Supplementary Data for Reseach Brief:

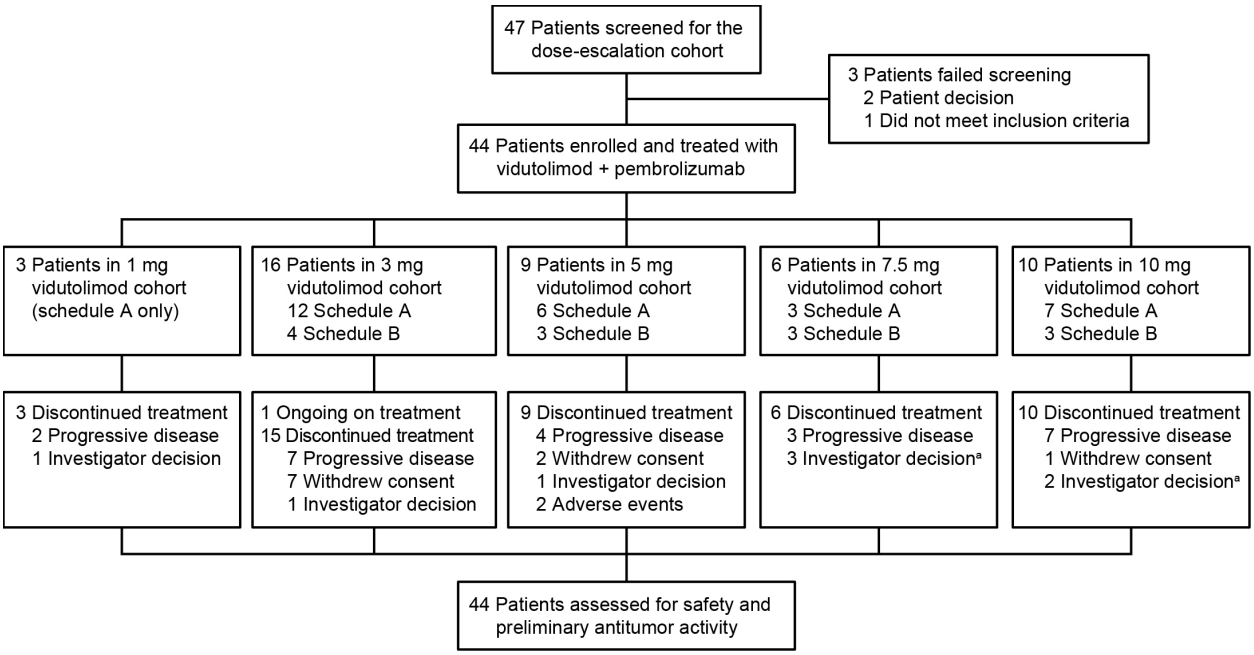
Overcoming PD-1 Blockade Resistance With CpG-A Toll-Like Receptor 9 Agonist Vidutolimod in Patients With Metastatic Melanoma

