency Disease • concurrent opportunistic infection • receiving systemic immunosuppressive therapy (>2 weeks), including oral steroid doses >10 mg/day of prednisone or equivalent during the 2 months prior to enrollment
Active herpetic skin lesions or prior complications of HSV-1 infection (eg, herpetic keratitis or encephalitis)
Requires intermittent or chronic systemic (intravenous or oral) treatment with an antiherpetic drug (eg, acyclovir), other than intermittent topical use
Previous treatment with talimogene laherparepvec
Currently receiving treatment with another investigational device or drug study, or <28 days since ending treatment with another investigational device or drug study(s)
Other investigational procedures while participating in this study are excluded
Known to have acute or chronic active hepatitis B infection
Known to have acute or chronic active hepatitis C infection
Known to have human immunodeficiency virus infection
History of other malignancy within the past 3 years with the following exceptions: • malignancy treated with curative intent and with no known active disease present for ≥3 years before enrollment and felt to be at low risk for recurrence by the treating physician • adequately treated non-melanoma skin cancer without evidence of disease • adequately treated cervical carcinoma *in situ* without evidence of disease • adequately treated breast ductal carcinoma *in situ* without evidence of disease • prostatic intraepithelial neoplasia without evidence of prostate cancer • adequately treated urothelial papillary noninvasive carcinoma or carcinoma *in situ*
Patient has known sensitivity to any of the products or components to be administered during dosing
Patient likely to not be available to complete all protocol-required study visits or procedures, and/or to comply with all required study procedures to the best of the patient's and investigator's knowledge
History or evidence of any other clinically significant disorder, condition, or disease (with the exception of those outlined above) that, in the opinion of the investigator or Amgen medical monitor, if consulted, would pose a risk to patient safety or interfere with the study evaluation, procedures, or completion
Patient previously has entered this study
Female patient is pregnant or breast-feeding, or planning to become pregnant during study treatment and through 3 months after the last dose of talimogene laherparepvec
Female patient of childbearing potential who is unwilling to use acceptable method(s) of effective contraception during study treatment and through 3 months after the last dose of talimogene laherparepvec *Note*: Women not of childbearing potential are defined as: Any female who is postmenopausal (age ≥55 years with cessation of menses for ≥12 months or <55 years with postmenopausal status confirmed by FSH in the postmenopausal range), or who have had a hysterectomy, bilateral salpingectomy, or bilateral oophorectomy
Sexually active patients and their partners unwilling to use male or female latex condom to avoid potential viral transmission during sexual contact while on treatment and within 30 days after treatment with talimogene laherparepvec

ALT, alanine amino transferase; AST, aspartate amino transferase; CT, computed tomography, ECOG, Eastern Cooperative Oncology Group; FSH, follicle-stimulating hormone; HSV, human simplex virus; INR, international normalization ratio; LDH, lactate dehydrogenase; MRI, magnetic resonance imaging; PT, prothrombin time; PTT, partial thromboplastin time; ULN, upper limit of normal.

Supplementary Table 2 Schedule of assessments

	Screening	Treatment period[a]									Follow-up period	
Cycle	**≤28 days**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Cycle 8 and beyond**	**Safety[b]**	**Survival[c]**
Day		**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**30 (+7) days**	**Every 12 (±28 days) weeks**
General and safety assessments												
Informed consent and review of medical/surgical history/eligibility	X											
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	
Adverse and serious adverse events	X	X	X	X	X	X	X	X	X	X	X	
Adverse events thought to be related to talimogene laherparepvec		X	X	X	X	X	X	X	X	X	X	X
Physical exam	X	X					X			Q6C	X	
Vital signs	X	X	X		X		X		X	Q2C	X	
ECOG performance status	X	X					X			Q6C	X	
Survival assessment												X
Anticancer therapy for melanoma											X	X
Local laboratory assessments												
Urine or serum pregnancy test	X										X	
Hematology and chemistry	X	X	X	X				X		Q4C	X	
LDH, PT (or INR), and PTT	X											
Central laboratory assessments												
Archived tumor tissue for biomarker analysis and *BRAF*$^{V600E/K}$		Within 28 days after enrollment										
Swab of herpetic lesion for qPCR		Within 3 days of occurrence of suspected lesion of herpetic origin									X	X

Blood for HSV serostatus	Within 3 days before dose at day 1 of week 1, week 6, week 12											
Blood for biomarker analysis	Within 3 days before dose at day 1 of week 1, week 6, week 12, week 24											
Tumor biopsy for biomarker analysis[d]	Within 5 days prior to dose at day 1 or week 1, ≤7 days before dose at week 6, and ≤7 days after disease progression and treatment discontinuation											
Tumor/response assessments												
Clinical and radiological tumor assessments	X	Day 1 of week 12 and then every 12 weeks until disease progression/start of new anticancer therapy									X	X
Photographs of all visible tumor lesions (selected sites only)		X	X		X		X		X	Q2C		
Dosing												
Talimogene laherparepvec administration[e]		X	X	X	X	X	X	X	X	Q2C		
Reporting exposure to talimogene laherparepvec												
Exposure of household member, healthcare provider, or close contact		X	X	X	X	X	X	X	X	X	X	
Reporting pregnancy/lactation												
Reporting pregnancy/lactation		X	X	X	X	X	X	X	X	X	X	X

[a] During the treatment period assessments and procedures were performed within 3 days of the planned visit unless otherwise specified.