Prior therapies received for the patient shown in Fig. 1E

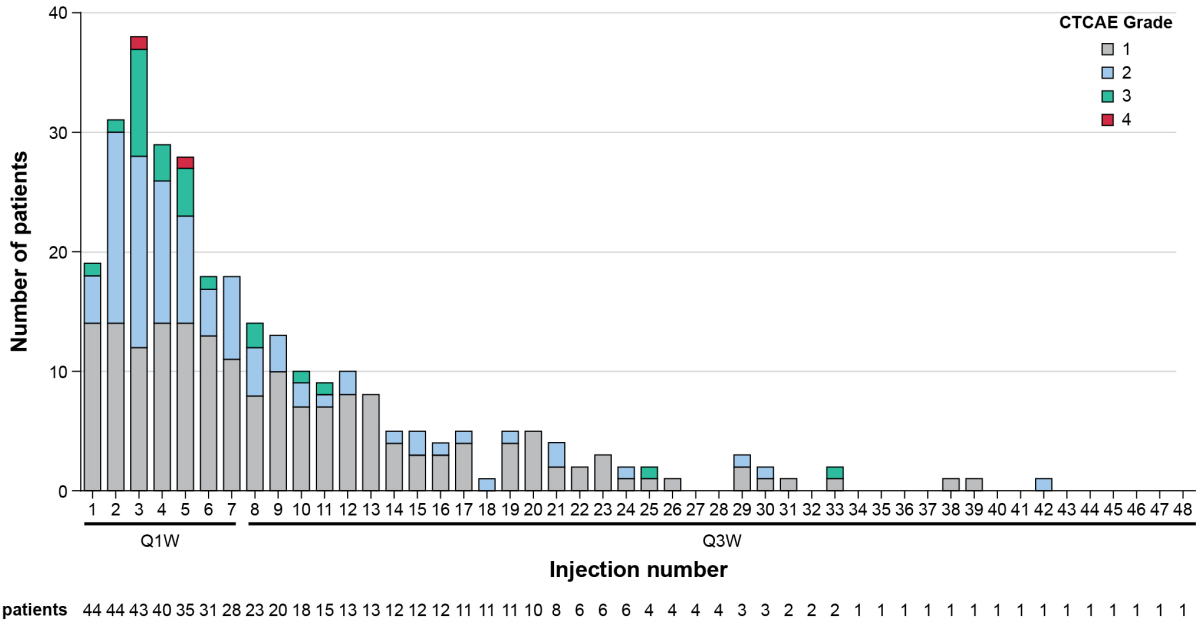
- Ipilimumab (adjuvant setting)
- Interferon-α (adjuvant setting)
- Pembrolizumab (best response of stable disease; last response of progressive disease)
- Aflibercept (best response of stable disease)
- Interleukin-2 (best response of stable disease)

Prior therapies received for the patient shown in Fig. 1F

- Interferon-α (best response of progressive disease)
- Ipilimumab (best response of progressive disease)
- Dabrafenib and trametinib (best response of partial response; last response of progressive disease)
- Pembrolizumab (best response of progressive disease)
- Dabrafenib and trametinib as a temporising measure immediately prior to study entry (response unknown)

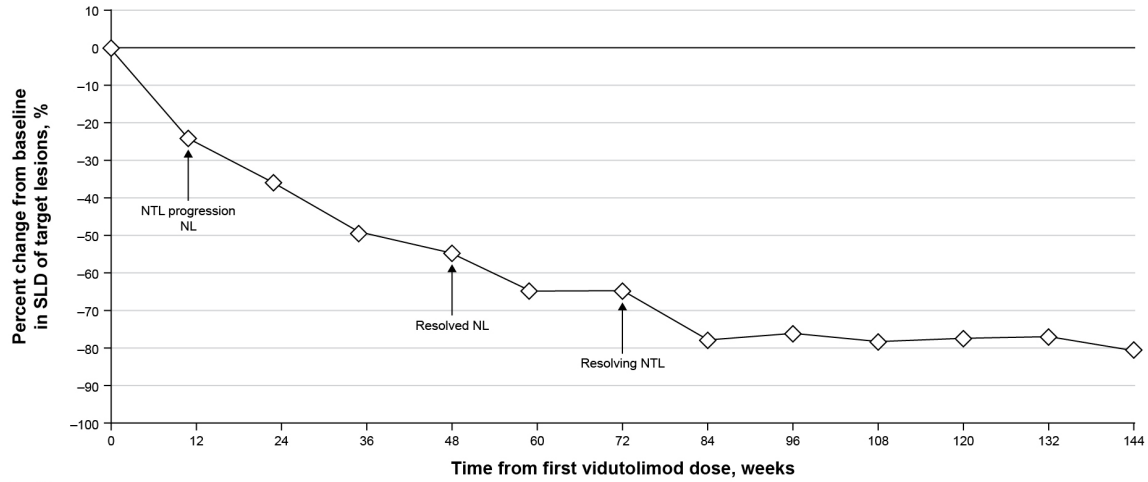


Supplementary Figure 1. Study flow diagram. Patient enrollment into each vidutolimod dose level, schedule of vidutolimod administration, and patient disposition at data cutoff are shown. The 1-mg vidutolimod dose level was evaluated with schedule A only; all other doses were evaluated with both dosing schedules. [a]Two patients (one in the 7.5-mg cohort and one in the 10-mg cohort) discontinued treatment based on investigator decision due to a lack of injectable tumor lesions, which was confirmed by negative biopsy.