[b] Safety follow-up was performed 30 (+7) days after the last dose of talimogene laherparepvec.

[c] Follow-up occurred every 12 weeks (±28 days) following the safety follow-up visit until death, withdrawal of consent, or up to 24 months after the last patient enrolled in the study.

[d] Tumor biopsy was collected (within 5 days prior to first talimogene laherparepvec administration) from one lesion at day 1 of week 1 [Cycle 1] and, if there were ≥2 lesions at baseline and one is left non-injected, from a non-injected lesion within 7 days prior to dose at day 1 of week 6 [Cycle 3]. Also within 7 days after documentation of disease progression followed by treatment discontinuation from the available cutaneous, subcutaneous, or nodal lesion responsible for disease progression and easily accessible for biopsy with or without ultrasound guidance. *Note*: Non-injected lesion biopsied at day 1 of week 6 [Cycle 3] had to be a different lesion from that biopsied at day 1 of week 1 [Cycle 1].

[e] The first cycle of talimogene laherparepvec was 21 (+5) days. Subsequent cycles were 14 (±3) days. On day 1 of Cycle 1**,** the first dose of talimogene laherparepvec was up to 4.0 mL of 106 PFU/mL. The second dose up to 4.0 mL of 108 PFU/mL was administered 21 (+5) days after the initial injection. Subsequent injections up to 4.0 mL of 108 PFU/mL were given every 14 (±3) days. Patients will be treated with talimogene laherparepvec until complete response, all injectable tumors

disappeared, clinically significant (resulting in clinical deterioration or requiring change of therapy) disease progression beyond 6 months of treatment, per modified WHO Response Criteria, or intolerance of study treatment, whichever occurs first.

BRAF, v-raf murine sarcoma viral oncogene homolog B1; ECOG, Eastern Cooperative Oncology Group; HSV, herpes simplex virus; INR, international normalization ratio; LDH, lactate dehydrogenase; PT, prothrombin time; PTT, partial thromboplastin time; Q2C, every 2nd cycle; Q4C, every 4th cycle; Q6C, every 6th cycle; qPCR, quantitative polymerase chain reaction.

Supplementary Table 3 Immunophenotypes for cell populations of interest using multiparameter immunofluorescence

Cell type, markers
CTL expressing CTLA-4, $CD3^+$ $CD8^+$ $CTLA4^+$
CTL expressing PD-1, $CD3^+$ $CD8^+$ $PD\text{-}1^+$
CTL expressing PD-L1, $CD3^+$ $CD8^+$ $PD\text{-}L1^+$
CTL, $CD3^+$ $CD8^+$
Effector CTL, $CD3^+$ $CD8^+$ granzyme B^+
Helper T-cells, $CD3^+$ $CD4^+$
Macrophage expressing PD-L1, $CD3^-$ $CD68^+$ $PD\text{-}L1^+$
Macrophage, $CD68^+$
Memory CTL, $CD3^+$ $CD8^+$ $CD45RO^+$
Proliferating CTL, $CD3^+$ $CD8^+$ $Ki67^+$

CTL, cytotoxic T-lymphocyte; CTLA-4, cytotoxic T-lymphocyte antigen-4; PD-1, programmed death-1; PD-L1, programmed death-ligand 1.

Supplementary Table 4 Patient disposition

	n (%)	
Enrolled patients	112 (100)	
Talimogene laherparepvec treatment		
	Primary analysis (median FU 59 weeks)	**Longer-term analysis (median FU 108 weeks)**
Patients who never received talimogene laherparepvec	1 (0.9)	1 (0.9)
Patients who received talimogene laherparepvec	111 (99.1)	111 (99.1)
Patients continuing to receive talimogene laherparepvec	17 (15.2)	4 (3.6)
Patients who discontinued talimogene laherparepvec	94 (83.9)	107 (95.5)
• Disease progression[a]	46 (48.9)	53 (49.5)
• Protocol-specific criteria[a, b]	20 (21.3)	21 (19.6)
• Requirement for alternative therapy[a]	13 (13.8)	15 (14.0)
• Patient request[a]	7 (7.4)	9 (8.4)
• Adverse event[a]	2 (2.1)	2 (1.9)
• Death[a]	2 (2.1)	2 (1.9)
• Other[a]	4 (4.3)	5 (4.7)
Study completion		
	Primary analysis	**Longer-term analysis**
Patients who completed study	0 (0)	55 (49.1)
Patients continuing study	79 (70.5)	5 (4.5)
Patients who discontinued study	33 (29.5)	107 (95.5)
• Death[c]	26 (78.8)	41 (38.3)
• Withdrawal of consent [c]	6 (18.2)	9 (8.4)
• Lost to follow-up[c]	1 (3.0)	1 (0.9)
• Protocol-specific criteria[c]	–	1 (0.9)

[a] Denominator uses 'Patients who discontinued talimogene laherparepvec'.

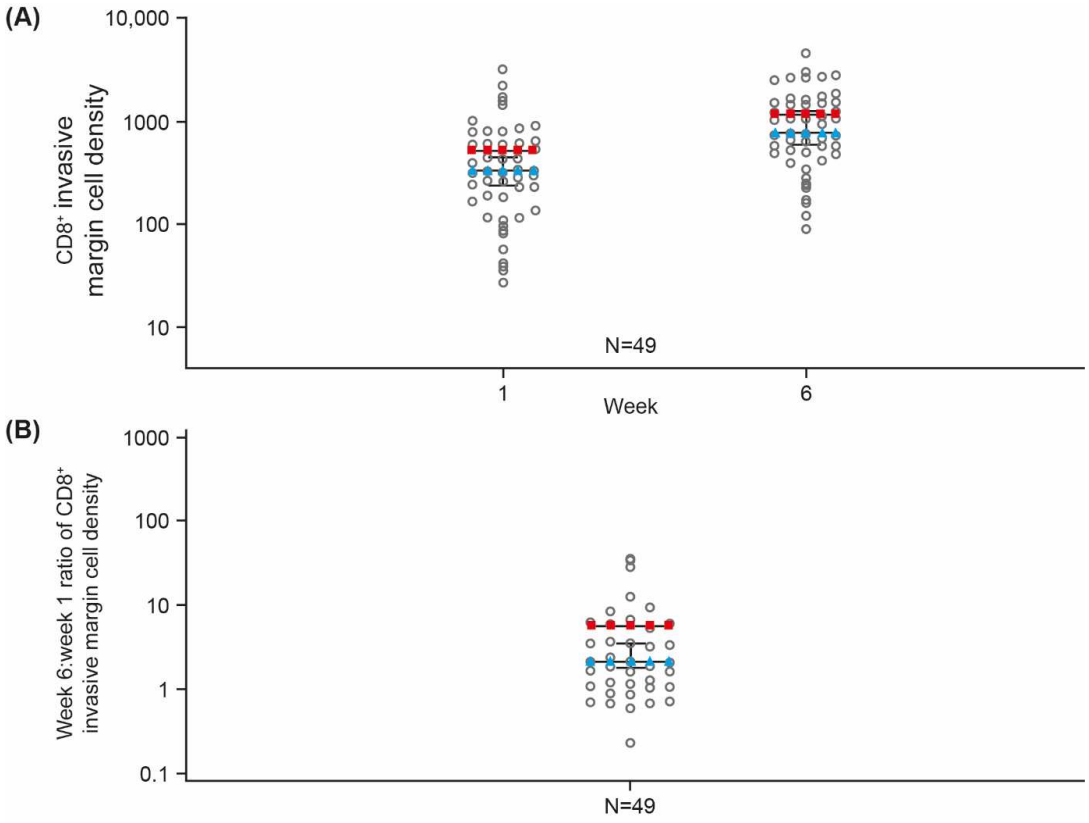
[b] Complete response for 14 patients and no injectable lesions for 6 patients.

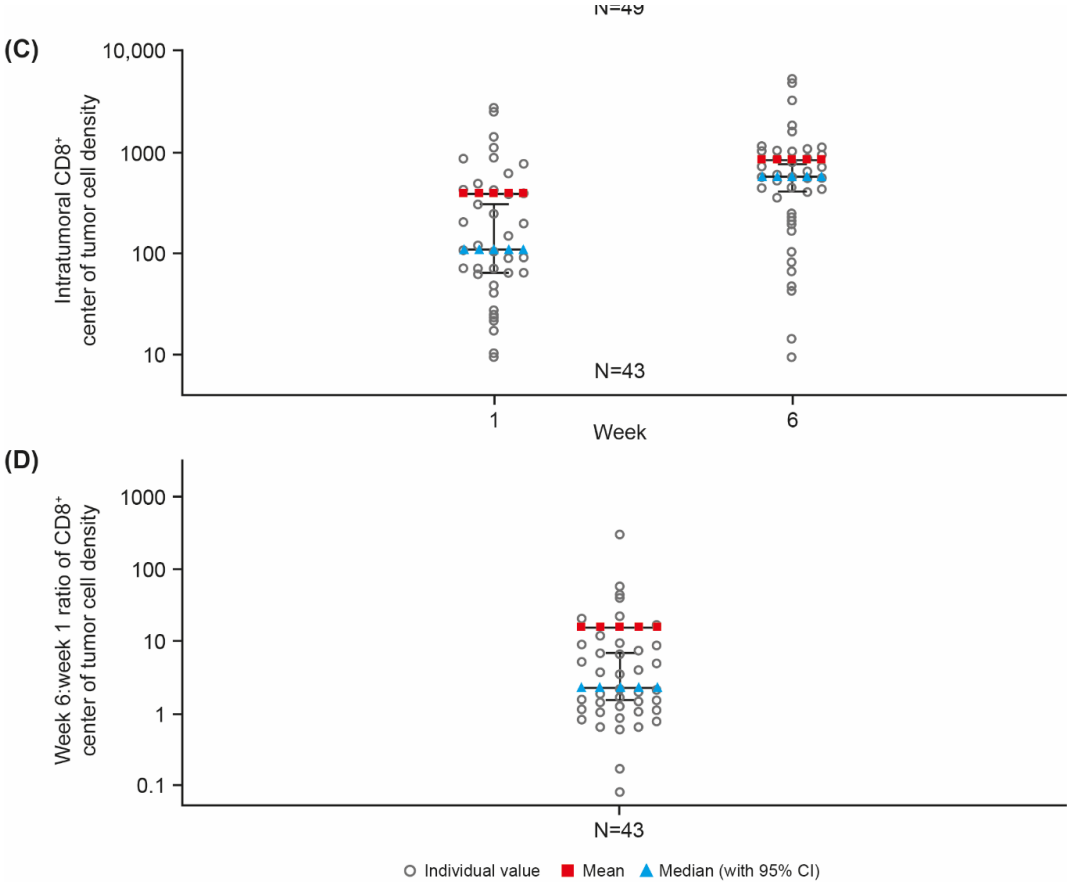
[c] Denominator uses 'Patients who discontinued study'.