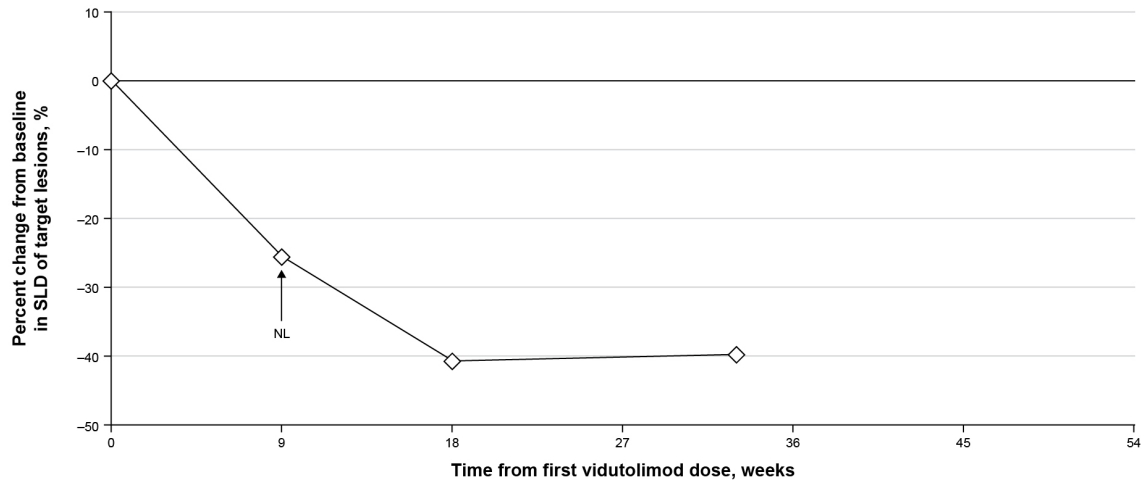
Supplementary Figure 2. Incidence of TRAEs with vidutolimod plus pembrolizumab treatment by vidutolimod injection number. Incidence of TRAEs during each injection cycle from the start of vidutolimod plus pembrolizumab therapy. Patients were counted only once for the highest-grade event within each injection cycle. All AEs shown were treatment emergent. CTCAE, Common Terminology Criteria for Adverse Events; Q1W, every week; Q3W, every 3 weeks.