FU, follow-up.

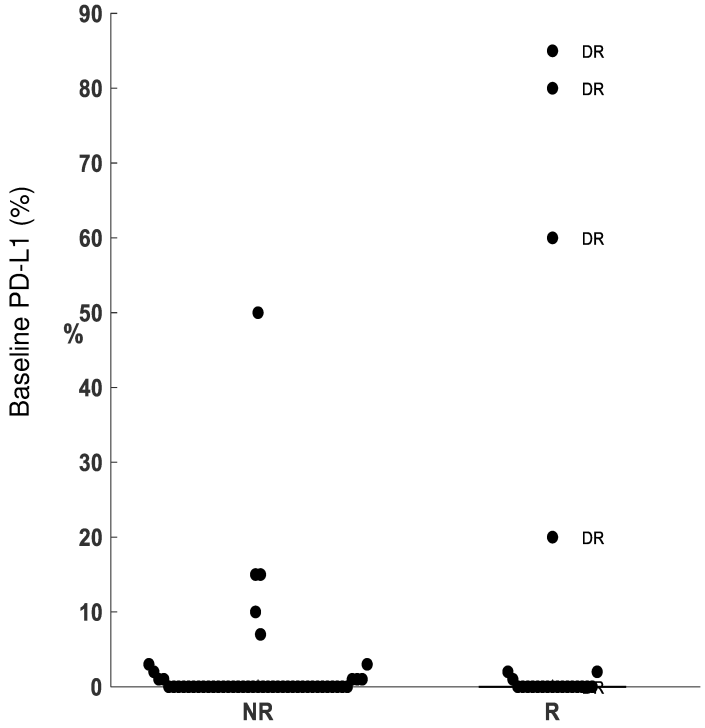
Supplementary Figure 1 Changes in intratumoral CD8⁺ T-cell density in the invasive margin and center of tumor areas (biomarker evaluable analysis set for non-injected lesions for CD8⁺ invasive margins [n=49] and for CD8⁺ center of tumor [n=43]).

(A) Scatter graph showing intratumoral CD8⁺ T-cell density in invasive margin areas at baseline and week 6 in non-injected lesions. (B) Scatter graph showing week 6/week 1 ratio of intratumoral CD8⁺ T-cell density in invasive margin areas in non-injected lesions. (C) Scatter graph showing intratumoral CD8⁺ T-cell density in center of tumor areas at baseline and week 6 in non-injected lesions. (D) Scatter graph showing week 6/week 1 ratio of intratumoral CD8⁺ T-cell density in center of tumor areas in non-injected lesions. Biomarker valuable analysis set for non-injected lesions includes all subjects in the safety analysis set who had the intratumoral CD8⁺ cell density recorded at baseline and week 6, and the week 6 measurements from the injected lesion. CI, confidence interval.




(C)
Intratumoral CD8+ center of tumor cell density
10,000
1000
100
10
N=49
N=43
1
6
Week
(D)
Week 6:week 1 ratio of CD8+ center of tumor cell density
1000
100
10
1
0.1
N=43
Individual value
Mean
Median (with 95% CI)

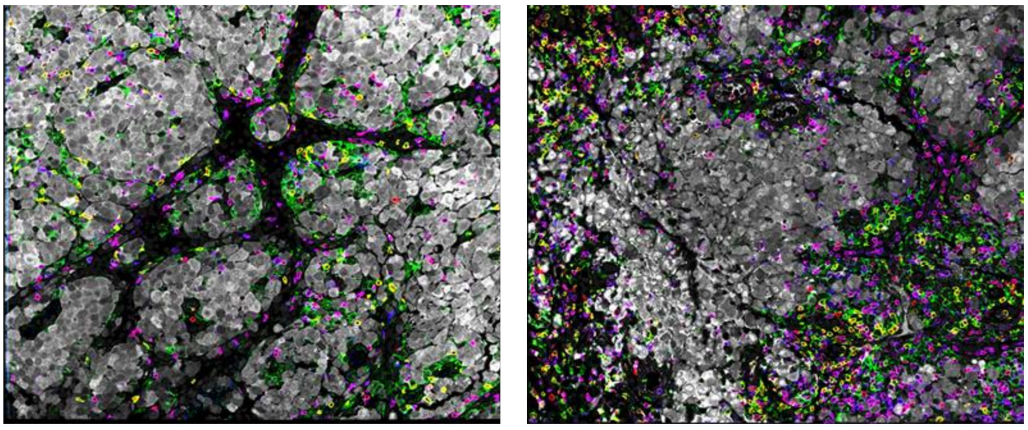
Supplementary Figure 2 Association between baseline PD-L1 %+ and response to T-VEC.



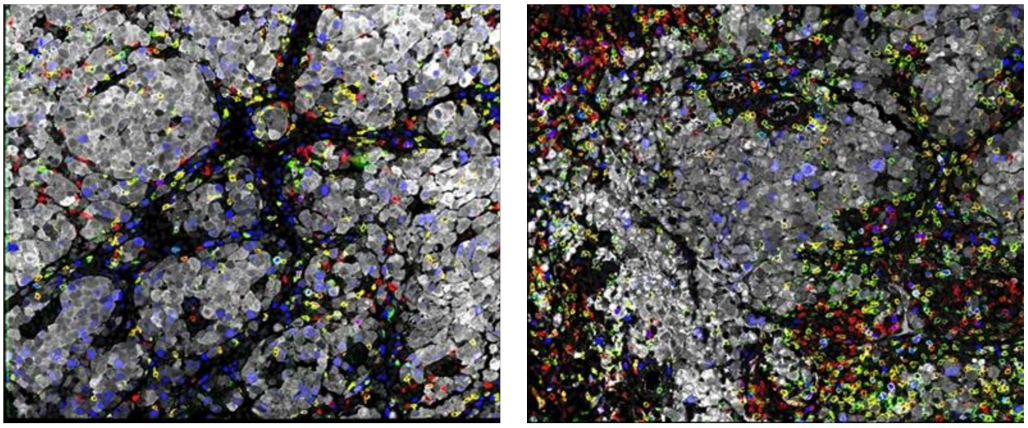
PD-L1 results were not transformed prior to analysis. The change from baseline (week 6%+ – week 1%+) was modeled by linear regression, with baseline PD-L1 as a covariate. A sensitivity analysis was performed by including lesion distance from injection site and fixation time as covariates. DR, durable responder; NR, non-responder; PD-L1, programmed death- ligand 1; R, responder; T-VEC, talimogene laherparepvec.

Supplementary Figure 3 Additional examples of changes in intratumoral immune-cell subsets from baseline to week 6 in non-injected lesions from four patients (A–D). Images on the left are from baseline. Images on the right are at week 6. T-VEC, talimogene laherparepvec.

(A) Images from a T-VEC-treated patient who achieved partial response

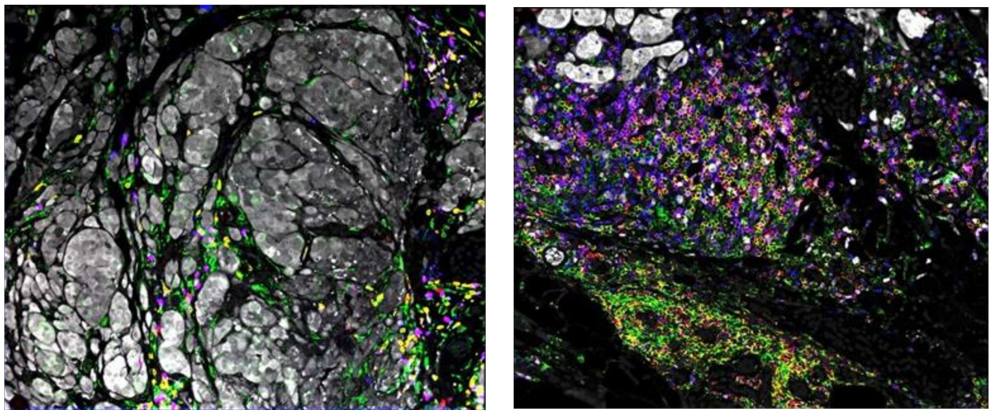


S100 (gray), CD3 (red) , CD4 (green), CD8 (blue)

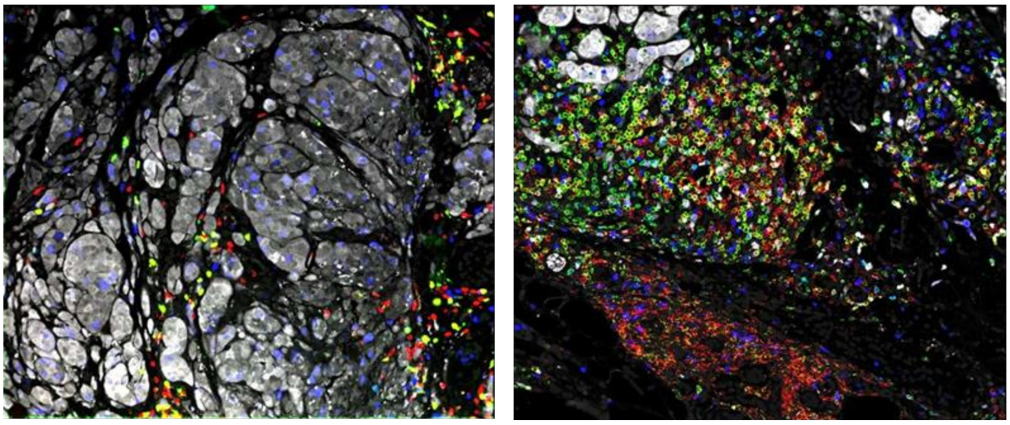


S100 (gray), CD3 (red) , CD8 (green), Ki67 (blue)