A



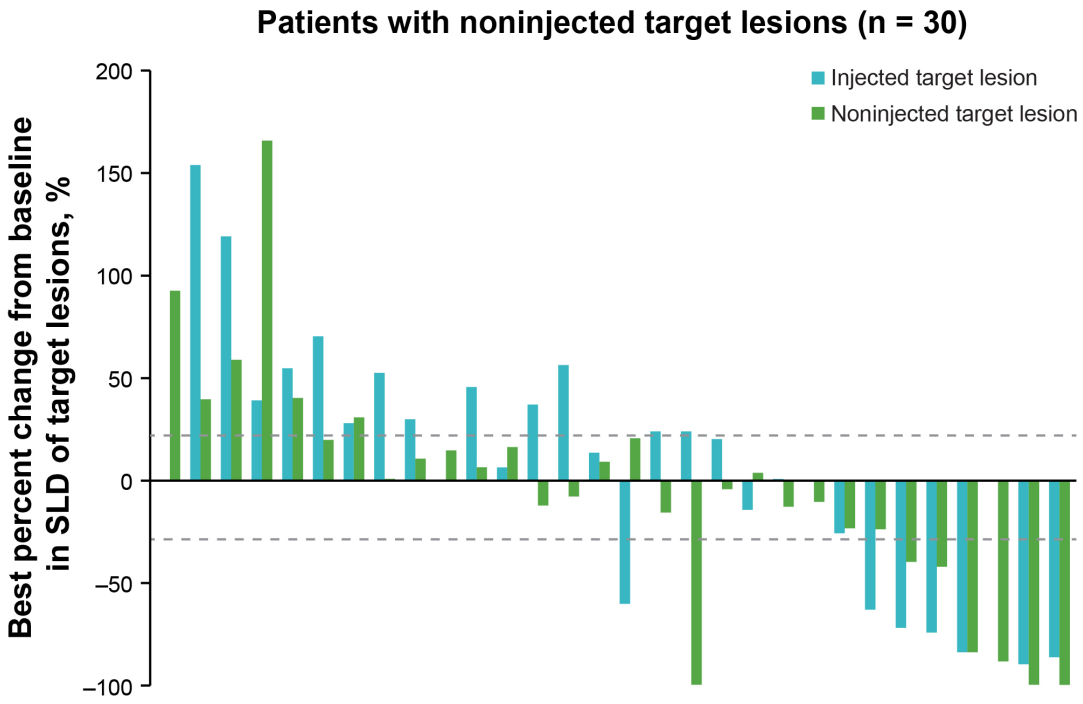
Target lesion size (percent change from baseline)	Baseline	Week 11	Week 23	Week 35	Week 48	Week 59	Week 72	Week 84	Week 96	Week 108	Week 120	Week 132	Week 144
T01	29.7	22.5 (–24.1%)	19.1 (–35.6%)	15.0 (–49.3%)	13.5 (–54.6%)	10.6 (–64.3%)	10.5 (–64.4%)	6.7 (–77.3%)	7.1 (–75.9%)	6.5 (–78.0%)	6.8 (–77.2%)	6.9 (–76.8%)	5.9 (–80.2%)
Nontarget lesions													
NTL01	Present	Progression	Progression	Progression	Stable	Stable	Resolved	Resolved	Resolved	Resolved	Resolved	Resolved	Resolved
NTL02	Present	Progression	Progression	Stable	Stable	Stable	Resolved	Resolved	Resolved	Resolved	Resolved	Resolved	Resolved
NTL03	Present	Progression	Progression	Stable	Stable	Stable	Resolved	Resolved	Resolved	Resolved	Resolved	Resolved	Resolved
NTL04	Present	NE	NE	NE	NE	NE	NE	Stable	Stable	Stable	Stable	Stable	Stable
New lesion													
NL01		Present	Stable	Stable	Resolved	Resolved	Resolved	Resolved	Resolved	Resolved	Resolved	Resolved	Resolved
Time response assessments													
RECIST	Undefined	PD	PD	PD	PR	PR	PR	PR	PR	PR	PR	PR	PR
iRECIST	Undefined	iUPD	iUPD	iUPD	iPR	iPR	iPR	iPR	iPR	iPR	iPR	iPR	iPR