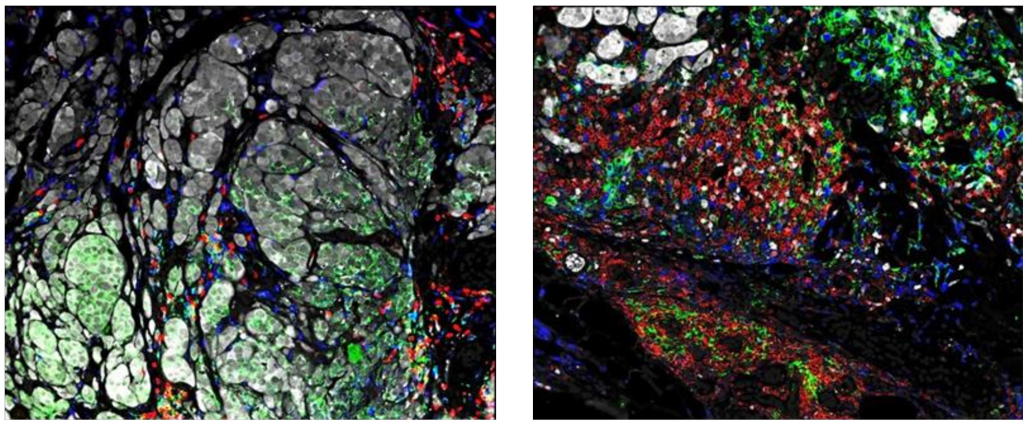
(B) Images from a T-VEC-treated patient who achieved durable response



S100 (gray), CD3 (red) , CD4 (green), CD8 (blue)

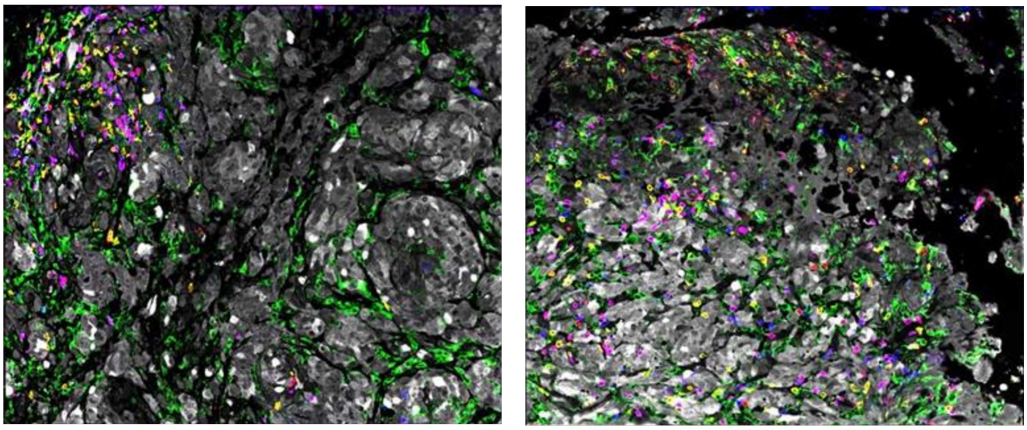


S100 (gray), CD3 (red) , CD8 (green), Ki67 (blue)

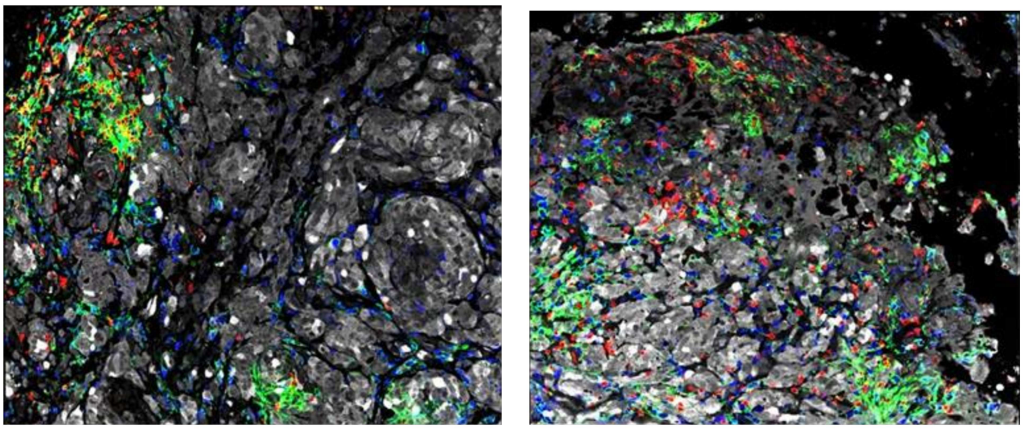


S100 (gray), CD3 (red) , PD-L1 (green), CD68 (blue)

(C) Images from a T-VEC-treated patient who achieved partial response



S100 (gray), CD3 (red) , CD4 (green), CD8 (blue)



S100 (gray), CD3 (red) , PD-L1 (green), CD68 (blue)