B

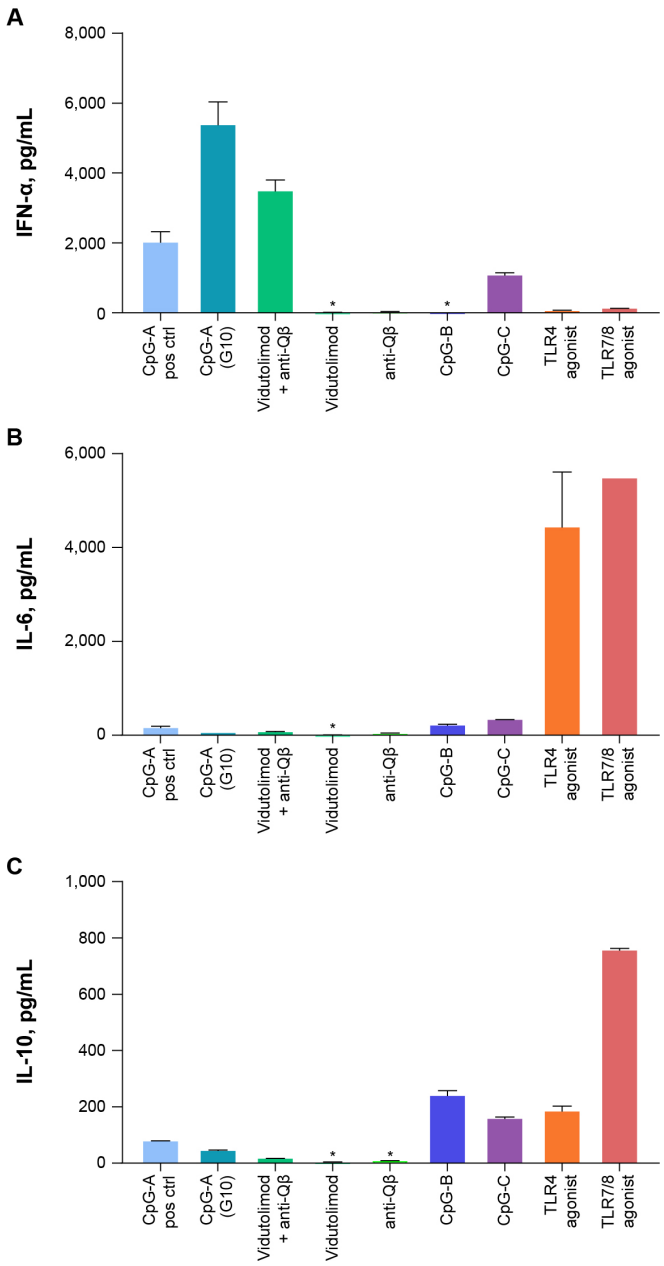


Target lesion size (percent change from baseline)	Baseline	Week 9	Week 18	Week 33	Week 42	Week 54
T01	16.2	0.0 (–100.0%)	0.0 (–100.0%)	0.0 (–100.0%)	0.0 (–100.0%)	0.0 (–100.0%)
T02	76.4	69.0 (–9.7%)	54.7 (–28.4%)	55.6 (–27.2%)	NE	NE
Target sum of diameters	92.6	69.0 (–25.5%)	54.7 (–40.9%)	55.6 (–39.9%)	NE[a]	NE[a]
Nontarget lesions						
NTL01	Present	Stable	Stable	Stable	NE	NE
NTL02	Present	Stable	Stable	Stable	NE	NE
NTL03	Present	Stable	Stable	Stable	Stable	Stable
NTL04	Present	Resolved	Resolved	Resolved	Resolved	Resolved
New lesion						
NL01		Present	Resolved[b]	Resolved[b]	Resolved[b]	Resolved[b]
Time response assessments						
RECIST	Undefined	PD	PR	PR	NE	NE
iRECIST	Undefined	iUPD	iPR	iPR	NE	NE

Supplementary Figure 3. iRECIST response in two patients with initial PD per RECIST v1.1. **(A)** The percent change in target lesion diameter and the status of nontarget lesions and new lesions over time in a patient with PR by iRECIST as assessed by blinded central review. This patient previously received granulocyte macrophage colony-stimulating factor (adjuvant setting), ipilimumab (adjuvant setting), and pembrolizumab (best response of CR), and this patient had PD as last response when vidutolimod plus pembrolizumab therapy began. **(B)** The percent change in the sum of target lesion diameters and the status of non-target lesions and new lesions over time in another patient with PR by iRECIST as assessed by blinded central review. This patient previously received ipilimumab (best response of PR), melphalan/dactinomycin (best response of PD), biochemotherapy (best response of PD), temozolomide (best response of PD), pembrolizumab (best response of PD), MLN-2480 (best response of PD), SD-101 (a CpG-C oligodeoxynucleotide) plus pembrolizumab (best response of PD), and entinostat plus pembrolizumab (best response of PD). [a]The target lesion used in central review was not included in field of view and therefore was not evaluable at week 42 and week 54 of follow-up. [b]New brain lesion underwent surgical resection. At week 12, the patient developed an acute central nervous system bleed and was found to have a metastasis. Since no baseline or prescreening head imaging had been performed, this lesion was designated as new, per RECIST v1.1, and was deemed as PD. Upon resection, the pathology showed significant necrosis and hemorrhage. No new central nervous system lesions appeared during subsequent therapy. iPR, PR by iRECIST; iRECIST, modified RECIST v1.1 for immune-based therapeutics; iUPD, unconfirmed PD by iRECIST; NE, not evaluable; NL, new lesion; NTL, nontarget lesion.

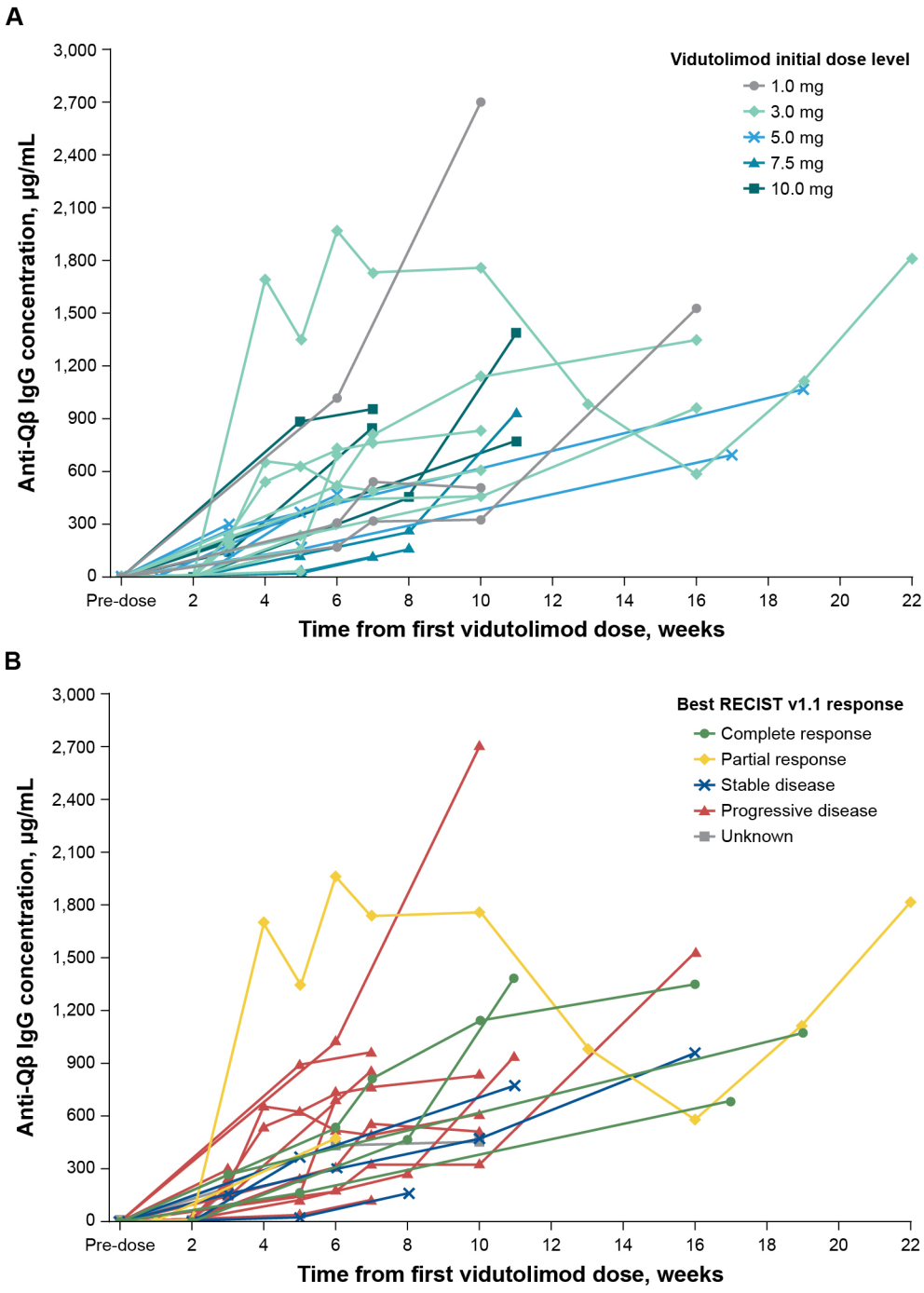


Supplementary Figure 4. Change in injected and noninjected target lesions. Percent change from baseline in the SLD of injected and noninjected target lesions for all dose-escalation patients with non-injected target lesions (*n* = 30).