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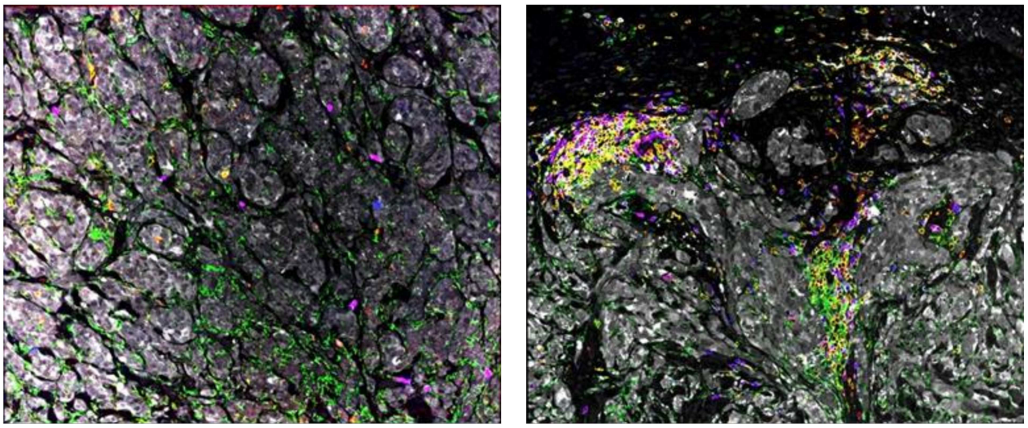
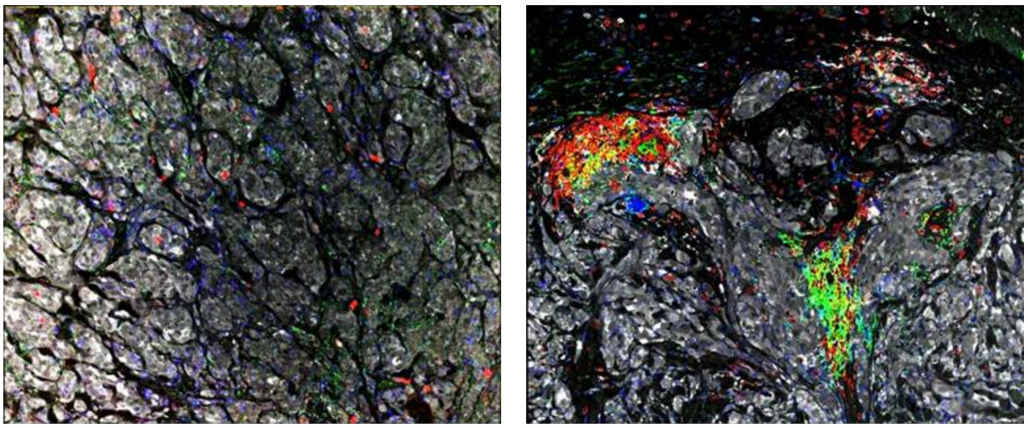
(D) Images from a T-VEC-treated patient who achieved partial response



S100 (gray), CD3 (red) , CD4 (green), CD8 (blue)



S100 (gray), CD3 (red) , PD-L1 (green), CD68 (blue)

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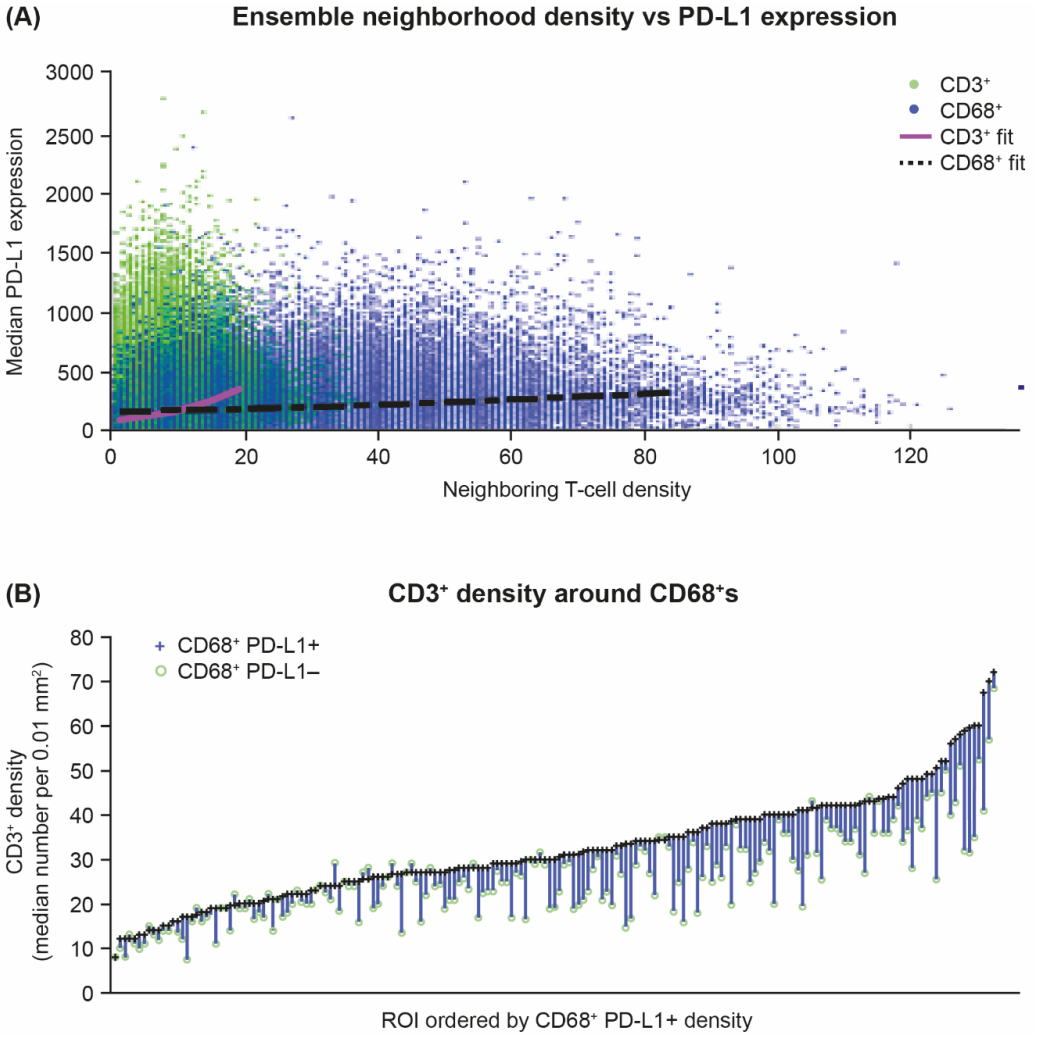
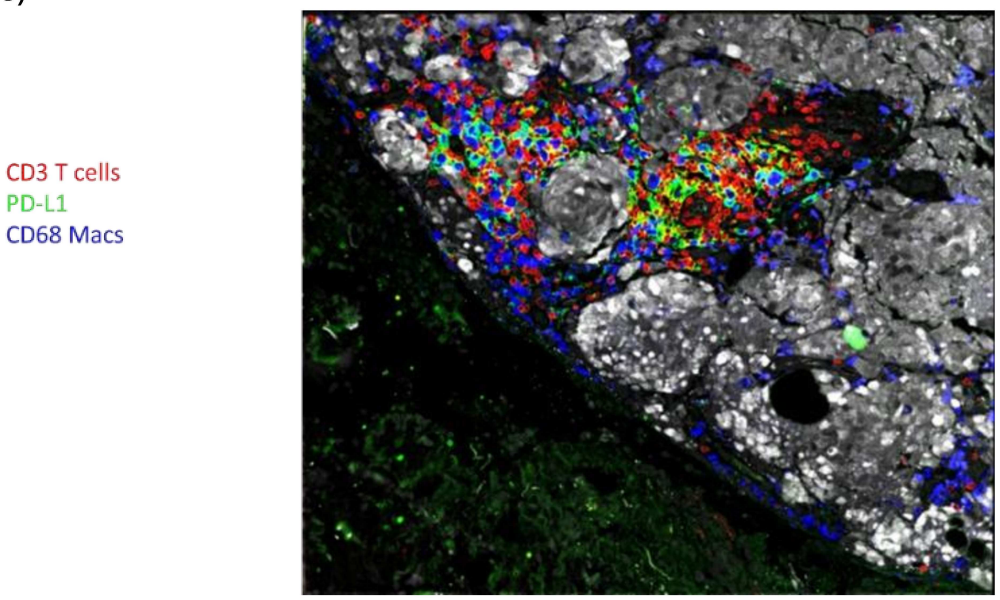
Supplementary Figure 4 Spatial analysis demonstrated that T-cells were often in close proximity to macrophages (Macs) expressing programmed death-ligand 1 (PD-L1).