Supplementary Figure 5. Cytokine induction. **(A)** Mean (± SD) concentration of IFN-α, **(B)** IL-6, and **(C)** IL-10 within PBMC supernatant after treatment with a CpG-A positive control (2216), G10 (naked CpG-A from vidutolimod), vidutolimod (G10+VLP) + anti-Qβ, vidutolimod alone, anti-Qβ alone, a CpG-B positive control (2006), a CpG-C agonist (SD-101), a TLR4 agonist (MPL), and a TLR7/8 agonist (R-848) in vitro. All samples were run in duplicate. The experiment

shown was one of three performed with similar results. * Values out of range (below the limit of quantitation).



Supplementary Figure 6. Immune response to vidutolimod. **(A)** The kinetics of anti-Qβ antibody induction by vidutolimod dose cohort are shown. No association between anti-Qβ antibody concentration and vidutolimod dose level was observed. **(B)** The lack of association of anti-Qβ antibody concentration with clinical response is depicted. Patients with unknown best

RECIST v1.1 response did not have a postbaseline scan. Analysis for both panels includes patients with available anti-Qβ data (*n* = 35). IgG, immunoglobulin G .

Supplementary Table 1. Baseline disease sites and objective response rate by site of baseline disease

Baseline disease sites	**No. of patients, N (% of N=44)**	**ORR by site of baseline disease, % (n/N)**
Skin only	3 (7)	67 (2/3)
Lymph nodes ± skin	6 (14)	33 (2/6)
Soft tissue ± skin and lymph nodes	7 (16)	29 (2/7)
Bone metastases without visceral metastases	2 (5)	0 (0/2)
Any visceral metastases	26 (59)	19 (5/26)
Lung metastases without other visceral metastases	8 (18)	25 (2/8)
Other visceral metastases ± lung metastases	17 (39)	18 (3/17)
Any liver metastases ± other visceral metastases	10 (23)	20 (2/10)
Any brain metastases ± other visceral metastases	1 (2)	0 (0/1)

Supplementary Table 2. Overall safety summary[a]

	Vidutolimod					
	1-mg Cohort (*n* = 3)	**3-mg Cohort (*n* = 16)**	**5-mg Cohort (*n* = 9)**	**7.5-mg Cohort (*n* = 6)**	**10-mg Cohort (*n* = 10)**	**All Patients (*N* = 44)**
Patients with at least one						
Any-grade AE, n (%)	3 (100)	16 (100)	9 (100)	6 (100)	10 (100)	44 (100)
Treatment-related	3 (100)	15 (94)	9 (100)	6 (100)	10 (100)	43 (98)
Grade 3/4 AE,[b] n (%)	1 (33)	11 (69)	6 (67)	3 (50)	6 (60)	27 (61)
Treatment-related	1 (33)	8 (50)	4 (44)	2 (33)	5 (50)	20 (45)
Grade 5 AE,[c] n (%)	0	0	1 (11)	0	0	1 (2)
Treatment-related	0	0	0	0	0	0
Serious AE, n (%)	2 (67)	9 (56)	4 (44)	1 (17)	4 (40)	20 (45)
Treatment-related	0	5 (31)	3 (33)	1 (17)	3 (30)	12 (27)
AE leading to treatment discontinuation,[d] n (%)	0	1 (6)	2 (22)	0	1 (10)	4 (9)

[a]All AEs shown were treatment emergent.

[b]AEs were manageable and transient.

[c]One pateint had a fatal AE (respiratory failure) that was assessed as unrelated to treatment.

[d]Two patients discontinued treatment because of TRAEs and two because of AEs related to disease progression.

Supplementary Table 3. Most common TRAEs[a]

Incidence, n (%)	Vidutolimod											
	1-mg Cohort (n=3)		3-mg Cohort (n=16)		5-mg Cohort (n=9)		7.5-mg Cohort (n=6)		10-mg Cohort (n=10)		All Patients (N=44)	
	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4
Patients with ≥1 TRAE[b]	3 (100)	1 (33)	15 (94)	8 (50)	9 (100)	4 (44)	6 (100)	2 (33)	10 (100)	5 (50)	43 (98)	20 (45)
TRAEs with ≥10% incidence in all patients												
Chills	2 (67)	0	11 (69)	1 (6)	6 (67)	0	5 (83)	1 (17)	10 (100)	0	34 (77)	2 (5)
Fever	2 (67)	0	12 (75)	1 (6)	7 (78)	1 (11)	1 (17)	0	7 (70)	0	29 (66)	2 (5)
Nausea	0	0	7 (44)	0	7 (78)	0	3 (50)	0	9 (90)	0	26 (59)	0
Fatigue	1 (33)	0	7 (44)	1 (6)	4 (44)	0	4 (67)	0	5 (50)	0	21 (48)	1 (2)
Vomiting	2 (67)	0	6 (38)	0	5 (56)	0	1 (17)	0	5 (50)	0	19 (43)	0
Headache	0	0	7 (44)	0	4 (44)	0	1 (17)	0	5 (50)	0	17 (39)	0
Hypotension[b]	0	0	7 (44)	3 (19)	4 (44)	2 (22)	2 (33)	1 (17)	4 (40)	1 (10)	17 (39)	7 (16)
Injection-site pain	0	0	5 (31)	0	3 (33)	0	3 (50)	0	4 (40)	0	15 (34)	0

Incidence, n (%)	Vidutolimod											
	1-mg Cohort (n=3)		3-mg Cohort (n=16)		5-mg Cohort (n=9)		7.5-mg Cohort (n=6)		10-mg Cohort (n=10)		All Patients (N=44)	
	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4
Diarrhea	1 (33)	0	3 (19)	0	3 (33)	0	3 (50)	0	3 (30)	0	13 (30)	0
Arthralgia	1 (33)	0	3 (19)	1 (6)	4 (44)	0	2 (33)	0	1 (10)	1 (10)	11 (25)	2 (5)
Decreased appetite	0	0	2 (13)	0	2 (22)	0	3 (50)	0	3 (30)	0	10 (23)	0
Dizziness	0	0	1 (6)	0	1 (11)	0	3 (50)	0	3 (30)	0	8 (18)	0
Rash	0	0	2 (13)	0	2 (22)	0	1 (17)	0	3 (30)	0	8 (18)	0
Constipation	1 (33)	0	2 (13)	0	1 (11)	0	2 (33)	0	1 (10)	0	7 (16)	0
Cough	1 (33)	0	1 (6)	0	3 (33)	0	0	0	1 (10)	0	6 (14)	0
Dyspnea	0	0	0	0	0	0	0	0	6 (60)	0	6 (14)	0
Injection-site erythema	0	0	1 (6)	0	2 (22)	0	2 (33)	0	1 (10)	0	6 (14)	0
Pruritus	0	0	1 (6)	1 (6)	2 (22)	0	2 (33)	0	1 (10)	0	6 (14)	1 (2)
Anemia	0	0	3 (19)	2 (13)	1 (11)	0	0	0	1 (10)	0	5 (11)	2 (5)

Incidence, n (%)	Vidutolimod											
	1-mg Cohort (n=3)		3-mg Cohort (n=16)		5-mg Cohort (n=9)		7.5-mg Cohort (n=6)		10-mg Cohort (n=10)		All Patients (N=44)	
	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4
Flushing	0	0	2 (13)	0	2 (22)	0	0	0	1 (10)	0	5 (11)	0

[a]All AEs shown were treatment emergent.

[b]No treatment-related grade 5 AEs (deaths) occurred.

[c]In response to unexpected grade ≥3 hypotension events in the 3-mg vidutolimod dose cohort, the cohort was expanded to 16 patients and a second dosing schedule was added.

Supplementary Table 4. Clinical response by on-study steroid use

On-Study Steroid Use[a]	All Patients (N=44)	
	Patients With CR or PR, n/N	ORR by RECIST v1.1, % (95% CI)
No steroids	5/24	21 (7–42)
≥1 administration of <10 mg prednisone equivalent per day	1/4	25 (1–81)
≥1 administration of >10 mg prednisone equivalent per day	5/16	31 (11–59)
≥1 administration of >10 mg and <30 mg prednisone equivalent per day	1/3	33 (1–91)
≥1 administration of >30 mg prednisone equivalent per day	4/13	31 (9–62)

CI, confidence interval.

[a]Patients counted once at their highest level of steroid use.