T-cell proximity to PD-L1-expressing macrophages was assessed by reviewing each macrophage in each region of interest (ROI) for its PD-L1 expression level and the local density of T-cells, and vice versa. (A) Macrophage PD-L1 expression was correlated with local density of T-cell, and vice versa. (B) All regions of interest in the tumor (ROI) were analyzed for T-cell (CD3⁺) density around macrophages (CD68⁺ cells) either expressing PD-L1 or not. PD-L1+ macrophages (+) generally have more neighboring T-cells than PD-L1- macrophages (o). (C–E) PD-L1 expression on T-cells and macrophages increases when they are near each other. Example pseudocolor overlay images are presented with CD3⁺, PD-L1, and CD68⁺ colored red, green, and blue, respectively. S100 expression is colored white.



(C)



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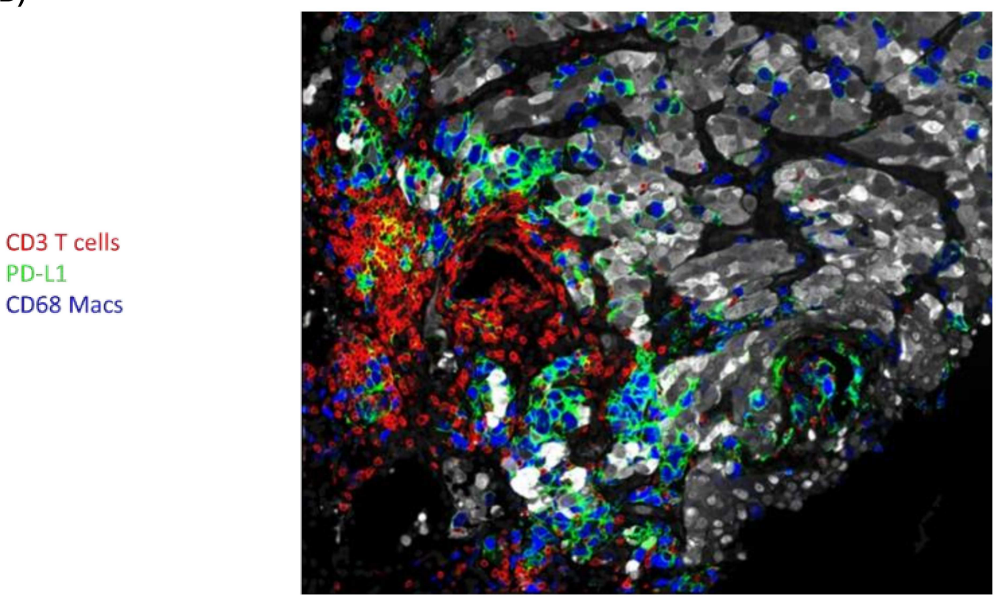

(E)

CD3 T cells
PD-L1
CD68 Macs